Serial Measurements of Mesothelioma Serum Biomarkers in Asbestos-Exposed Individuals

A Prospective Longitudinal Cohort Study

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Introduction: Soluble mesothelin (SM) and megakaryocyte potentiating factor (MPF) are serum biomarkers of mesothelioma. This study aims to examine the longitudinal behavior of SM and MPF in controls to gain insight in the optimal use of these biomarkers in screening.

Methods: Asbestos-exposed individuals, with no malignant disease at inclusion, were surveilled for 2 years with annual measurements of SM and MPF. Fixed thresholds were set at 2.10 nmol/L for SM and 13.10 ng/ml for MPF. Longitudinal biomarker analysis, using a random intercept model, estimated the association with age and glomerular filtration rate (GFR), and the intraclass correlation. The latter represents the proportion of total biomarker variance accounted for by the between-individual variance.

Results: A total of 215 participants were included, of whom 179 and 137 provided a second sample and third sample, respectively. Two participants with normal SM and MPF levels presented afterward with mesothelioma and lung cancer, respectively. Participants with elevated biomarker levels were typically older and had a lower GFR. During follow-up, biomarker levels significantly increased. Longitudinal analysis indicated that this was in part due to aging, while changes in GFR had a less pronounced effect on serial biomarker measurements. SM and MPF had a high intraclass correlation of 0.81 and 0.78, respectively, which implies that a single biomarker measurement and fixed threshold are suboptimal in screening.

Conclusions: The longitudinal behavior of SM and MPF in controls indicates that a biomarker-based screening approach can benefit from the incorporation of serial measurements and individual-specific screening rules, adjusted for age and GFR. Large-scale validation remains nevertheless mandatory to elucidate whether such an approach can improve the early detection of mesothelioma.

Key Words: Soluble mesothelin, Megakaryocyte potentiating factor, Mesothelioma, Screening, Asbestos.

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Malignant pleural mesothelioma is a rare asbestos-related malignancy with a fatal outcome.1 Because of its nonspecific presenting symptoms, patients often suffer a substantial diagnostic delay, resulting in a more advanced disease at diagnosis. An adequate screening program and subsequent earlier detection might improve patient outcome.2 Current guidelines on mesothelioma management do, however, not advocate the use of screening and recommend that the efficacy of any screening tool should be further evaluated in high-risk populations.1 Although serum biomarkers such as CA125 and cytokeratins were found to be ineffective in mesothelioma screening,2 the advent of more accurate serum biomarkers of mesothelioma, such as soluble mesothelin (SM) and megakaryocyte potentiating factor (MPF),3,4 could change prospects. We have recently shown that these two biomarkers, which both originate from the mesothelin precursor protein,5 are highly correlated and have an equivalent diagnostic performance.6 While MPF is not yet evaluated in mesothelioma screening, the use of a single SM measurement and a fixed threshold reveals disappointing results in retrospective screening studies.7–9 Nevertheless, it has been demonstrated, especially in ovarian cancer screening, that a single biomarker measurement and fixed screening threshold are suboptimal in the presence of a high between-individual biomarker variance, and screening can be improved by incorporating serial biomarker measurements and individual-specific screening rules.10,11
To determine whether SM and MPF are suitable for such screening approach, it is important that the normal (longitudinal) behavior of these biomarkers is first ascertained.10 Serial SM and MPF measurements have, however, not been prospectively assessed in controls. In addition, although we and others have recently shown that both age and GFR affect single SM and MPF measurements12–14 (Hollevoet et al., unpublished data), it is unclear to what extent longitudinal changes of these clinical covariates affect serial biomarker measurements and should be accounted for.

The aim of this prospective study is to examine the normal longitudinal behavior of SM and MPF, including biomarker variance and the effect of age and GFR, to gain insight in the optimal use of these biomarkers in screening. We, therefore, surveilled a cohort of asbestos-exposed individuals, with no malignant disease at inclusion, for 2 years with annual biomarker measurements.

