

## Detection and Quantitation of Serum Mesothelin, a Tumor Marker for Patients with Mesothelioma and Ovarian Cancer

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**Abstract Purpose:** To determine whether mesothelin, a cell surface protein highly expressed in mesothelioma and ovarian cancer, is shed into serum and if so to accurately measure it.

**Experimental Design:** We developed a sandwich ELISA using antibodies reacting with two different epitopes on human mesothelin. To quantitate serum mesothelin levels, a standard curve was generated using a mesothelin-Fc fusion protein. Sera from 24 healthy volunteers, 95 random hospital patients, 56 patients with mesothelioma, and 21 patients with ovarian cancer were analyzed. Serum mesothelin levels were also measured before and after surgical cytoreduction in six patients with peritoneal mesothelioma.

**Results:** Elevated serum mesothelin levels were noted in 40 of 56 (71%) patients with mesothelioma and in 14 of 21 (67%) patients with ovarian cancer. Serum mesothelin levels were increased in 80% and 75% of the cases of mesothelioma and ovarian cancer, respectively, in which the tumors expressed mesothelin by immunohistochemistry. Out of the six patients with peritoneal mesothelioma who underwent surgery, four had elevated serum mesothelin levels before surgery. Out of these four patients, three had cytoreductive surgery and the serum mesothelin level decreased by 71% on postoperative day 1 and was undetectable by postoperative day 7.

**Conclusions:** We developed a serum mesothelin assay that shows that mesothelin is elevated in patients with mesothelioma and ovarian cancer. The rapid decrease in mesothelin levels after surgery in patients with peritoneal mesothelioma suggests that serum mesothelin may be a useful test to monitor treatment response in mesothelin-expressing cancers.

Mesothelin is a cell surface protein present on normal mesothelial cells lining the body cavities. It is highly expressed in several cancers, including mesotheliomas, ovarian and pancreatic cancers, and some squamous cell carcinomas (1–4). Human mesothelin is made as a 69 kDa polypeptide with a hydrophobic sequence at the carboxyl end that is removed and replaced by phosphatidylinositol. This glycosyl-phosphatidylinositol linkage anchors mesothelin to the cell membrane (1, 5). After glycosylation at one or more of its four putative glycosylation sites, it is probably cleaved by the protease furin to yield a 32 kDa soluble protein called megakaryocyte potentiating factor (MPF) and a 40 kDa cell membrane bound protein called mesothelin. However, the proteolytic cleavage of

mesothelin by furin has not been clearly shown for human tumors. MPF has been shown to potentiate megakaryocyte proliferation in mouse bone marrow cultures in the presence of interleukin-3 (6, 7). No data is available to show if MPF has megakaryocyte potentiating activity against human bone marrow precursors. The normal biological function of cell membrane-bound mesothelin is not known and mice with a knockout of the mesothelin gene have no obvious phenotype (8). A recent study presented evidence that mesothelin binds CA125 and may, therefore, play a role in the dissemination of ovarian cancer in the peritoneal cavity (9).

Cell-bound mesothelin is a promising target for antibody-based treatment of cancers overexpressing mesothelin and clinical trials of an antimesothelin immunotoxin are currently under way (5). Mesothelin is also an immunogenic protein and antimesothelin antibodies are frequently detected in patients with mesotheliomas and ovarian cancer (10). Also, a T-cell immune response to mesothelin has been noted in patients with pancreatic cancer treated with irradiated, whole pancreatic cancer cell lines transduced with granulocyte macrophage colony-stimulating factor (11).

Although mesothelin is attached to the cell membrane, it could be shed like many other cell membrane proteins (12). Scholler et al. (13) and Robinson et al. (14) have described a 42 to 44 kDa protein, called soluble mesothelin/MPF-related (SMR) protein, that they detected in sera from patients with ovarian carcinoma and malignant pleural mesothelioma. However, the precise relationship of the SMR protein to

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membrane-bound mesothelin is not currently clear and reactivity of the antibodies used to measure SMR protein with mesothelin has not been shown.

