

Research Article

Serum Mesothelin for Early Detection of Asbestos-Induced Cancer Malignant Mesothelioma

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Abstract

Background: Malignant mesothelioma is an aggressive, almost uniformly fatal tumor, primarily caused by exposure to asbestos. Since the recent discovery that serum mesothelin is a sensitive and highly specific biomarker for mesothelioma, one of the key issues raised is whether mesothelin levels represent a useful screening test for asbestos-exposed at-risk individuals. In this study, soluble mesothelin was determined in sequential serum samples collected from asbestos-exposed individuals before the development of mesothelioma.

Methods: Archival serum samples from 106 individuals who developed mesothelioma, 99 asbestos-exposed individuals from the Wittenoom Cancer Surveillance Program, and 109 non-asbestos-exposed individuals from the Busselton Health Survey were identified. Serum mesothelin concentrations were determined using the MESOMARK assay.

Results: Longitudinal mesothelin levels determined in healthy asbestos-exposed individuals over a period of 4 years were stable (Pearson's $r = 0.96$; $P < 0.0001$). There was no correlation between mesothelin concentration and cumulative asbestos exposure. Mesothelin concentrations were greater than the threshold value of 2.5 nmol/L in the penultimate serum sample before the diagnosis of mesothelioma in 17 of 106 people. Using an increase above the 95% confidence interval of the mean of a given individual's longitudinal mesothelin results, 33 of 82 individuals had increasing mesothelin levels before diagnosis.

Conclusion: In a population with a high pretest probability of developing mesothelioma, the serum biomarker mesothelin is elevated in absolute terms in 15% and in relative terms in 40% of the group.

Impact: Future studies examining a combination of biomarkers could improve sensitivity of screening. *Cancer Epidemiol Biomarkers Prev*; 19(9); 2238–46. ©2010 AACR.

Introduction

Malignant mesothelioma (MM) is an aggressive, asbestos-induced tumor that is almost uniformly fatal (1, 2). Overall population survival has improved very little over time. Patients who are treated with supportive care have a median survival of 9 months (3); those treated with the best available chemotherapy (pemetrexed and cisplatin) have an increase in survival on average

of 10 weeks (4). In selected patients with epithelial, early-stage disease who undergo extrapleural pneumonectomy followed by adjunct chemotherapy and radiotherapy, 5-year survival rates of 46% have been reported (5). However, in most patients, median survival remains between 9 and 14 months (6).

MM is a tumor of the serosal cavities, occurring predominantly in the pleura. It is generally widespread throughout the cavity by the time of presentation and is generally diagnosed late in its course. A diagnostic test capable of detecting disease at an early stage could be useful and might allow effective therapy at an earlier stage.

Recently, we have described a new serum-based marker for MM known as soluble mesothelin-related protein (SMRP) or serum mesothelin. Mesothelin is expressed in normal mesothelial cells and in various cancers including MM, pancreatic adenocarcinoma, ovarian, and lung cancer (7). Differential posttranscriptional and posttranslational processing of the mesothelin gene produces four protein products: MPF, mesothelin variant 1, mesothelin variant 2, and SMRP (8). The major isoform of mesothelin, variant 1 is a ~40-kDa glycosylated protein mainly found anchored to the cell surface of normal mesothelial cells, but also present in solution (9). SMRP is a

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soluble protein with an identical NH₂-terminal sequence to mesothelin but a unique COOH terminal (10). Using ELISA, which detects both mesothelin variant 1 and SMRP, we found that patients with MM had significantly higher mean levels of this protein in their blood than normal healthy individuals. Soluble mesothelin is elevated in more than half of patients at diagnosis, making it a potential useful addition to the diagnostic repertoire of tests for this disease (11, 12).

Our preliminary examination of seven samples from a random pool of 40 healthy, asbestos-exposed controls that were mesothelin-positive revealed that three individuals had developed MM at 15, 26, and 69 months after the sample had been taken, whereas none of the other 33 asbestos-exposed individuals with normal mesothelin levels went on to develop MM or other cancer in the 8 years of follow-up (11). Given the considerable interest that exists in the development of screening tests for asbestos-exposed populations, especially those with high levels of exposure whose risk of MM is high, we extended those initial studies and evaluated mesothelin levels in pre-diagnosis serum samples collected prospectively as part of a cohort study.

Individuals exposed to blue asbestos (crocidolite) through their employment or residence in Wittenoom Township have been monitored since 1995. Approximately 7,000 individuals who worked for the Australian Blue Asbestos Company (13) and ~5,000 people who were resident in the township (14) have been identified. The risk of MM in this group is markedly increased and is one of the highest in the world (15), with the estimated lifetime risk of MM in the workers being ~17% (16). A subset of these people have taken part in a cancer prevention program since 1991 (17, 18).