PATIENTS AND METHODS

Participants

Between November 2007 and July 2010, individuals with professional asbestos exposure, and no malignant disease, were consecutively enrolled and surveilled for 2 years at either the Belgian Occupational Diseases Fund, the four participating departments of Respiratory Medicine, or two companies with a history of asbestos processing. At inclusion, a blood sample was taken, and participants were appropriately examined to assess the presence of a benign asbestos-related condition and exclude malignant disease. After approximately 1 and 2 years, participants were invited, by written notice and telephone conversation, for a follow-up visit, at which blood sampling was repeated and a brief health inquiry was conducted. Because of privacy regulation, no motivation was requested when participants declined the invitation. If the participant reported no complaints, no additional examinations were performed. Serum samples were coded and stored in aliquots at −80°C. This study was approved by the ethics committee of all participating hospitals, and all participants gave written informed consent before inclusion. Biomarker test results were handled in a double-blinded fashion and not communicated to participants and responsible physicians.

Characteristics

Age and GFR (ml/min/1.73 m²) were recorded at inclusion and follow-up. GFR was estimated with the Chronic Kidney Disease Epidemiology Collaboration equation,15 based on serum creatinine levels. A GFR above 90 ml/min/1.73 m² is considered normal, whereas a lower GFR represents a decrease in renal function.15 The quantity of past asbestos exposure was estimated in fiber years. Participants were then interviewed by trained staff of the Occupational Diseases Fund using a standardized questionnaire.16 One fiber year equals the exposure of 10⁶ fibers/m³ during 1 year.

Biomarker Assays

Serum SM (nmol/L) and MPF levels (ng/ml) were measured using the Mesomark (Cis bio International, Gif sur Yvette, France) and Human MPF ELISA kit (Medical & Biological Laboratories, Nagano, Japan), respectively, according to the manufacturer’s instructions.4,17 Analyses were performed in a single laboratory, blinded to the coded sample data, shortly after sample collection. SM and MPF levels were considered elevated when exceeding 2.10 nmol/L and 13.10 ng/ml, respectively. These fixed thresholds were obtained by differentiating the baseline biomarker levels of the asbestos-exposed participants and 106 patients with mesothelioma at a specificity of 95% (Hollevoet et al., unpublished data).

Statistical Analysis

Variables are reported as median with 25th to 75th percentiles. Biomarker levels were compared between independent and dependent groups using the nonparametric Mann-Whitney U and Wilcoxon test, respectively. Spearman’s rank analysis determined the correlation between the different variables. Longitudinal analysis of SM and MPF was done using residual maximum likelihood estimation under a random intercept model with a residual autoregressive covariance structure, with age and GFR as covariates (allowing for both within- and between-individual age and GFR effects). This allowed to adequately account for dropouts and differences in sampling time among participants. As we focused on the biomarker behavior in controls, participants who developed a malignancy during follow-up were excluded from this analysis. Biomarker levels were transformed on the common logarithmic scale to improve model fit. To enable interpretation on the original scale, the reported model’s coefficients were exponentiated (i.e., raised to the 10th power). The variances of the random intercept (σ²b) and the residual error term (σ²ε) represent the between- and within-individual variance of the logarithmic biomarker levels, respectively. The 95% prediction intervals at a given age and GFR stretch out up to a factor \( \exp(\pm 1.96 \times \sigma) \) from the corresponding geometric mean, where \( \sigma^2 = \sigma^2_b + \sigma^2_e \). Intraclass correlation, the proportion of total variance in biomarker levels accounted for by the between-individual variance, was estimated as \( \sigma^2_b / (\sigma^2_b + \sigma^2_e) \). A high intraclass correlation is consequently suggestive of a substantial between-individual biomarker variance, relative to the total variance in the data. This implies that a substantial improvement in screening can be obtained when using information from serial biomarker measurements, as compared to using a single measurement and fixed threshold.10,11 All hypothesis tests were performed two sided at the 5% level of significance. Statistical analysis was done with statistical software SPSS (version 17.0, Chicago, IL) and SAS (version 9.2, SAS Institute, Cary, NC).