Using mesothelin-deficient mice, we have recently developed antihuman mesothelin monoclonal antibodies (mAb) that react with different epitopes on human mesothelin (15). To detect mesothelin that might be shed into the serum, we developed a double determinant ELISA using one of our newly generated antihuman mesothelin mAbs and the Fv portion of an immunotoxin that reacts with a different epitope on human mesothelin. Our results show that serum mesothelin levels are elevated in patients with mesothelioma and ovarian cancer compared with normal healthy volunteers and decrease after surgical therapy for mesothelioma. The assay accurately measures serum mesothelin levels and may be useful as a tumor marker for diagnosis and to follow response to treatment in patients with mesothelin-expressing cancers.

## Materials and Methods

**Human subjects.** Peripheral blood was obtained from patients who were evaluated for possible participation in the phase I clinical trials of the antimesothelin immunotoxin SS1P being conducted at the National Cancer Institute (NCI; ref. 5). The clinical trials were approved by the NCI Institutional Review Board and patients signed an informed consent. Serum from 56 patients with mesothelioma and 21 patients with ovarian cancer were evaluated in this study. The median age of patients with mesothelioma was 57 years (range 26-79 years). The median age of patients with ovarian cancer was 61 years (range 35-79 years). In addition, serum from 24 healthy volunteers was tested. Also, as a control for the assays, serum from 95 random hospital patients was evaluated. Initially, 43 leftover serum specimens in the clinical laboratory were collected without obtaining any patient information, such as age, sex, or diagnosis. Because some of these specimens could have included patients with cancer, we obtained a second set of leftover serum in the clinical laboratory from 52 random hospitalized patients without a diagnosis of cancer. Very limited clinical information was obtained regarding these patients and included diagnosis (to exclude cancer patients) and renal function. The sera from healthy volunteers and random hospital patients were obtained on an Institutional Review Board–approved protocol.

**Serum samples from patients with peritoneal mesothelioma.** Peripheral blood was obtained from six patients with peritoneal mesothelioma who were treated on Surgery Branch protocols at the NCI. These patients underwent tumor cytoreduction with intraoperative continuous hyperthermic peritoneal perfusion with cisplatin (16). Blood samples were obtained before surgery and at different time points after surgery. These patients were treated on an Institutional Review Board–approved protocol at the NCI.

**Mesothelin, SS1P, and mAb MB.** The mesothelin protein used as a standard in the ELISA was made as a mesothelin-Fc fusion protein in mammalian cells. The extracellular domain of the human mesothelin was expressed as a fusion protein with rabbit IgG Fc in HEK 293T cells. The rabbit Fc mesothelin fusion protein was harvested from the culture supernatant and purified on a Hi-Trap protein A column (15).

Purified SS1(dsFv)PE38 (SS1P) is a recombinant immunotoxin consisting of an antimesothelin Fv linked to a truncated *Pseudomonas* exotoxin that mediates cell killing (5, 17). SS1P (0.250 mg/mL) was obtained from the Laboratory of Molecular Biology, NCI, and kept frozen at  $-80^{\circ}\text{C}$  before use.

mAb MB is a mouse antimesothelin mAb that was obtained by immunizing mesothelin-deficient mice with human mesothelin cDNA (Genbank accession no. AR119934) followed by a single boost of a recombinant mesothelin-Fc fusion protein. This recombinant protein

consists of the 40 kDa COOH-terminal mesothelin fused to rabbit Fc (15). mAb MB has been shown to detect mesothelin by immunohistochemistry, fluorescence-activated cell sorting, and ELISA, as well as by Western blotting and has a  $K_d$  of 1 nmol/L (15).

**ELISA.** A 96-well Immulon microtiter plate (Dynatech Laboratories, Chantilly, VA) was coated with 1  $\mu\text{g}$  of SS1P in 100  $\mu\text{L}$  of PBS for 4 hours at room temperature. The plate was then washed five times with 350  $\mu\text{L}$  of wash solution (PBS + 0.05% Tween 20) per well. Next the plate was blocked overnight at  $4^{\circ}\text{C}$  with 350  $\mu\text{L}$  of blocking buffer (SuperBlock buffer + 0.05% Tween 20; Pierce, Rockford, IL) and washed five times with wash solution.

