MESOMARK™: A Potential Test for Malignant Pleural Mesothelioma

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Abstract

BACKGROUND

Malignant mesothelioma (MM) is a relatively rare and highly aggressive neoplasm, arising primarily from the surface serosal cells of the pleura, pericardium, or peritoneum. Exposure to asbestos fibers is the primary cause of disease. While several biomarker studies have been reported in the literature for mesothelioma, there are currently no blood tests commercially available. We developed the MESOMARK™ assay as a forward sandwich Enzyme Linked ImmunoSorbent Assay (ELISA) for the quantitative measurement of Soluble Mesothelin Related Peptides (SMRP): a family of proteins previously described as biomarkers for malignant pleural mesothelioma (MPM).

METHODS

The MESOMARK assay is a two-step immunoenzymatic assay in a standard microplate ELISA format. The analyte signal is read from a 6-point calibration curve spanning 0-32 nM. Analytical performance characterization of the MESOMARK assay included precision, dilution linearity, spike recovery, sample processing, interfering substances, and establishment of a normal reference value. In addition, blinded longitudinal serum samples (preoperative to 47 month range) from 31 patients having cytoreduction and identical adjuvant therapy were investigated for post surgery SMRP levels. The results were correlated with computerized tomographic (CT) follow-up scans.

RESULTS

A 20-day Precision study was performed using 3 samples of defribrinated human plasma spiked with SMRP antigen and 2 controls, in which the assay exhibited within-run CVs ranging from 1.1% to 5.3% and total imprecision ranging from 4.0% to 11.0%. The mean dilution recovery for five samples was 109% and ranged from 99% to 113%. Interference studies with potential interferents yielded grand mean recoveries ranging from 96% to 109% for each sample across a range of interferent levels. Potential interferents included relevant chemotherapeutic agents, elevated triglycerides, hemoglobin, human anti-mouse antibodies (HAMA), rheumatoid factor (RF), bilirubin, and elevated protein levels. Antigen stability in sample collection and storage conditions yielded percent recoveries ranging from 94% to 107%. Analyte spike recovery studies were performed on five serum samples, across the range of the MESOMARK assay and grand percent recoveries were 103% to 113%. SMRP values in serum samples from healthy normal individuals (n=409) were not significantly different from those in a population with known exposure to asbestos (n=61). Of the 409 healthy subjects, 99% had SMRP levels at or below 1.5 nM. SMRP concentrations were significantly elevated in serum from 88 patients with diagnosed MPM (mean=7.5 nM, 95% CI: 2.8 to 12.1 nM). In longitudinal serum samples collected from 31 of these patients, we found 20/31 (65%) showed longitudinal trends in SMRP level that agreed well with the known clinical information.

CONCLUSION

These data suggest when interpreted in conjunction with all other available clinical and laboratory data, that the MESOMARK assay values may be useful for early detection of recurrence and to measure response to therapy in patients with MPM.

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Introduction

ASBESTOS EXPOSURE AND MESOTHELIOMA

- As many as 80% of Mesothelioma patients were exposed to asbestos fibers
- Up to 8 million people in the U.S. have been occupationally exposed to asbestos in the last 5 decades
- 10 to 15% of all schools today contain asbestos
- Latency period of 30 to 40 years

KEY RATES

- U.S. 3000 new cases/year
- Incidence of 8/100,000 in Scotland and England
- Rate of 6/100,000 in Australia

BACKGROUND

A two site sandwich ELISA assay was developed previously for research purposes at the Pacific Northwest Research Institute. This assay was used to detect soluble mesothelin related peptides in serum and to demonstrate that such peptides are elevated in serum from patients with several types of cancer including mesothelioma, ovarian cancer and pancreatic cancer. In contrast, SMRP levels are not elevated in serum from patients with other types of cancer and are typically low in healthy subjects and patients with non-malignant diseases.

Robinson et al. extended these studies and demonstrated that SMRP levels are elevated in patients with mesothelioma. They also showed that some patients exposed to asbestos have elevated serum concentrations of SMRP; in some cases SMRP was found to be elevated prior to detection of mesothelioma by conventional means.

These data demonstrated that in patients with mesothelioma the serum concentration of SMRP is elevated above the background level found in healthy subjects, and that the assay is relatively selective for this disease. In addition, these data suggest that the SMRP assay may have important clinical utility in the management of patients with this deadly disease.

Assay Description

The MESOMARK assay is a two-step immunoassay used to quantitate the presence of the Soluble Mesothelin Related Peptides (SMRP) in human serum using Enzyme Immunoassay technology with colorimetric detection in a standard ELISA microplate sandwich assay format. Two separate monoclonals are used (4H3 and OV569); one for capturing SMRP, the other for detection of SMRP. Detection is accomplished by the addition of a standard chromogenic substrate that binds to the HRP-labeled monoclonal antibody. A direct relationship exists between the amount of SMRP in sample and the Optical Density (OD) detected by the spectrophotometric microtiter plate reader.