RESULTS

Participants

A total of 215 individuals were included, of whom 126 (59%) had a benign asbestos-related condition, including pleural plaques (n = 71), diffuse pleural thickening (n = 39), and other lesions, mainly asbestos (n = 16). Eighty-nine (41%) had no radiologically obvious asbestos-related lesions. A second sample and third blood sample were obtained in 179 (84%) and 137 (64%) individuals (Table 1), respectively,
with a median follow-up time after inclusion of 12.2 months (11.8–13.0) and 24.2 months (22.0–24.6), respectively.

### Biomarker Levels

At inclusion, SM and MPF levels positively correlated with age \( (r_{\text{SM}} = 0.44, r_{\text{MPF}} = 0.31, p < 0.001) \) and inversely with GFR \( (r_{\text{SM}} = -0.32, r_{\text{MPF}} = -0.24, p < 0.001) \). When using the fixed SM and MPF thresholds, 20 and 21 participants, respectively, had an elevated level, either at baseline or follow-up, of whom 14 had both biomarker levels above threshold (Table S1, Supplementary data). In all participants with an elevated SM and MPF level and follow-up samples available, respectively, 10 and nine, biomarker levels remained above the threshold. At each sampling point, those with an elevated biomarker level were significantly older and had a lower GFR than those with normal biomarker levels \( (p < 0.001, \text{Table S1, Supplementary data}) \).

### Severe Events

During the course of the study, seven severe events, including four deaths, were reported, all in individuals with a benign asbestos-related condition at inclusion (Table 2). Three participants presented a malignancy during follow-up, including one malignant pleural mesothelioma. After his initial inclusion in May 2008, the second blood sample was only obtained approximately 2 years later, because the patient did not respond to the first follow-up invitation. At this follow-up, the patient reported pleurisy and a persistent cough. A pleural effusion was found, and thoracoscopy biopsy further established the diagnosis of a stage III epithelioid mesothelioma. Interestingly, this patient had normal and stable biomarker levels. Elevated biomarker levels were only observed in the patient with a prostate cancer and the individual who had a paralyzing stroke. Noteworthy, these two were among the elder of the seven participants and had the lowest GFR. Similarly, the individuals with the lowest biomarker levels were also the youngest and had the highest GFR (Table 2).

### Asbestos Exposure

Fiber years were estimated in 204 participants (95%). In patients with a benign asbestos-related condition, fiber years did not differ with the type of condition \( (p = 0.66) \) but were significantly higher \( (40.0 \text{ fiber years}, 22.0–116.8) \) than in the healthy asbestos-exposed \( (15.8 \text{ fiber years}, 6.1–40.6, p < 0.001) \). At baseline, fiber years positively correlated with SM \( (p < 0.05, r = 0.15) \) and age \( (p < 0.001, r = 0.37) \), inversely with GFR \( (p < 0.01, r = -0.21) \), and not with MPF \( (p = 0.13, r = 0.11) \). A multiple (forward) linear regression analysis with fiber years as response variable revealed an independent association with age \( (p < 0.05) \), whereas no association with SM \( (p = 0.73) \), MPF \( (p = 0.98) \), or GFR \( (p = 0.44) \) was found.

### Longitudinal Behavior of the Biomarker Levels

The biomarker levels were strongly correlated across the three sampling points (Figure 1). Nevertheless, in the 137 participants of whom all three samples were available, SM and MPF levels significantly increased \( (p < 0.001) \) during follow-up (Figure 2). Of interest, the longitudinal biomarker analysis revealed a significant effect of aging on the serial measurements \( (p_{\text{SM}} < 0.001, p_{\text{MPF}} < 0.001) \) (Table 3). For example, a 1-year increase in age increased geometric mean SM and MPF levels with 18% \((95\% \text{ CI} = 15–20\%)\) and 14% \((95\% \text{ CI} = 12–17\%)\), respectively. In contrast, the effect of GFR was less pronounced and only significant in SM \( (p_{\text{SM}} < 0.001, p_{\text{MPF}} = 0.39) \) (Table 3). For example, a decrease of 10