These populations have justifiable anxiety about their risk of developing asbestos-related malignancies, including MM as well as lung cancer (19). They represent an ideal cohort for analysis of this biomarker as a screening tool for MM.

Materials and Methods

Case-control identification

Individuals who took part in the cancer prevention program (17, 18) were identified as those who had serum samples available from the time before they developed symptoms or evidence of MM. All MM cases had the diagnosis confirmed by the Western Australian Mesothelioma Registry, which was established in 1960, and reviews and verifies all MM cases diagnosed in the state, where all cancers are compulsorily notified (20).

Control subjects were randomly selected from asbestos-exposed individuals in the cancer prevention program who had a serum sample available in 1994 and who had a 10-year malignancy-free period of follow-up. Follow-up of the cohort has been described previously (13).

In addition, other control samples were studied; stored serum from non-asbestos-exposed healthy subjects were obtained from the Busselton Population Medical Research Foundation (21). Cases were selected as having a serum sample available from 1994 and a 10-year malignancy-free period of follow-up. Equal numbers of males and females were randomly selected from within age groups 35 to 44, 45 to 54, 55 to 64, 66 to 74, and 75+ years. Control individuals were only studied if their serum samples had been stored under similar conditions and for similar lengths of time to that of the MM cases.

This project was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee.

Asbestos exposure data

Cumulative asbestos exposure was estimated in fiber years per milliliter: for all former workers at the Australian Blue Asbestos (ABA) company and residents of Wittenoom (22).

Mesothelin concentrations

Mesothelin concentrations were determined following the manufacturer's instructions using the MESOMARK assay (Fujirebio Diagnostics). This double determinant sandwich ELISA uses the monoclonal antibodies OV569 and 4H3, and was initially described by Scholler et al. (10). All assays were done at least in duplicate on coded samples by investigators who were unaware of the patient's diagnosis. The limit of detection of the assay was 0.3 nmol/L, unless otherwise stated, and samples below the limit of detection were assigned an arbitrary value of 0.1 nmol/L.

Clinical information

A retrospective review of the available clinic and clinician's notes, and radiological data were undertaken for individuals who developed MM. Symptoms such as weight loss, breathlessness, and chest pain were recorded without knowledge of the serum mesothelin levels. For radiological data, the X-ray taken at the time of collection of blood for testing was compared with the most recent X-ray at least 6 months prior. X-ray information was based on International Labour Organization classification of radiographs for pneumoconiosis and reviewed by more than two trained readers (23).

Statistical analysis

To test for statistically significant differences, biomarker levels were transformed to the logarithmic scale on which normal theory statistical estimates (mean, SD) and tests (*t* tests or ANOVA) were applied. All reported *P* values are two-sided. A level of *P* < 0.05 was accepted as significant. Pearson's correlation coefficient and χ^2 tests were used to assess relationships between mesothelin values (log scale) and patient characteristics.

To estimate the average within-individual variability in mesothelin concentrations, the SD of log-normalized data was calculated. Samples below the limit of detection of

Table 1. Characteristics of mesothelioma cases

Characteristic	Values
Age at diagnosis (y)	
Mean \pm SD	66 \pm 9.2
Range	32-84
Site (no. of cases)	
Pleural	87
Peritoneal	17
Other	2
Histology (no. of cases)	
Epithelioid	36
Biphasic	22
Sarcomatoid	7
Not specified	41
Survival (mo)	
Median	12
Range	0-122

the assay were removed from the analysis so as not to unduly bias the variability analysis by introducing the constant 0.1 nmol/L value.

Fisher's exact test was used to determine if there was a significant association between patient symptoms and/or changes observed on X-ray and mesothelin test status.

A logistic regression model was used to examine the possible relationship between mesothelin and the time difference from blood sampling and MM diagnosis; variables included in the model were sex, age, tumor histology, site of tumor, and symptoms at sampling including weight loss, breathlessness, pain, radiological change, or a consolidated parameter that included any new symptoms.

To determine if mesothelin levels significantly increased over time, the penultimate sample before diagnosis was compared with previous samples. If the penultimate sample was greater than the mean + 1.96 (SD) of the previous samples, the mesothelin level was said to have increased before diagnosis. For cases with only two pre-diagnostic samples, the threshold was calculated using the within-subject variation of the asbestos-exposed con-

trols, and only cases in which two or more samples were available were analyzed. Only cases in which the penultimate sample was within 24 months of diagnosis were analyzed. Statistical analysis was done using SPSS 15.0 and GraphPad Prism (version 4.03).

Results

Study population characteristics

Serum samples from 3,738 individuals from the Wittenoom cohort were available with an average of seven annually collected serum samples per individual (range, 1-19). From 1994 to 2006, there were 118 confirmed cases of MM, and of these, 106 had serum samples available before they were diagnosed with MM (99 male and 7 female). The majority of cases occurred in the pleural cavity and were of epithelial histology; the median survival for this group was 12 months (Table 1). Control individuals were of a similar age to MM patients. The gender distribution of asbestos-exposed controls was similar to MM patients, although as expected, a greater percentage of females were noted in the non-asbestos-exposed control individuals (Table 2).

