Serum Levels of Soluble Mesothelin-Related Peptides in Malignant and Nonmalignant Asbestos-Related Pleural Disease: Relation with Past Asbestos Exposure

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Abstract

Background: Malignant pleural mesothelioma (MPM) results from malignant transformation of mesothelial cells. Past asbestos exposure represents a major risk factor for MPM and other benign pleural disease. Soluble mesothelin-related peptides (SMRP) have been regarded as a promising serum biomarker for MPM. The aim of this study was to investigate serum levels of SMRP in malignant and nonmalignant asbestos-related pleural disease.

Patients: Four groups of patients were investigated: group 1 composed of 48 healthy subjects, group 2 composed of 177 patients with previous asbestos exposure and no pleural disease, group 3 composed of 36 patients with MPM, and group 4 composed of 101 patients with previous asbestos exposure and benign pleural disease. Serum SMRP levels were determined by ELISA.

Results: Serum SMRP levels were significantly higher among group 3 than the other three groups. There were no differences in SMRP concentrations between groups 2 and 4. Subjects exposed to asbestos had higher SMRP concentrations than normal control subjects regardless of the presence of pleural disease. The area under the receiver operating characteristic curve for SMRP values was 0.75 (95% confidence interval, 0.68-0.83). The SMRP level at 0.55 nmol/L/L was determined as the most optimal cutoff value with resulting sensitivity and specificity of 72% and 72% for the diagnosis of MPM.

Conclusions: These data attest to good diagnostic sensitivity and specificity of SMRP for the diagnosis of malignant mesothelioma. We have also shown that serum SMRP levels might serve as a marker of asbestos exposure.

Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with a poor survival rate arising from the mesothelial cells lining the pleura (1, 2). MPM is usually associated with history of chronic asbestos exposure (1). Unfortunately, early symptoms of MPM are generally nonspecific and can lead to a delay in a diagnosis. In this context, there has been an ever-growing effort focusing on the identification of biological indicators for the diagnosis of this malignancy (3). In recent years, soluble mesothelin-related peptides (SMRP) have emerged as a promising biomarker for MPM diagnosis (4, 5). Mesothelin, a 40-kDa glycosyl phosphatidylinositol-linked cell surface glycoprotein, is normally expressed at low levels in mesothelial cells but has been found to be overexpressed in MPM (3). Notably, SMRP can be detected in serum and are highly increased in patients with MPM and ovarian tumors (3, 6). Robinson et al. have also reported increased SMRP levels 1 to 5 years before the onset of MPM in some individuals exposed to asbestos, suggesting the potential usefulness of SMRP as a screening tool for MPM (4). In addition, elevated SMRP level in serum has been recently linked to a poor prognosis in MPM patients (7). The aim of this study was to investigate serum levels of SMRP in nonmalignant asbestos-related pleural abnormalities and MPM. We also wanted to assess whether serum SMRP concentrations might serve as a marker of asbestos exposure.

Materials and Methods

Study Design. This is a prospective, multicenter, cross-sectional study. In case of MPM, we used samples that were both collected prospectively and from our serum bank of patients diagnosed of MPM.

Four groups of patients were investigated: group 1 composed of 48 healthy comparison subjects, group 2 composed of 177 patients with previous asbestos exposure and no pleural disease, group 3 composed of 36 patients with MPM, and group 4 composed of 101 patients with previous asbestos exposure and benign pleural disease. All patients diagnosed with MPM
between January 2006 and December 2007 at our centers were recruited in group 3. Tests were also done with a bank of serum samples from Spanish MPM patients. Finally, a total of 36 serum samples of MPM were available, 20 prospectively and 16 from the bank. The criteria for diagnosis of MPM in these patients was the same for the entire group. It was established at surgical biopsy, image-guided biopsy, immunohistochemistry, and/or fluid cytologic analysis following our study protocol.

The diagnostic methodology, histologic types (epithelial, sarcomatous, mixed), radiologic stage, and survival time were carefully recorded in all MPM patients.