**TABLE 1. Baseline and Follow-Up Characteristics**

<table>
<thead>
<tr>
<th>Number</th>
<th>First Follow-Up</th>
<th>Second Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>(female)</td>
<td>215 (18)</td>
<td>179 (16)</td>
</tr>
<tr>
<td>Healthy/ARD (%)</td>
<td>41/59</td>
<td>49/51</td>
</tr>
<tr>
<td>SM (nmol/L)</td>
<td>0.96 (0.74–1.37)</td>
<td>1.18 (0.96–1.54)</td>
</tr>
<tr>
<td>MPF (ng/ml)</td>
<td>6.64 (5.20–8.88)</td>
<td>7.60 (6.16–10.00)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>55.7 (51.9–66.1)</td>
<td>55.8 (52.4–64.8)</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>79.5 (66.2–90.8)</td>
<td>78.5 (66.7–88.5)</td>
</tr>
</tbody>
</table>

**TABLE 2. Severe Events and Characteristics**

<table>
<thead>
<tr>
<th>Condition at Inclusion</th>
<th>Event</th>
<th>SM (nmol/L)</th>
<th>MPF (ng/ml)</th>
<th>Age (yr)</th>
<th>GFR (ml/min/1.73 m²)</th>
<th>Available Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaques</td>
<td>Epithelioid pleural mesothelioma</td>
<td>1.76</td>
<td>10.56</td>
<td>75.9</td>
<td>47.7</td>
<td>1.61</td>
</tr>
<tr>
<td>Asbestosis and plaques</td>
<td>Fatal lung cancer</td>
<td>0.64</td>
<td>5.04</td>
<td>64.0</td>
<td>85.3</td>
<td>—</td>
</tr>
<tr>
<td>DPT</td>
<td>Prostate cancer</td>
<td>3.51</td>
<td>26.48</td>
<td>79.7</td>
<td>33.1</td>
<td>—</td>
</tr>
<tr>
<td>DPT and plaques</td>
<td>Paralyzing stroke</td>
<td>2.98</td>
<td>17.28</td>
<td>78.9</td>
<td>19.4</td>
<td>—</td>
</tr>
<tr>
<td>DPT and plaques</td>
<td>Fatal cardiac failure</td>
<td>0.85</td>
<td>5.32</td>
<td>66.9</td>
<td>88.9</td>
<td>—</td>
</tr>
<tr>
<td>Asbestosis</td>
<td>Fatal cardiac failure</td>
<td>1.52</td>
<td>9.28</td>
<td>74.0</td>
<td>53.3</td>
<td>—</td>
</tr>
<tr>
<td>Plaques</td>
<td>Deceaseda</td>
<td>1.76</td>
<td>8.12</td>
<td>84.6</td>
<td>61.7</td>
<td>2.09</td>
</tr>
</tbody>
</table>

a Unknown cause.

DPT, diffuse pleural thickening; SM, soluble mesothelin; MPF, megakaryocyte potentiating factor; GFR, glomerular filtration rate.
ml/min/1.73 m² resulted in an increase of 4% (95% CI = 1–6%) in geometric mean SM levels, respectively. Furthermore, aging accounted for 22% and 15% of the within-individual SM and MPF variance, respectively, whereas changes in GFR explained only 1% for both biomarkers. Combining between- and within-individual biomarker variance resulted in a high intraclass correlation of 0.81 for SM and 0.78 for MPF.

FIGURE 1. Spearman rank correlation plots between baseline and first follow-up levels of (A) soluble mesothelin (SM) and (C) megakaryocyte potentiating factor (MPF); and between baseline and second follow-up levels of (B) SM and (D) MPF.

FIGURE 2. Box plot of (A) soluble mesothelin (SM) and (B) megakaryocyte potentiating factor (MPF) levels of the 137 participants in whom a sample at baseline, follow-up visit 1 (FU 1), and follow-up visit 2 (FU 2) was available. Biomarker levels significantly increased over time, ***p < 0.001.