Mesothelin levels in controls

Mesothelin concentrations in archived serum of asbestos-exposed individuals ranged from nondetectable to 4.0 nmol/L. Mesothelin concentrations in archived serum of non-asbestos-exposed persons ranged from nondetectable to 2.68 nmol/L. Mesothelin levels were not significantly different in people with no asbestos exposure with a median (interquartile range) of 0.701 (0.383-1.117), compared with people with a history of asbestos exposure and no subsequent malignant disease [median, 0.638 (0.386-0.972); Fig. 1A]. There was no sex-associated difference in median mesothelin level in these groups (Fig. 1B). There was a small, yet significant, correlation of mesothelin with the individual's age in non-asbestos-exposed group ($r = 0.363$, $P = 0.0001$; Fig. 1D); however, this relationship was not observed in the asbestos-exposed group ($r = 0.097$, $P = 0.35$; Fig. 1C).

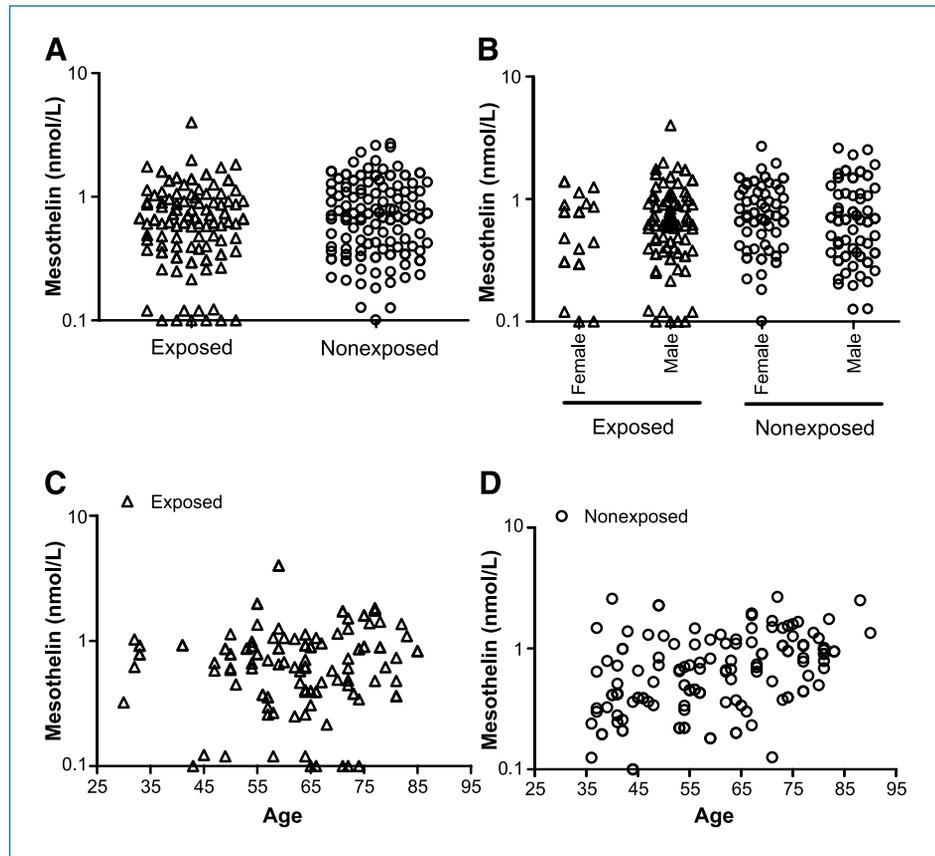
In 77 of the 99 asbestos-exposed individuals, estimates of cumulative asbestos exposure were available. Asbestos

Table 2. Characteristics of study groups

	No.	YOB median (range)	Sex male/female	Asbestos exposure (CF)	Average no. serum samples
Non-asbestos-exposed controls	109	1934 (1904-1958)	54 M/55 F	ND	1
Asbestos-exposed controls	99	1937 (1909-1973)	79 M/20 F	19 (\pm 48)	7.6
MM cases	106	1933 (1909-1960)	99 M/7 F	46 (\pm 81)	8

Abbreviations: YOB, year of birth; CF, cumulative fiber/mL; ND, not determined.

Figure 1. Mesothelin levels in individuals with 10-y malignancy-free follow-up. A, individuals with a history of asbestos exposure (exposed) and those without a history of asbestos exposure (nonexposed). B, mesothelin levels plotted versus sex and asbestos exposure status. C and D, mesothelin levels plotted against the individual's age at the time of serum sampling for (C) asbestos-exposed and (D) nonexposed individuals.



exposure for this group ranged from 0.1 to 253 fiber-years/mL. There was no correlation between mesothelin concentration and estimated degree of asbestos exposure (Fig. 2).