The standard curve was made by making dilutions of the mesothelin-Fc fusion protein with blocking buffer to generate working standards with concentrations of 50, 25, 10, 5, 2.5, 1, 0.5, and 0 ng/mL. The concentration of a mesothelin-Fc stock solution used to make the standards was determined by a bicinchoninic acid protein assay. One hundred microliters of each standard in duplicate was used to make the standard curve. Samples were prepared by diluting 10  $\mu\text{L}$  of serum with 90  $\mu\text{L}$  of blocking buffer and were analyzed in duplicate. After addition of the samples or standards, the microtiter plate was incubated for 2 hours at room temperature and then washed five times with wash solution. Next, 100  $\mu\text{L}$  of a 1  $\mu\text{g}/\text{mL}$  biotinylated MB antibody dissolved in blocking buffer was added and incubated for 2 hours at room temperature. Following five wash steps, 100  $\mu\text{L}$  of a  $10^5$ -fold dilution of a streptavidin-peroxidase conjugate (Pierce) in blocking buffer was added and incubated for 30 minutes at room temperature. The plate was washed five times with wash solution and 100  $\mu\text{L}$  of TMBE substrate (Rockland, Gilbertsville, PA) in PBS was added and incubated for 15 minutes at room temperature. The microtiter plate was read at 450 nm with a EL340 BioKinetic plate reader (Bio-TEK Instruments, Winooski, VT) after the addition of 50  $\mu\text{L}$  of stop solution (0.5 N HCl). The standard curve was fitted using a four-point variable curve-fitting program.

**Immunohistochemistry.** Tissue sections obtained from archival paraffin-embedded tumor blocks from the original biopsies of patient or surgical resection were evaluated for mesothelin expression. Immunohistochemical detection of mesothelin was done by a pathologist (M. Willingham, Wake Forest University School of Medicine, Winston-Salem, NC) using the antimesothelin mAbs K1 or 5B2 (5). Mesothelin positivity was identified by brownish staining of the surface of the tumor cells and graded as positive if at least 30% of accessible tumor cells were labeled. This cutoff was based on the requirement for the clinical trial of the antimesothelin immunotoxin, SS1P, for which these patients were being screened (5). Similar cutoffs for antigen expression have been used for other immunotoxin studies (18). However, it is possible that patients who were defined as having mesothelin-negative tumors could have mesothelin expression in a small percentage of tumor cells.

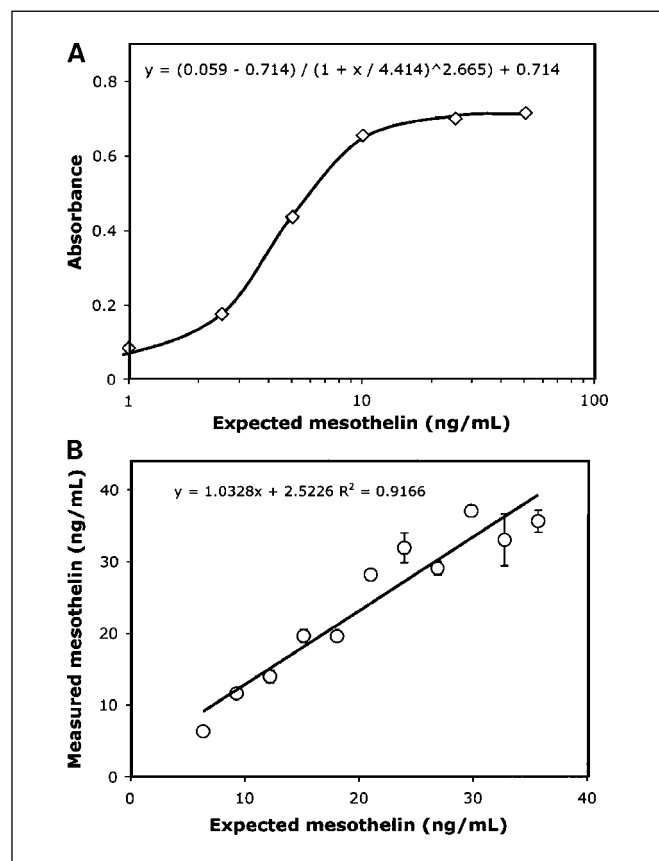
**Statistical analysis.** The statistical analysis of the ELISA data was done using JMP and SAS version 8 software (SAS Institute, Inc., Cary, NC). Analytic sensitivity was determined as  $\pm 3$  SD from the 0 calibrator. The minimum functional sensitivity (coefficient of variation  $<20\%$ ) was determined from the precision of the linearity study.