DISCUSSION

To examine the longitudinal behavior of SM and MPF in controls, a cohort of asbestos-exposed individuals, with no malignant disease at inclusion, was surveilled for 2 years with annual biomarker measurements. Past asbestos exposure of these participants, estimated in fiber years, was weakly correlated with SM levels. In contrast, regression analysis found
TABLE 3. Random Intercept Model Estimates of the Within-Individual Effect of Age and GFR on Biomarker Levels

<table>
<thead>
<tr>
<th>Biomarker Parameter</th>
<th>Coefficient</th>
<th>SE</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log(SM) Age</td>
<td>0.071</td>
<td>0.005</td>
<td>0.062 to 0.081</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GFR</td>
<td>-0.067</td>
<td>0.005</td>
<td>-0.077 to -0.057</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log(MPF) Age</td>
<td>0.058</td>
<td>0.005</td>
<td>0.048 to 0.067</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GFR</td>
<td>-0.001</td>
<td>0.005</td>
<td>-0.002 to 0.001</td>
<td>0.390</td>
</tr>
</tbody>
</table>

SM, soluble mesothelin; MPF, megakaryocyte potentiating factor; GFR, glomerular filtration rate; SE, standard error; CI, confidence interval.

no independent association, and the observed correlation was likely due to the interrelationship between fiber years, age, GFR, and SM. Biomarker levels were consequently not useful to estimate an individual’s past asbestos exposure. For SM, this was in agreement with previous findings, except for one report that suggested an association with asbestos exposure, based on a difference in SM levels between healthy and asbestos-exposed individuals. These groups, however, differed substantially in age, and it is possible that the reported effect was age related, rather than asbestos related. For MPF, the relationship with past asbestos exposure had not been reported previously.

In contrast to MPF, the use of a single SM measurement and a fixed threshold in screening for mesothelioma has been evaluated. In a first retrospective study by Robinson et al., three of seven asbestos-exposed individuals with elevated SM levels were later diagnosed with mesothelioma. In addition, two of eight patients with mesothelioma had elevated SM levels before diagnosis. Large retrospective studies did, however, not confirm these promising findings. Using a threshold of 2.3 nmol/L, Roe et al. found that only 1 of 77 prediagnostic samples (1%) had an elevated SM level. Creaney et al. demonstrated, using a 2.5 nmol/L threshold, that 17 of 106 patients with mesothelioma (16%) had an elevated SM level before diagnosis. Most recently, Gube et al. found that 2 of 20 patients with mesothelioma (10%) had a prediagnostic SM level above a threshold of 1.5 nmol/L. In a first prospective study, 538 individuals were surveilled for 1 year, whereas SM levels were only measured at baseline. From the 15 (3%) who had a level above 2.5 nmol/L, one had chronic renal failure but no malignancy, another had an early-stage lung cancer, and a third had a suspected cardiac tumor. In the 12 other individuals, no malignancies were observed. Two participants with normal SM levels died during the course of the study due to lung and pancreatic cancer, respectively.

Altogether, these disappointing results seem not in favor of incorporating SM in screening for mesothelioma. SM deserves, however, some credit, as the design of these studies often hampered an appropriate evaluation of its use. First, typical of retrospective studies, a too long period (≥1 year) between analyzed “prediagnostic” samples and the actual presentation of mesothelioma could underestimate the efficacy of SM. Second, in the prospective study, the size of study population and duration of the follow-up were not in proportion with the low incidence and long latency period of mesothelioma. This was also the case in our series. The finding that a participant with normal biomarker levels presented a mesothelioma is consequently of limited relevance. Third, the high intraclass correlation of SM and MPF in our series clearly indicated that the use of a single biomarker measurement and fixed screening threshold is suboptimal. Fourth, none of these studies took age or GFR into account. The association of these covariates with SM and MPF has been evaluated. In a first retrospective study by Robinson et al., 538 individuals were surveilled for 1 year, with a too long period (≥1 year) between analyzed “prediagnostic” samples and the actual presentation of mesothelioma. Consequently, a too long period (≥1 year) between analyzed “prediagnostic” samples and the actual presentation of mesothelioma could underestimate the efficacy of SM. Second, in the prospective study, the size of study population and duration of the follow-up were not in proportion with the low incidence and long latency period of mesothelioma. This was also the case in our series. The high intraclass correlation of SM and MPF indicated that screening can benefit from incorporating serial biomarker measurements. Our report is the first to prospectively examine the longitudinal behavior of such SM and MPF measurements in controls. Although the number of follow-up samples decreased after initial inclusion, the retrieved study population still allowed for significant and critical observations. Biomarker levels were strongly correlated across the sampling points but surprisingly increased during follow-up. Analysis of the within-individual biomarker variance revealed that a substantial proportion of this increase was due to aging. The impact of GFR was limited, most likely because GFR changes little in 2 years. It is probable that larger differences in GFR, occurring over a longer period of time, can have a more pronounced effect on serial biomarker measurements. Assay variability and other within-individual changes accounted for the remaining with-individual biomarker variance.