Mesothelin levels were determined in a total of 664 samples from 99 asbestos-exposed controls; on average, 7.6 annual samples were available for each individual. There was no difference in variance observed in mesothelin levels between the years of sample collection (Fig. 3A). The within-subject variation was estimated to be 0.181; that is, if a subject's baseline mesothelin was 1.0 nmol/L, then 95% of the time, the mesothelin levels would vary between 0.645 and 1.354 nmol/L. A representative figure is presented for longitudinal mesothelin concentrations from seven individuals (Fig. 3B). The within-individual stability of mesothelin concentrations was determined in a two-point analysis in a subset of the subjects in which samples were available 4 years apart, giving a Pearson's correlation of 0.96 ($P < 0.0001$; Fig. 3C). One individual in the asbestos-exposed control group displayed increasing mesothelin concentrations over time (Fig. 3D). A review of the patient's notes revealed progressive elevation of serum creatinine levels of 150 $\mu\text{mol/L}$ in 1989, 210 $\mu\text{mol/L}$ in 1990, 250 $\mu\text{mol/L}$ in 1991, and 628 $\mu\text{mol/L}$ in 1994; the patient began peritoneal dialysis in September 1994.

A cutoff value for normal mesothelin concentrations was chosen as the mesothelin level that was three times the SD of the mean, above the mean of the non-asbestos-exposed healthy group, that is, at a mesothelin concentration of 2.5 nmol/L [i. e., $0.8285 + (3 \times 0.5562)$].

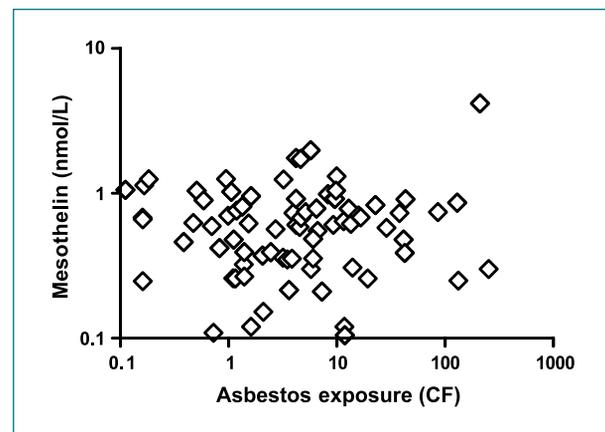


Figure 2. Mesothelin levels in asbestos-exposed individuals with 10-y malignancy-free follow-up and asbestos exposure.