Patients in groups 2 and 4 were recruited via a specific consultancy service for workers who have been exposed to asbestos (8). These subjects were required to have at least 1 year of exposure. This timeframe was chosen because shorter periods are rarely associated with development of a measurable respiratory risk (1). Subjects in group 2 had no evidence of pleural disease by chest radiographs and pulmonary function tests. Subjects in group 4 had radiologic evidence of nonmalignant pleural disease. Asbestos-related pleural abnormalities were classified in accordance to the Programme of the Spanish Ministry of Health and Consumer Affairs (9). A total of 48 healthy subjects without exposure to asbestos served as controls (group 1). Control subjects were recruited mainly from healthy volunteers. The research ethics board at our hospital approved this study, and informed consent was obtained from all participants.

Data Collection. The following data were collected in all participants: age, gender, area of residence, working history, medical history, and history of exposure to asbestos. The accepted methodology to quantitate the degree of exposure are the years exposed to asbestos (year of first exposure, year of last exposure, and type of industry in which the exposure occurred).

Table 1. General characteristics of the study participants

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>48</td>
<td>177</td>
<td>36</td>
</tr>
<tr>
<td>Age ± SD, y</td>
<td>30 ± 7</td>
<td>52 ± 10</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46 (95.6)</td>
<td>170 (96)</td>
<td>28 (77.77)</td>
</tr>
<tr>
<td>Female</td>
<td>2 (4.2)</td>
<td>7 (4)</td>
<td>8 (22.23)</td>
</tr>
<tr>
<td>Time of exposure, y (mean ± SD)*</td>
<td>Not applicable</td>
<td>12 ± 10</td>
<td>18 ± 12 data of 15 from total patients</td>
</tr>
<tr>
<td>Type of exposure, n (%)*</td>
<td>Not applicable</td>
<td>Asbestos cement 5 (2.8)</td>
<td>Asbestos cement 3 (20)</td>
</tr>
<tr>
<td></td>
<td>Shipyard 72 (40.8)</td>
<td>Shipyard 10 (66)</td>
<td>Shipyard 66 (65.3)</td>
</tr>
<tr>
<td></td>
<td>Insulation 41 (23.16)</td>
<td>Building 2 (14)</td>
<td>Insulation 14 (13.8)</td>
</tr>
<tr>
<td></td>
<td>Building 17 (9.6)</td>
<td></td>
<td>Building 9 (8.9)</td>
</tr>
<tr>
<td></td>
<td>Others 42 (23.72)</td>
<td></td>
<td>Others 10 (10.1)</td>
</tr>
<tr>
<td>Type of disease, n (%)</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>Pleural effusion 20 (19.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Asbestosis 11 (10.8)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Pleural plaques 22 (21.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Asbestosis + pleural plaques 14 (13.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pleural fibrosis 26 (25.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rounded atelectasis 8 (7.9)</td>
</tr>
</tbody>
</table>

NOTE: Group 1: Healthy comparison subjects. Group 2: Patients with previous asbestos exposure and no pleural disease. Group 3: Patients with MPM. Group 4: Patients with previous asbestos exposure and benign pleural disease.

*Data in group 3 about time and type of exposition are incomplete because of retrospective collection in some cases. We present time and type of exposure from 15 patients.

Assay of SMRP Levels. Sera were stored at −20°C until the level of SMRP was determined with a commercial ELISA kit (Mesomark; Fujirebio Diagnostics) according to the manufacturer’s instructions (6). All measurements were done in duplicate and in random order. Results are expressed in nmol/L. Laboratory personnel were blinded to clinical status of the study participants.