Our findings allowed to speculate on the optimal design of a biomarker-based screening approach for mesothelioma. Although the use of a single baseline biomarker measurement alone is unlikely to be effective, it could act as a first triage and risk-stratification step. An alternative for a fixed threshold could be the implementation of age- and GFR-adjusted biomarker reference values. After this initial triaging, further follow-up would then be guided by changes in serial biomarker measurements, accounted for aging and changes in GFR. Importantly, such biomarker-based screening approach will only be effective if serial SM and MPF measurements increase relatively more in patients who will develop mesothelioma compared with those who remain disease free. Two recent retrospective studies have examined the behavior of serial prediagnostic SM measurements. Creaney et al. reported a serial biomarker-based screening algorithm, which detected 33 of the 88 (38%) patients with mesothelioma, up to 2 years before diagnosis. This was substantially higher than the 16% detected when applying a single measurement at a fixed threshold and illustrates the potential of a serial biomarker-based approach. Nevertheless, when extrapolating this algorithm to our series, 26% (35/137) would have
tested positive at the third follow-up visit. As indicated by the authors, their algorithm is possibly skewed by the retrospective sample analysis, in which assay variability was minimized. This can explain the high fraction of “positives” in our series and illustrates the influence of a prospective collection on biomarker variance. Most recently, Gube et al. evaluated serial measurements of a panel of serum biomarkers, including SM, in screening for mesothelioma but did not find a clear trend in 20 prediagnostic samples. In this study, the time considered between the prediagnostic samples and presentation of the tumor was up to 11 years, likely underestimating the efficacy of serial measurements. While these two reports illustrate the challenges in developing and validating an efficient algorithm, neither of them accounted for age or GFR. Further research is consequently mandatory to ascertain whether a serial biomarker-based screening approach is suitable for the early detection of mesothelioma. This requires the analysis of a large enough amount of retrospective samples, collected in a serial manner in cohorts in which a sufficient number of individuals developed mesothelioma, followed by prospective validation.

Besides the accuracy of a screening tool, other issues are equally important in early detection. For a rare malignancy such as mesothelioma, a positive predictive value of 10% can be considered sufficient for a screening approach. Nevertheless, when considering, for example, a biomarker with a sensitivity of 60% and a specificity of 95%, a positive predictive value of 10% requires a prevalence of at least 1 of 100, emphasizing that screening is only worthwhile in a population with a very high probability of disease. Furthermore, for screening to be justifiable, treatment of early-stage disease should improve outcome, and it is still uncertain whether this is the case for mesothelioma. In addition, although a detectable preclinical phase of the disease should ideally be present, a tumor must reach a certain size before it can be detected by imaging modalities such as computed tomography and positron emission tomography, and very early changes are difficult to observe and nonspecific among other asbestos-related lesions. Progress in these fields is consequently mandatory before large-scale screening efforts can make a true difference.

In conclusion, this prospective study reveals important insights on the longitudinal behavior of SM and MPF, which can aid future screening approaches. The impact of age and GFR, both on single and serial biomarker measurements, together with the substantial biomarker variance, indicates that a single biomarker measurement and fixed threshold are suboptimal in screening. A biomarker-based screening approach can consequently benefit from incorporating serial measurements and individual-specific screening rules. Large-scale validation remains nevertheless mandatory to elucidate whether such an approach can improve the early detection of mesothelioma.

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