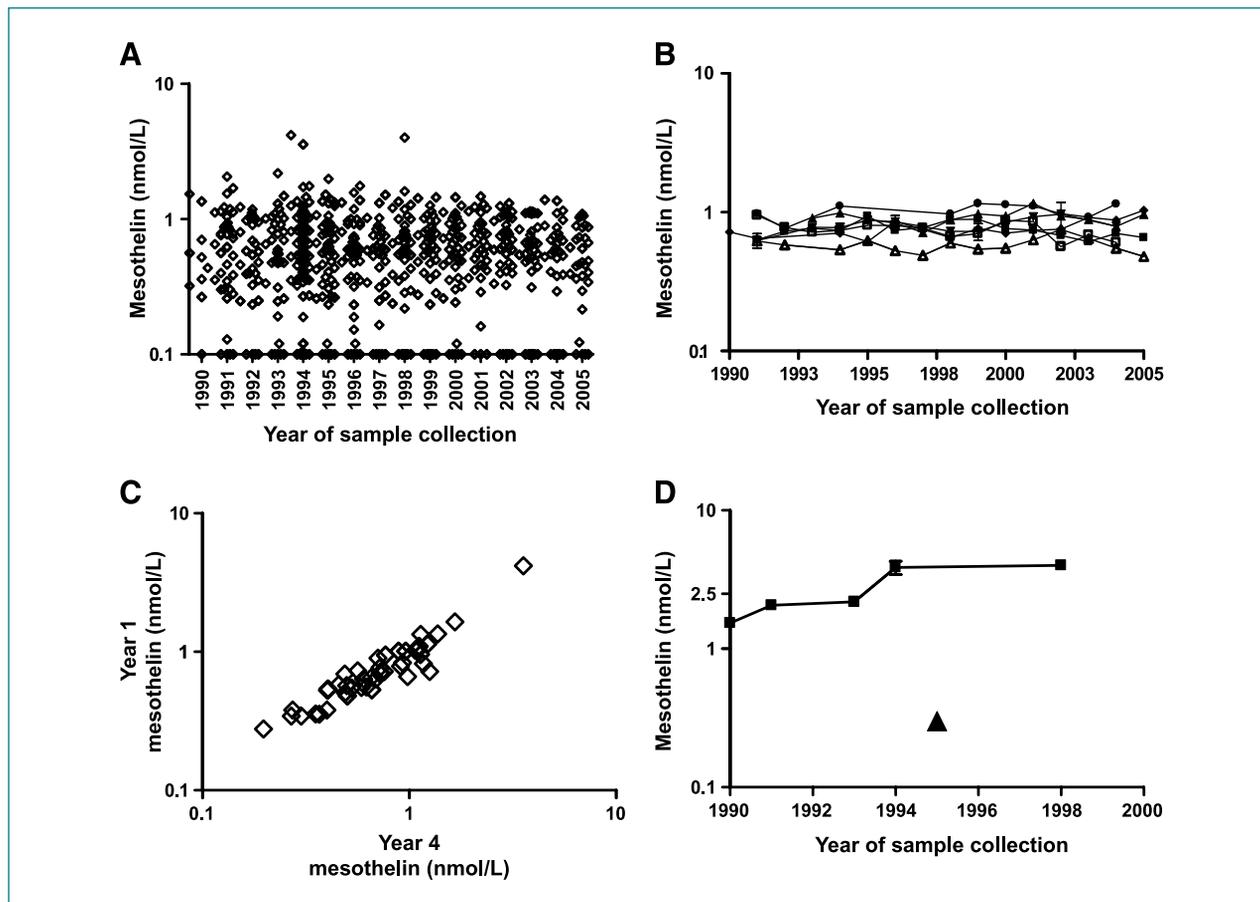


Figure 3. Mesothelin levels in asbestos-exposed individuals with 10-y malignancy-free follow-up. A, all samples analyzed over the study period. B, representative plots of seven asbestos-exposed individuals. C, results plotted for 44 individuals with samples available 4 y apart. D, an asbestos-exposed individual whose creatinine level was 210 $\mu\text{mol/L}$ at the end of 1990 and increased to over 600 $\mu\text{mol/L}$ in September 1994 when he began peritoneal dialysis (indicated by closed triangle).

Elevated serum mesothelin levels before MM diagnosis

Mesothelin concentrations in the penultimate prediagnostic serum sample of subjects who developed MM ranged from nondetectable to 9.63 nmol/L. Mesothelin levels were significantly different in the premorbid sample with a median concentration of 1.556 nmol/L (0.600-1.802), compared with people with a history of asbestos exposure and no subsequent malignant disease ($P < 0.0001$; Fig. 4A). Using the cutoff value determined above, 17 of the 106 individuals had elevated mesothelin levels before pathologic diagnosis. The median timing of this sample was 8 months before diagnosis, with the mean \pm SD being 16.8 ± 26.5 months (range, 1 wk to 13 y; Fig. 4B). Seven of the 43 individuals (16%) with a serum sample available within 6 months of diagnosis were mesothelin positive (Fig. 4C).

There was a significant association between an individual having new symptoms of pain, but not weight loss or shortness of breath, at the time of blood sample collection and the mesothelin level in that sample being elevated

or within the reference range (Table 3). The time of sampling from diagnosis was not a significant determinant of mesothelin status [odds ratio, 1.011; 95% confidence interval (95% CI), 0.98-1.04]. None of the other variables examined (sex, age, tumor histology, site of tumor, radiological change, or a consolidated parameter which included the total of new symptoms) were significantly associated with mesothelin status.

Longitudinal changes in mesothelin pre-MM diagnosis

Given that there is a reference range of mesothelin levels in healthy individuals, we examined whether progressive increases in mesothelin levels, rather than absolute increases, might uncover more early MM cases in this cohort. Sequential levels were determined on 488 prediagnostic samples from 106 individuals who developed MM. Examining the penultimate sample in relation to the prior samples collected longitudinally for the subject and a 95% CI threshold, mesothelin levels increased above the baseline level in 33 of 82 analyzable

subjects. That is, an increase in mesothelin concentration could be determined in a given individual compared with their own longitudinally collected samples in ~40% of the group studied (for examples, see Fig. 5A and B). In those 49 subjects who did not exhibit increased

mesothelin levels before diagnosis, 26 showed increasing mesothelin levels postdiagnosis (Fig. 5C), 6 did not have increased mesothelin levels observed (e.g., Fig. 5D), and 17 had unanalyzable samples.