Serum samples for 56 mesothelioma and 21 ovarian cancer patients were obtained from patients who were screened for participation in clinical trials of SS1P. The serum samples for 95 random hospitalized patients were obtained from leftover serum in the clinical laboratory the day the ELISA was done. In addition, serum samples from 24 healthy volunteers were used as a control. We compared the serum mesothelin levels in patients with mesothelioma and ovarian cancer with levels in healthy volunteers and random hospital patients. In addition, serum mesothelin levels were compared in mesothelin-positive and mesothelin-negative tumors. This statistical analysis was done using an exact Wilcoxon rank sum test. The  $P$  values within each group were adjusted for the multiple evaluations done within each group by the Hochberg method (19). Two-tailed  $P < 0.05$  after adjustment were considered significant.

## Results

**Development of a mesothelin ELISA.** A mesothelin ELISA was developed using two different binding reagents that react with different epitopes on mesothelin. First, mesothelin was captured onto the ELISA plates with SS1P, an antimethelin immunotoxin (5). The bound mesothelin was then detected with MB, a mAb with high affinity for mesothelin (15). The MB antibody was biotinylated and the assay signal was generated by using a streptavidin-peroxidase conjugate and peroxidase substrate. A mesothelin-Fc fusion protein was used as a standard for generating a calibration curve (Fig. 1A). The calibration curve was fitted with a four-point variable curve-fitting algorithm and the absorbance units were converted into mesothelin mass units. A linearity study of the assay was done by mixing a serum specimen with elevated mesothelin, with a serum specimen with a low level of mesothelin (5 ng/mL; Fig. 1B). The assay was found to be linear from 5 to 50 ng/mL, with a functional sensitivity of at least 5 ng/mL. The analytic sensitivity of the assay, as assessed from the signal from the 0 calibrator, was 1 ng/mL. The interassay coefficient of variation of the assay was ~12% at a concentration of 31 ng/mL ( $n = 20$ ).

**Serum mesothelin levels in healthy volunteers and random hospitalized patients.** Mesothelin levels were evaluated in



**Fig. 1.** ELISA for serum mesothelin. *A*, standard curve for serum mesothelin. X axis, the indicated concentrations of mesothelin-Fc fusion protein measured by ELISA. Points, absorbance. *B*, linearity study of mesothelin ELISA. A positive serum specimen containing 38 ng/mL of mesothelin was mixed in various proportions with a low serum sample (5 ng/mL) to produce the expected concentrations of serum mesothelin (X axis). Points, mean of triplicates; bars, SD.

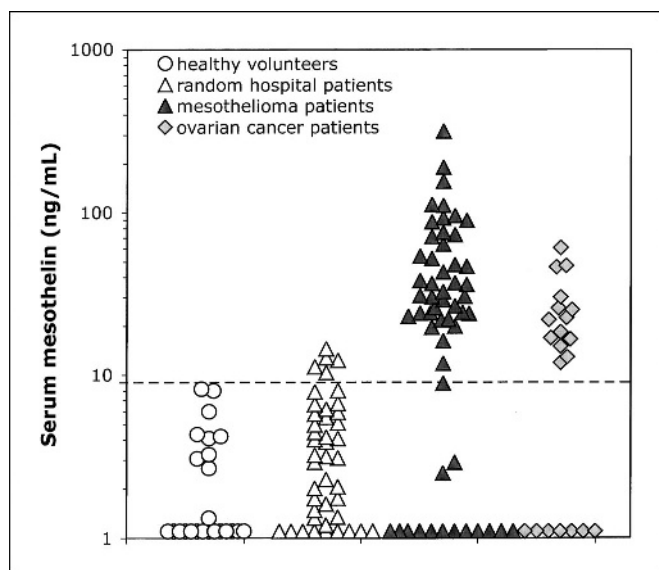
serum obtained from 24 healthy volunteers. In 14 (58%) of these healthy volunteers, no mesothelin was detected, whereas 7 (29%) healthy volunteers had levels <5 ng/mL and 3 (13%) had levels between 5 and 9 ng/mL (Fig. 2). Based on this analysis, a serum level of 9 ng/mL was used as the upper limit of the reference range for normal values of serum mesothelin.