Statistical Analysis. Data were expressed as means (SD), medians (interquartile ranges) and counts, as appropriate. Data of SMRP levels of four groups are not normally distributed. The Mann-Whitney U test was applied for two-group comparisons of serum SMRP levels. The Spearman’s test was applied for correlations between variables. Differences in normally distributed variables were analyzed by the unpaired Student’s t test. The ability of serum SMRP to differentiate between MPM and those exposed to asbestos was estimated based on sensitivity and specificity at various cutoff levels. We take MPM patients as true positives. The best statistical “cutoff” was calculated by minimizing the distance between the point with specificity = 1 and sensitivity = 1 and the points on the receiver operating characteristic (ROC) curve. Areas under ROC curves are reported with their 95% confidence intervals. An area under the ROC curve of 1.0 indicates perfect discrimination, whereas an area of 0.5 indicates that the test discriminates no better than chance. Results were considered significant if two-tailed P values < 0.05. All analyses were done with SPSS (release 14.0; SPSS).

Results

The general characteristics of the study participants are depicted in Table 1. Subjects with MPM were significantly older (mean age, 64.0 ± 5.0 years) than healthy comparison subjects (mean age, 30.7 ± 7.5 years; P < 0.001). In contrast, no significant difference with
Table 2. Serum SMRP levels in the study participants

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMRP (nmol/L/L)</td>
<td>0.23*</td>
<td>0.41</td>
<td>0.79†</td>
<td>0.40</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.35-0.38</td>
<td>0.30-0.65</td>
<td>0.46-1.74</td>
<td>0.31-0.63</td>
</tr>
<tr>
<td>Min-max</td>
<td>0.3-1.1</td>
<td>0.3-2.4</td>
<td>0.3-12.1</td>
<td>0.3-2.4</td>
</tr>
</tbody>
</table>


*P < 0.001, group 1 compared with groups 2 to 4.
†P < 0.001, group 3 compared with groups 1, 2, and 4.

regard to age was seen between patients exposed to asbestos with benign pleural pathology (mean age, 58.3 ± 6.6 years) and those exposed to asbestos and no evidence of pleural pathology (mean age, 52.3 ± 10.5 years). The mean duration of exposure to asbestos was significantly higher in patients exposed to asbestos with benign pleural pathology (mean duration, 17.9 ± 10.6 years) compared with those without (mean duration, 12.8 ± 10.1 years; P < 0.001). There was no significant gender difference in the study groups.

Serum SMRP levels in the study subjects are shown in Table 2 and Fig. 1. Patients with MPM had higher SMRP concentrations (0.79 ± 2.61 nmol/L) than patients exposed to asbestos with benign pleural pathology (0.40 ± 0.14 nmol/L; P < 0.001), subjects exposed to asbestos and no evidence of pleural pathology (0.41 ± 0.15 nmol/L; P < 0.001), and healthy comparison subjects (0.23 ± 0.07 nmol/L; P < 0.001). No differences were seen in SMRP values in patients exposed to asbestos with benign pleural pathology, compared with those without (P = 0.69). On the other hand, subjects exposed to asbestos, either with or without benign pleural pathology, had higher SMRP levels than controls (P < 0.001; Fig. 1). As can be seen in Fig. 2, the area under the ROC curve for SMRP values was 0.75 (95% confidence interval, 0.68-0.83). The SMRP level at 0.55 nmol/L was determined as the most optimal cutoff value with resulting sensitivity and specificity of 72% and 72% for the diagnosis of MPM.

Discussion

The incidence of MPM is increasing throughout several countries, and it is predicted that it will increase in the next 10 to 15 years as a result of widespread exposure to asbestos in past decades (10). Despite recent advances in assessment and treatment, the prognosis in MPM remains poor (1). In this context, early diagnosis offers the best hope for a favorable prognosis. There is a clear need for sensitive and reliable biological markers that are able to show the presence of MPM before an individual exposed to asbestos has reached the stage of clinical diagnosis (11, 12). Mesothelin, a cell surface protein highly expressed in mesothelioma (13), is shed by tumor cells and can be detected in the serum as SMRP by means of sandwich ELISA (3, 6).

The present study supports the growing evidence that serum SMRP levels are elevated in patients with MPM (14, 15), thereby confirming the potential usefulness of this biomarker in the clinical practice. Notably, we have also shown that serum SMRP concentrations are higher in patients with MPM than in people exposed to asbestos with benign pleural disease.