Discussion

The use of blood biomarkers for the early diagnosis of cancer has been the goal of many individual researchers and extensive research cooperatives such as the Early Detection Research Network (24, 25); although biomarkers have been described, few are in routine clinical practice (26). The main problems are low levels of specificity combined with low pretest probabilities, making positive predictive values a barrier to widespread utility, i.e., the proportion of positive results that are true positives and not anxiety- and investigation-provoking false positives. Asbestos-exposed individuals represent an ideal cohort to study because those with high levels of exposure are at high risk of MM, and their exposure can usually be quantified, i.e., the most at-risk individuals can be readily identified. Thus, their relatively high pretest probability compared with the unexposed population markedly improves the chances that any test will have reasonable predictive value. This can be seen in contrast with the use of CA125 to screen for diseases such as ovarian cancer, in which the low pretest probability makes screening the general population difficult because of the large number of false-positive results and resulting anxiety, and need for intervention (27).

MM is an ideal cancer to study for another reason—the prognosis is poor. Because it occurs in a hidden location (serosal cavities) and thus typically presents at a late stage, it is not surprising that surgery and other modalities are palliative only. Yet, MM is a tumor type that, unlike most other pulmonary malignancies, does not metastasize until late in the disease, and these metastases are rarely the cause of death. Thus, any test that could diagnose the disease at an early stage offers the prospect of early diagnosis (probably thoracoscopic) and early intervention (using standard multimodality approaches) at a potentially curative stage. It is unknown, of course, if such early therapeutic intervention for MM will improve responses because no such test has been available and thus such trials have never been possible. The data from this study, showing that serum mesothelin levels are absolutely elevated in ~15% of subjects, and are relatively elevated in 40% of individuals some months before diagnosis, raises the possibility that the benefits of early intervention could be studied in such individuals.

If single mesothelin measures potentially form the basis of a screening program, consideration must be given to what is an acceptable threshold/cutoff value. In this study, a value of 2.5 nmol/L was chosen to increase specificity, but obviously at the cost of sensitivity. Although this study did not take into account the renal function of healthy subjects, which is a confounder for the

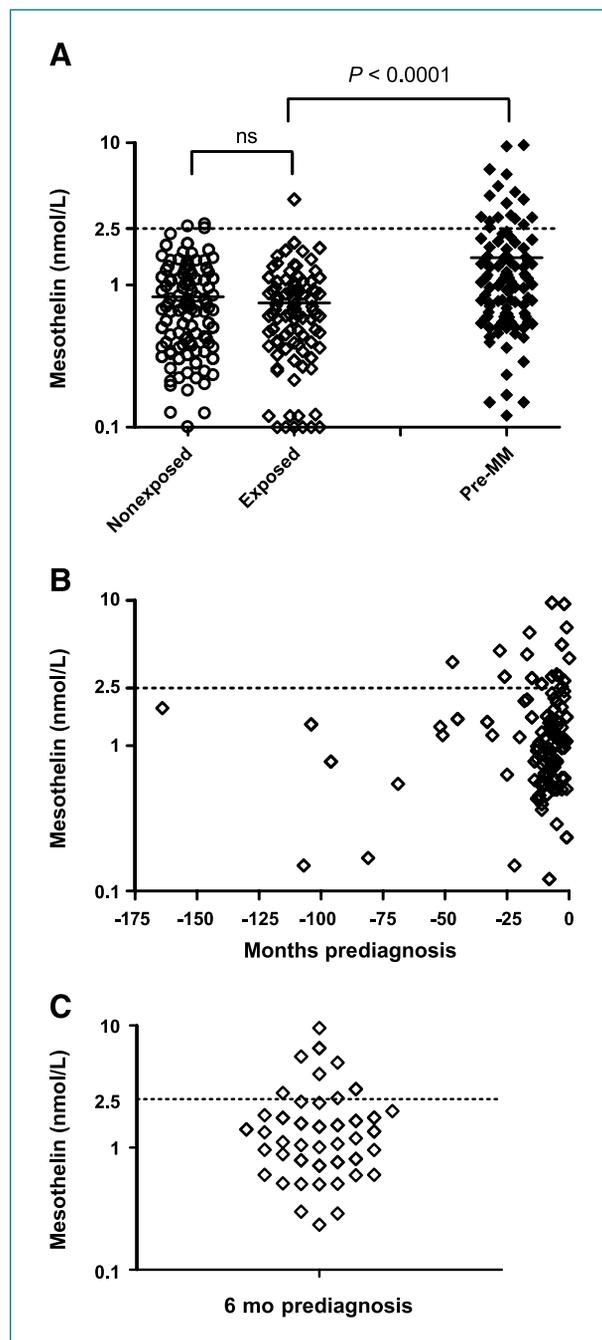


Figure 4. Mesothelin levels in the penultimate prediagnostic serum sample. A, mesothelin levels for individuals without asbestos exposure (nonexposed), with asbestos exposure (exposed), and in people who developed MM. Ns, not significant. B, mesothelin levels and months before MM diagnosis. C, mesothelin levels in samples obtained within 6 months of MM diagnosis.