In addition to healthy volunteers, we also evaluated mesothelin levels in serum obtained from 95 random hospital patients. Initially, we obtained serum from 43 random hospitalized patients at the Clinical Center, NIH, with no information regarding these patients including diagnosis. Out of these 43 patients, 38 (88%) had levels below 9 ng/mL and 5 (12%) had serum mesothelin levels between 9 and 15 ng/mL, which is slightly above the cutoff established from the normal healthy controls (Fig. 2). Because this group of random hospital patients could have included patients with cancers in which serum mesothelin levels are elevated, we obtained 52 additional serum samples from random hospital patients without the diagnosis of cancer. Out of these 52 patients, serum mesothelin levels above 9 ng/mL were seen in only seven patients (13%). Out of these seven patients, six had renal insufficiency with serum creatinine above the reference range (mean serum creatinine 3.9 mg/dL, range 1.5-6 mg/dL). The one patient with normal serum creatinine had a diagnosis of focal segmental glomerulosclerosis. These results suggest that mesothelin is probably excreted in the urine and its levels may be increased in patients with renal dysfunction.

**Serum mesothelin levels in patients with mesothelioma.** Sera from 56 patients (42 males and 14 females) with mesothelioma were evaluated for mesothelin. At the time of serum collection, all patients had advanced unresectable disease and had progressed on prior therapies. Out of these 56 patients, 40 (71%) had elevated serum mesothelin with levels  $\geq 9$  ng/mL (Fig. 2). More than 70% of patients with elevated serum mesothelin had levels >25 ng/mL (Table 1). Among the 15 patients with serum mesothelin levels >50 ng/mL, five had levels >100 ng/mL. Out of the 56 patients with mesothelioma, 43 had tumors that were mesothelin positive by immunohistochemistry and 13 cases were mesothelin negative (i.e., mesothelin expression by <30% of tumor cells). Of the 43 patients whose tumor expressed mesothelin, a high proportion (80% or 34) had elevated serum mesothelin levels, whereas in the 13 patients whose tissue was mesothelin negative only six (46%) had elevated serum mesothelin (Fig. 3; Table 2).

The median serum mesothelin level in patients with mesothelioma was 26.2 ng/mL, which was significantly different from healthy volunteers (median 0 ng/mL;  $P < 0.0001$ ) or levels in healthy volunteers plus random hospital patients (median 2.3 ng/mL;  $P < 0.0001$ ). The median serum mesothelin levels were lower (9 ng/mL) in mesotheliomas that did not express mesothelin by immunohistochemistry compared with mesotheliomas that expressed mesothelin (median 30.6 ng/mL) and this difference approached statistical significance ( $P = 0.062$ ).

**Serum mesothelin levels in patients with ovarian cancer.** Serum samples from 21 patients with ovarian cancer were evaluated for serum mesothelin. All patients had advanced ovarian cancer with stage III or IV disease at diagnosis. At the time serum samples were obtained, all patients had prior surgical tumor debulking and received several chemotherapeutic agents. In ovarian cancer, 14 (67%) of the 21 patients had elevated serum



**Fig. 2.** Scatter plot of serum mesothelin. Serum mesothelin levels (displayed using a log scale) in healthy volunteers ( $n = 24$ ), random hospital patients ( $n = 43$ ), mesothelioma patients ( $n = 56$ ), and ovarian cancer patients ( $n = 21$ ). Horizontal line, upper limit of normal (9 ng/mL). The median serum mesothelin levels in patients with mesothelioma was 26.2 ng/mL, which was significantly different from healthy volunteers ( $P < 0.0001$ ). The median serum mesothelin level in patients with ovarian cancer was 16.6 ng/mL, which was also significantly different compared with healthy volunteers ( $P = 0.0011$ ).

mesothelin levels (Fig. 1). Eight of the 14 patients had levels between 9 and 24 ng/mL, five patients had levels between 25 and 49 ng/mL, and one patient had a mesothelin level >50 ng/mL (Table 1). Of the 21 ovarian cancer patients whose serum was tested for mesothelin, 16 had tumor mesothelin expression by immunohistochemistry and five were negative. Out of the 16 patients whose tumor expressed mesothelin, 12 (75%) had elevated serum mesothelin levels whereas two (40%) of the five patients whose tumor was negative for mesothelin had elevated serum mesothelin (Fig. 3; Table 2).