Levels of SMRP as detected in our MPM patients were similar but lower than those reported by Scherpereel et al. (5), who found a median SMRP level of 2.05 nmol/L in malignant mesothelioma and 0.5 nmol/L in benign pleural disease. Their SMRP levels have a wider range (0-28.84 compared with 0.3-12.1 nmol/L) for MPM patients.

SMRP levels are not normally distributed, so the median value has high variability and could be influenced by data dispersion. In the study by Scherpereel et al. (5), which did not include a control group, the area under the ROC curve for SMRP levels was 0.77 (95% confidence interval, 0.68-0.85). In contrast, Pass et al. (16) found a median SMRP level of 17.27 nmol/L in MPM and 1.11 nmol/L in benign pleural disease. In that study published as abstract, there are some methodologic concerns: their SMRP values had a extremely high data dispersion (0.29-278.356 nmol/L), that is, 10- and 20-fold the upper limit found by Scherpereel et al. (5) and in our study, respectively.

The characteristics of the groups are not clearly described because these authors compared their MPM group versus a “normal volunteers” group that surprisingly presented pleural effusion. Therefore, our healthy group was much younger than the other three groups because we included only people without any other disease or possible exposition. This could raise concern about the group comparison, but we do not
believe that this could be a major confounding factor because SMRP levels are not age-related but asbestos exposure-related.

The SMRP level at 1.1 nmol/L was determined as the most optimal cutoff value with resulting sensitivity and specificity of 71.7% and 69.8% for the diagnosis of MPM (5). In the present study, a cutoff value at 1.1 nmol/L for SMRP concentrations would yield a 97.2% specificity but a low 24% sensitivity for the diagnosis of MPM. It is thus possible that high serum SMRP levels in patients exposed to asbestos and signs of pleural disease should be interpreted as suggestive of MPM; on the other hand, low SMRP concentrations should be interpreted cautiously.

In this study, there was a highly significant difference in serum SMRP values between healthy controls and patients exposed to asbestos with benign pleural pathology (0.23 versus 0.40 nmol/L respectively, \( P < 0.001 \)) or without pleural pathology (0.23 versus 0.41 nmol/L; \( P < 0.001 \)). These data indicate that an increased release of SMRP in the serum may occur as a consequence of asbestos exposure. It would be expected that there is a higher median SMRP value for the group with more years of exposure when we performed a correlation between time of exposition and SMRP levels (Fig. 3), which is very poor (Spearman correlation test 0.194; \( P = 0.50 \)). Maybe the explanation is because our study is not powered for that issue. The disease may occur up to 30 to 40 years after exposure, so we need a longitudinal study to elucidate that very important question. Robinson et al. found that 7 of the 40 asbestos-exposed individuals had increased serum concentrations of SMRP; 3 of those 7 developed MPM and 1 developed lung carcinoma within 1 to 5 years (4). These findings hold promise that serum SMRP concentrations might serve as a biomarker for screening of MPM in individuals exposed to asbestos (17, 18). In this study, however, no difference was seen in SMRP concentrations between patients exposed to asbestos either with or without benign pleural pathology (0.41 versus 0.40 nmol/L, respectively). The absence of a significant difference may be due to the low study power and to the long latency time between exposure to asbestos and the evidence of damage. Long-term longitudinal studies are needed to establish whether early diagnostic tests, including pleuroscopy and biopsy, are warranted in individuals exposed to asbestos and high SMRP levels.

In conclusion, our current data confirm the good diagnostic sensitivity and specificity of SMRP for the diagnosis of malignant mesothelioma. We have also shown that SMRP might also serve as a marker of asbestos exposure. Further longitudinal studies are needed to investigate SMRP as a biomarker of MPM development in individuals exposed to asbestos.

**Disclosure of Potential Conflicts of Interest**

All authors have no conflicts of interest to disclose.

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**References**

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