Table 3. Clinical and radiologic changes at the time of the penultimate serum sample before MM diagnosis

Mesothelin status*	No.	No. of cases with new symptoms†					Combined clinical and radiologic symptoms
		Weight loss	Breathlessness	Pain	Combined clinical symptoms	Radiologic change	
Elevated	17	1 (1)	4 (2)	6 (3)	9 (6)	5 (6)	11 (10)
Normal	89	5 (5) ^{ns}	11 (13) ^{ns}	13 (16)*	30 (33) ^{ns}	35 (34) ^{ns}	49 (50) ^{ns}

*The prediagnosis group was divided into two, depending on whether the mesothelin concentration in the penultimate sample was elevated or within the reference range.

†Results are number of cases observed. The expected number of cases are shown in parentheses. Significance determined by the χ^2 test. (*, $P < 0.05$).

mesothelin assay (28-30), it will be important to exclude renal dysfunction in any future cohort studies. An example of this occurred with the observed increase in mesothelin levels seen in the asbestos-exposed individual shown in Fig. 3D, which could clearly have been

mistaken as a false indicator of the presence of MM. A retrospective review of the MM patient's notes in this study ruled out renal dysfunction as a cause of their mesothelin-positive results, but in any future screening programs, renal function and age-associated

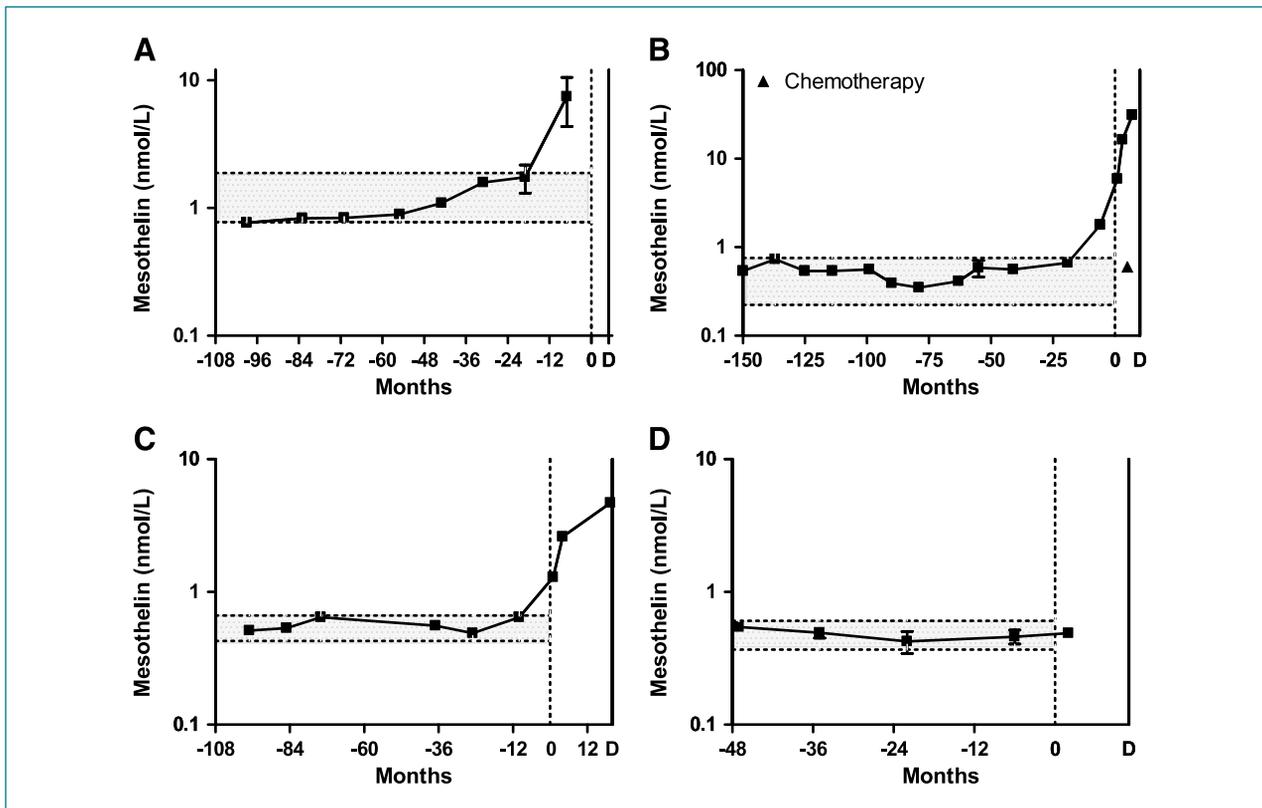


Figure 5. Longitudinal changes in mesothelin concentration. A, individual with a mesothelin concentration of >2.5 nmol/L in the penultimate prediagnosis serum sample. A longitudinal increase in mesothelin concentrations was also apparent over time, with the final sample having a concentration above the shaded 95% CI. B, an individual with a mesothelin concentration of 1.79 nmol/L (i.e., below the 2.5 nmol/L threshold) in the penultimate prediagnosis serum sample. The concentration of mesothelin in the final prediagnosis sample was above the shaded 95% CI. C, an individual with increasing mesothelin levels postdiagnosis. D, an individual whose mesothelin levels did not increase above the baseline in any of the available samples. Vertical dashed line, date of MM diagnosis; vertical solid line, date of death; horizontal shaded bar, 95% CI.

influences on mesothelin concentrations would clearly need to be corrected for.