The median serum mesothelin level in patients with ovarian cancer was 16.6 ng/mL, which was significantly different from healthy volunteers (median 0 ng/mL;  $P = 0.0011$ ) or levels in healthy volunteers plus random hospital patients (median 2.3 ng/mL;  $P = 0.0016$ ). The median serum mesothelin levels in ovarian tumors that did not express mesothelin by immunohistochemistry was 0 ng/mL compared with 17.5 ng/mL in tumors that expressed mesothelin. However, these levels were not statistically significantly different ( $P = 0.23$ ).

**Effect of tumor debulking on serum mesothelin levels in patients with peritoneal mesothelioma.** The effect of surgical cytoreduction on serum mesothelin levels was evaluated in six patients with peritoneal mesothelioma. The clinical characteristics and

serum mesothelin levels of these patients are summarized in Table 3. All patients except patient E had an epithelial type of mesothelioma and expressed mesothelin. Patient E had biphasic mesothelioma with a predominance of sarcomatous component and was mesothelin negative. Mesothelin expression is normally absent in sarcomatous mesotheliomas (5). Four of these six patients had a baseline elevation in serum mesothelin levels (mean 24.7 ng/mL; range 11.3-41.6 ng/mL). The serum mesothelin was undetectable immediately after surgical cytoreduction on postoperative day 1 in patient B and by postoperative day 7 in patients A and C. The serum mesothelin for patients A and B increased at follow-up 4 months after surgery. Patient C continued to have undetectable levels 45 days from surgery. At the time of surgical exploration, patient D was found to have extensive tumor spread and as a result underwent very limited tumor debulking. In this patient, postsurgical serum mesothelin levels did not change significantly from baseline. Patients E and F did not have baseline elevation in serum mesothelin and continued to have undetectable levels at follow-up after surgery. These results show that serum mesothelin levels can decrease significantly and become undetectable in some patients after tumor removal.

### Discussion

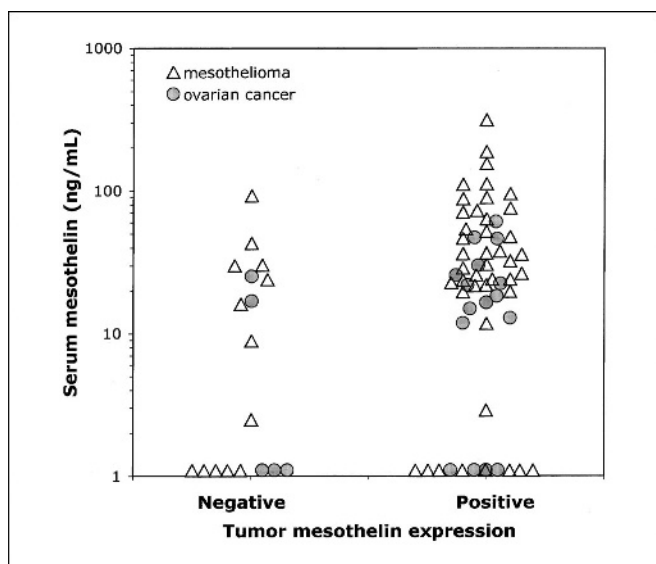
Identification of tumor markers and development of assays to measure them is an important goal in oncology. Such markers can be useful for early diagnosis, as well as to follow response to therapy (20-22). The goal of this study was to determine whether mesothelin, a cell surface protein highly expressed in mesotheliomas and ovarian cancer, is also shed into the serum and, if so, to accurately measure serum mesothelin levels. Using newly generated mAbs that specifically bind mesothelin with high affinity, a quantitative ELISA was developed for the measurement of serum mesothelin. We evaluated serum from patients with malignant mesothelioma and ovarian cancer for mesothelin because these tumors have high cell surface expression of mesothelin. Also, serum mesothelin levels were measured before and after tumor resection in patients with peritoneal mesothelioma.