Although these data are very encouraging for the prospect of detecting early-stage MM by monitoring longitudinal changes in an individual's mesothelin level, there are some limitations to this retrospective study of prospectively collected samples. The possibility of assay variation, which is stated by the manufacturer to be <15% of the coefficient of variation, was minimized in this study by performing the assay on each individual's longitudinal samples on the same day. This potential between-assay variation could, in practice, be quite high in a real-world screening program in which results would be compared from assays done 12 months previously, and possibly done by different laboratories. Furthermore, statistical variations are amplified in samples in which the baseline mesothelin concentrations are low.

Another potential issue is sample degradation due to different transport and storage conditions in different centers. Fortunately, mesothelin is a very stable molecule as evidenced by freeze-thaw experiments and long-term storage stability (31); however, there is always a possibility that concentrations of the molecule could have been affected by storage at -80°C for periods over 10 years. By including control samples that had been stored under identical or similar conditions, the influence of the effects of storage were minimized in our study, but this would have to be carefully controlled for any future multicenter study.

In addition to the sensitivity and specificity of the assay, the prevalence of the disease in a given population is an important issue. For MM, because of the short survival time, prevalence can be taken to be the same as incidence. In the high-risk Wittenoom cohort, the prevalence of MM is estimated to be ~ 700 in 100,000 (16); therefore, for the mesothelin assay with a sensitivity for early-stage disease of 40%, and a specificity of 98%, approximately 1 in 10 positive test results will in fact be due to MM. That means for every positive test result, nine bilateral thoracoscopies and/or laproscopies will be done for every case of MM detected. In the wider asbestos-exposed population of Australia where the prevalence of MM is lower and estimated to be ~ 50 in 100,000 (32), these calculations translate to 1 in 100 positive test results being MM. This would be unacceptable for an effective screening strategy. However, in populations with a reportedly high prevalence of MM, such as Libby, Montana (33) and Cappadocia, Turkey (34, 35), screening might be justified. For ovarian cancer in the high-risk postmenopausal population, a two-step screening strategy of serum CA125 followed by transvaginal sonography has an acceptable positive predictive value; however, the current imaging modalities available for MM, such as thoracic computed tomography scanning and positron emission tomography, are not likely to be sensitive enough to detect the earliest stages of MM in a screening program.

There have been two previous studies of mesothelin in healthy asbestos-exposed cohorts. In a retrospective analysis of the Norwegian Janus serum bank (36), there was no significant association between mesothelin, CA125, or CYFRA 21-1 levels and the risk of developing MM. However, the study was limited by the fact that the premorbid serum samples were collected, on average, 15 years before MM diagnosis (range, 1-30 y). In a prospective screening of 538 occupationally asbestos-exposed individuals followed for 12 months, Park et al. (37) found that 15 individuals ($\sim 3\%$) had absolute values of soluble mesothelin above 2.5 nmol/L. Of the 15 people with elevated mesothelin levels, one had chronic renal failure (which is known to cause elevated mesothelin levels) but no malignancy, another individual had early-stage lung adenocarcinoma, and a third patient had a suspected cardiac tumor. No malignancy was noted in the remaining 12 patients. Sequential studies were not performed. During the period of the study, two other individuals died, one from lung cancer and the other from pancreatic cancer but both had low soluble mesothelin levels. However, the pretest probability of asbestos-associated malignancy for the individuals in this study was low, and thus, in persons with elevated mesothelin, it was 1 out of 15.

This study has shown that in a population with a high pretest probability for mesothelioma, the mesothelin biomarker is elevated in between 15% and 40% of individuals before diagnosis. Although this is encouraging for those justifiably concerned about their asbestos exposure, there are many factors that need to be clearly addressed before large-scale mesothelin testing is encouraged. Those factors include, after optimizing the sample and assay conditions, assessing the acceptable rate of false-positive and false-negative results for that population and considering what the financial as well as psychological costs are of those results.

Disclosure of Potential Conflicts of Interest

B. Robinson: commercial research grant, Fujirebio Diagnostics, Inc.; consultant/advisory board, Fujirebio Diagnostics, Inc. J. Creaney: consultant/advisory board, Fujirebio Diagnostics, Inc.

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