Our results show that 71% and 67% of malignant mesothelioma and ovarian cancer patients, respectively, had elevated serum mesothelin levels. In both malignant mesothelioma and ovarian cancer patients, serum mesothelin levels were more frequently increased in patients whose tumor expressed mesothelin ( $\geq 30\%$  expression by tumor cells) by immunohistochemistry. However, 46% of mesotheliomas and 40% of ovarian cancers with absent mesothelin expression by immunohistochemistry had elevated serum mesothelin. The serum mesothelin levels were not statistically significantly different in patients with mesothelin-positive or mesothelin-negative

**Table 1.** Serum mesothelin levels in patients with mesothelioma and ovarian cancer

Diagnosis	n	Serum mesothelin level			
		Negative (<9 ng/mL)	9-24 ng/mL	25-49 ng/mL	>50 ng/mL
Mesothelioma	56	16 (29%)	11 (20%)	14 (25%)	15 (27%)
Ovarian cancer	21	7 (33%)	8 (39%)	5 (24%)	1 (5%)





**Fig. 3.** Comparison of serum mesothelin levels with tumor mesothelin expression. X axis, negative or positive tumor expression of mesothelin as measured by immunohistochemistry; Y axis, serum mesothelin levels (ng/mL). The median serum mesothelin levels were not statistically significantly different in patients with mesothelin-positive or mesothelin-negative tumors for both mesothelioma and ovarian cancer with *P* values of 0.062 and 0.23, respectively.

tumors for both mesothelioma and ovarian cancer with *P* values of 0.062 and 0.23, respectively. Because we used a cutoff of mesothelin expression by  $\geq 30\%$  of tumor cells to define tumor mesothelin positivity, some of the mesothelin-negative tumors could have weak mesothelin expression. Also, mesothelin negativity could be an artifact of the immunohistochemistry procedure with factors, such as tissue fixation, affecting antigen detection. Our results also show that serum mesothelin levels can decrease to undetectable levels after surgery in patients with peritoneal mesothelioma, suggesting that serum mesothelin levels may be useful to follow treatment response in mesothelin-expressing tumors.

Previously, Scholler et al. (13) have reported the development of an assay that detects SMR protein in sera of patients with ovarian cancer. Subsequent studies also showed that SMR protein is elevated in the serum of patients with malignant mesothelioma (14). However, the assay to measure SMR protein did not quantitate antigen levels (as ng/mL) because of the lack of purified antigen for construction of standard curves (13). The authors indicate that SMR protein (detected using the mAb OV569) could be a soluble member(s) of

the mesothelin/MPF family because "...the published gene sequence indicates that the membrane-associated part of mesothelin/MPF is not soluble, and the epitope to which OV569 binds is located to that part" (13). Using cDNA cloned from a prostate cancer cell line, they identified a clone that had an 82 bp insert in position 1,874 of MPF, which induced a frame shift of 212 bp. They state that "this frameshift codes for a new COOH terminus, which in contrast to MPF and mesothelin, shows a hydrophilic tail, suggesting that it is soluble" (13). Of interest, this clone with an 82 bp insert was obtained from a prostatic carcinoma cell line HE1P, a tumor type that does not express mesothelin (2, 23).

Although gene sequence analysis of the membrane-bound mesothelin suggests that it is not a soluble protein (13), it is likely that a small fraction of it is shed into the serum and was therefore detected by our assay. Mesothelin is a glycosyl-phosphatidylinositol-linked cell surface protein and such proteins are frequently shed into the serum and other body fluids. Release of glycosyl-phosphatidylinositol-anchored proteins from the cell surface can be mediated by phospholipase C or D, angiotensin-converting enzyme, or various proteases (12, 24, 25). Decay accelerating factor, a glycosyl-phosphatidylinositol-anchored complement regulatory protein present on the surface of blood cells is released from the cell surface and can be detected in various body fluids, including plasma, urine, tears, and saliva (26). Soluble folate-binding protein that is present in patients with ovarian cancer is an example of a glycosyl-phosphatidylinositol-linked tumor antigen shed into the serum (27). Carcinoembryonic antigen is another example of a glycosyl-phosphatidylinositol-anchored tumor antigen that is released into the serum of some patients with adenocarcinomas of the colon, rectum, stomach, pancreas, breast, and non-small cell lung cancer (28). Carcinoembryonic antigen is a useful marker for monitoring tumor recurrence especially in patients with colon cancer (29). A recent study has shown that cleavage of membrane-bound carcinoembryonic antigen by glycosyl-phosphatidylinositol-phospholipase D enhances the metastatic potential of colorectal carcinoma cells (30).

Because the antibodies used in our ELISA bind only to epitopes present on the COOH-terminal portion of mesothelin, our assay detect mesothelin that is released into the serum, most likely by proteolytic cleavage of the glycosyl-phosphatidylinositol anchor as is the case with several glycosyl-phosphatidylinositol-anchored cell surface proteins. Whether the released protein is the full-length 69 kDa protein or the 40 kDa COOH-terminal portion of mesothelin is not known. However, it is not the 30 kDa NH<sub>2</sub>-terminal portion of

**Table 2.** Serum mesothelin in relationship to mesothelin expression by tumors

Tumor type	Tumor mesothelin expression by immunohistochemistry*	<i>n</i>	Elevated serum mesothelin <sup>†</sup> (%)
Mesothelioma	+	43	34 (80%)
	-	13	6 (46%)
Ovarian cancer	+	16	12 (75%)
	-	5	2 (40%)

\*Tumors were considered mesothelin positive (+) if  $\geq 30\%$  of tumor cells had cell surface expression of mesothelin and negative (-) if  $< 30\%$  of tumor cells expressed mesothelin.

<sup>†</sup>Serum mesothelin levels were considered elevated if  $\geq 9$  ng/mL.

**Table 3.** Summary of clinical characteristics and serum mesothelin levels in six patients with peritoneal mesothelioma who underwent surgical debulking and continuous hyperthermic peritoneal perfusion with cisplatin

Patient	Gender	Age	Pathology	Mesothelin expression	Ascites	Debulked (%)	Mesothelin levels (ng/mL)		
							Baseline	POD 1	POD 7
A	M	68	Epithelial	Yes	Yes	95	22.2	5.5	<1
B	M	63	Epithelial	Yes	Yes	70	24	<1	<1
C	F	52	Epithelial	Yes	No	80	11.3	11.2	<1
D	F	31	Epithelial	Yes	Yes	10	41.6	34.4	26
E	M	46	Biphasic	No	No	90	<1	<1	<1
F	M	51	Epithelial	Yes	No	100	<1	<1	<1

Abbreviation: POD, postoperative day.

mesothelin (MPF) because the antibodies used in our assay do not bind that portion of the molecule. Because the SMR protein has not been purified, it is difficult to determine the relationship between it and mesothelin detected in our assay. It is possible that the SMR protein is mesothelin released into serum by cleavage of the glycosyl-phosphatidylinositol anchor or it could represent another protein related to mesothelin as has been suggested by Scholler et al. (13).

In summary, a quantitative ELISA for serum mesothelin was developed and serum mesothelin was found to be elevated in 71% of mesothelioma and 67% of ovarian cancer patients. These results have identified serum mesothelin as a potential biomarker for these cancers, which could be useful for diagnosis as well as for following response to therapy. However, studies involving much larger patient samples are needed to fully characterize the sensitivity and specificity of the assay. Our preliminary studies in patients with peritoneal mesothelioma do suggest that the serum mesothelin assay may be useful for monitoring treatment response. Presently, there are no routine laboratory tests or radiographic approaches for accurately assessing the extent of mesothelioma tumor burden.

Future studies correlating serum mesothelin with detailed clinical information, such as tumor stage, tumor bulk, and response to treatment, will also have to be done to determine if serum mesothelin has prognostic significance and also if it can be used as a biomarker to assess response to other therapies besides surgery. In addition, use of serum mesothelin in combination with the ovarian tumor marker CA125 may be useful for improving diagnostic sensitivity and specificity compared with either marker alone. Because mesothelin is expressed by virtually all pancreatic adenocarcinomas, it will be important to study if mesothelin levels are elevated in these patients (3). If mesothelin levels are indeed elevated, it could be a valuable test to diagnose and monitor pancreatic cancer. Finally, it will be important to clarify the relationship between serum mesothelin detected by our assay and the SMR protein.

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