CALIBRATION	Four-Parameter Logistic Curve Fit; Y-Weighted
CALIBRATORS	0, 2, 4, 8, 16, and 32 nM
CONTROLS	4.5 and 13.5 nM

PRECISION

Precision was evaluated following NCCLS Protocol EP5-A. Two replicates each of three panels were assayed in two separate runs on each of 20 days, at two separate sites. The evaluation was performed using two different lots of reagents. The data is shown in the table below and is indicative of assay performance.

PANEL MEMBER	REAGENT LOT	SITE	n	MEAN CONC. (nM)	WITHIN RUN SD	WITHIN RUN %CV	TOTAL SD	TOTAL %CV
1	1	1	80	3.85	0.07	1.9	0.21	5.5
		2	80	4.26	0.23	5.3	0.43	10.1
	2	1	80	4.17	0.08	1.8	0.18	4.4
		2	80	4.47	0.21	4.8	0.45	10.0
2	1	1	80	7.44	0.08	1.1	0.30	4.0
		2	80	8.16	0.28	3.4	0.46	5.6
	2	1	80	8.00	0.15	1.9	0.38	4.7
		2	80	8.59	0.20	2.3	0.48	5.6
3	1	1	80	16.54	0.31	1.9	1.31	7.9
		2	80	18.01	0.39	2.2	0.91	5.0
	2	1	80	17.97	0.31	1.7	0.86	4.8
		2	80	19.02	0.43	2.3	2.09	11.0

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ANALYTICAL SENSITIVITY

The average analytical sensitivity for the MESOMARK assay is 0.2 nM. Analytical sensitivity corresponds to the upper limit of the 95% confidence interval and represents the lowest concentration of antigen that can be distinguished from zero. It was determined as the two standard deviation limit of 20 measurements of a sample containing no analyte using two different lots. Data is shown below:

	LOT 1	LOT 2
Mean (O.D.)	0.057	0.063
Standard Deviation (O.D.)	0.014	0.012
Mean + 2 St Dev (O.D.)	0.085	0.087
Measured Analytical Sensitivity (nM)	0.16	0.16

DILUTION LINEARITY

Dilution Linearity was evaluated with a study modeled after NCCLS Protocol EP6-A. Five (5) samples with known elevated SMRP levels were diluted with assay diluent. The SMRP level was determined for each dilution and the percent recovery was calculated.

The average recovery across the five diluted samples was 109% (Range was 99% to113%). Representative data from the study are presented below:

SAMPLE	DILUTION FACTOR	EXPECTED VALUE (nM)	OBSERVED VALUE (nM)	PERCENT RECOVERY**
1	Undiluted	26.69	26.69	N/A
	1:1.1	24.02	24.21	101
	1:1.4	18.68	20.05	107
	1:2	13.35	15.01	112
	1:2.5	10.68	12.39	116
	1:3.3	8.01	9.11	114
	1:5	5.34	6.16	115
	1:10	2.67	3.29	123
2	Undiluted	26.53	26.53	N/A
	1:1.1	23.88	24.15	101
	1:1.4	18.57	19.39	104
	1:2	13.26	15.14	114
	1:2.5	10.61	12.24	115
	1:3.3	7.96	9.19	115
	1:5	5.31	6.26	118
	1:10	2.65	2.78	105
3	Undiluted	25.91	25.91	N/A
	1:1.1	23.31	21.58	93
	1:1.4	18.13	17.93	99
	1:2	12.95	11.28	87
	1:2.5	10.36	10.60	102
	1:3.3	7.77	7.19	93
	1:5	5.18	5.33	103
	1:10	2.59	3.10	120

INTERFERING SUBSTANCES

Mean assay recoveries in the presence of interfering substances are 100 ± 15%. Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera.

INTERFERING SUBSTANCE

TEST COMPOUND	TEST CONCENTATION
Bilirubin	20 mg/dL
Hemoglobin	500 mg/mL
Total Protein	12 g/dL
Triglycerides	3 g/dL

CHEMOTHERAPEUTIC AGENTS

TEST COMPOUND	TEST CONCENTATION
Cisplatin	1.53 mg/mL
Carboplatin	0.34 mg/mL
Alimta	1.3 mg/mL
Gemcitabine	300 µM

POTENTIALLY INTERFERING CLINICAL CONDITION

The MESOMARK assay was evaluated using specimens with Human Anti-mouse Antibodies (HAMA) and Rheumatoid factor (RF) to further assess the assay specificity. Ten specimens positive for HAMA and five specimens positive for RF were evaluated for percent recovery with 569-reactive antigen spiked into each specimen at 5 and 12.5 nM; mean percent recovery results are summarized in the following table:

CLINICAL PERCENT	NUMBER OF SPECIMENS	MEAN PERCENT RECOVERY (RANGE)
HAMA	10	99% (86% to 112%)
RF	5	105% (100% to 112%)

RECOVERY

Known concentrations of 569-reactive antigen were added to five (5) independent normal human serum samples throughout the range of the assay. The concentration of 569-reactive antigen was determined using the MESOMARK assay, and the resulting percent recovery was calculated. The average percent recovery at each level of added analyte was 107% (Range was 103% to 113%). Representative data from the study are summarized below:

SAMPLE	ENDOGENOUS ASSAY VALUE (nM)	569-REACTIVE ANTIGEN (nM)	OBSERVED VALUE (nM)	PERCENT RECOVERY**
1	3.96	0	3.96	N/A
		3.2	7.49	105
		6.4	10.39	100
		16.0	21.25	106
		24.0	31.11	111
2	4.25	0	4.25	N/A
		3.2	8.65	116
		6.4	10.03	94
		16.0	20.95	103
		24.0	27.62	98
3	3.35	0	3.35	N/A
		3.2	7.39	113
		6.4	10.02	103
		16.0	21.20	110
		24.0	31.50	115

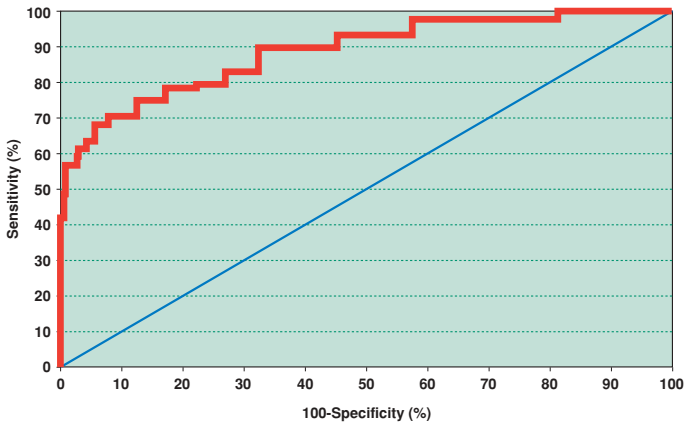
Distribution of SMRP in Different Clinical Conditions

- The distribution of SMRP levels determined in 1,086 specimens is shown in the table below.
- In this study, 99% of the healthy subjects had SMRP levels at or below 1.5 nM.

CATEGORY	N	0.0 - 1.4 nM	1.5 - 2.9 nM	3.0 - 9.9 nM	≥10.0 nM
APPARENTLY HEALTHY					
Normal Female	163	98.8%	1.2%	0.0%	0.0%
Normal Male	246	98.8%	1.2%	0.0%	0.0%
MALIGNANT CONDITIONS					
Mesothelioma Pre-Op	88	43.2%	26.1%	17.0%	13.6%
Ovarian Cancer	111	92.8%	4.5%	2.7%	0.0%
Lung Cancer	174	82.1%	11.5%	2.3%	4.0%
Colon Cancer	50	94.0%	6.0%	0.0%	0.0%
Pancreatic Cancer	52	90.4%	7.7%	1.9%	0.0%
Endometrial Cancer	25	96.0%	4.0%	0.0%	0.0%
NON-MALIGNANT CONDITIONS					
Hypertension	100	87.0%	13.0%	0.0%	0.0%
Asbestos Exposed	61	95.0%	4.9%	0.0%	0.0%
Endometriosis	16	100.0%	0.0%	0.0%	0.0%
Total	1,086				

ROC ANALYSIS

RECEIVER OPERATOR CHARACTERISTICS: MESOTHELIOMA V. HEALTHY NORMALS
AUC=0.871 (95% CI: 0.824-0.918)



ROC ANALYSIS TABLE: MESOTHELIOMA V. HEALTHY NORMALS

% SPECIFICITY	MESOMARK VALUE (> Cut Off)	% SENSITIVITY	95% CI
99	1.5 nM	57	45.8 - 67.3
97	1.3 nM	59	48.1 - 69.5
94	1.1 nM	64	52.7 - 73.6
92	1.0 nM	68	57.4 - 77.7
88	0.9 nM	70	59.8 - 79.7

Using a cut off of 1.0 nM the test was able to identify 68% of mesothelioma samples and 92% of healthy normal controls

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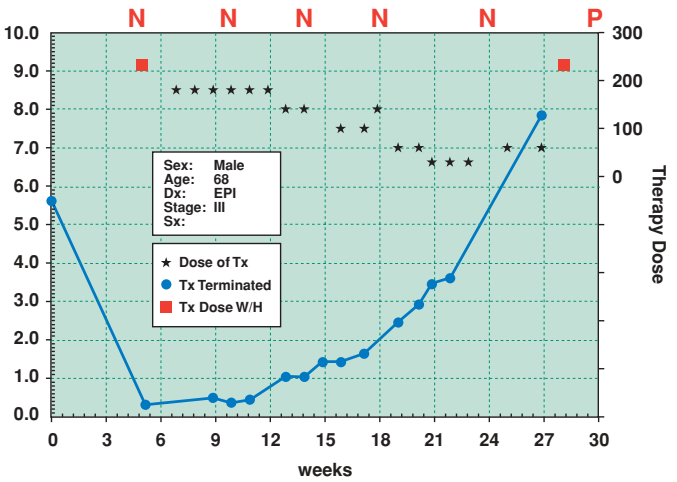
LONGITUDINAL ANALYSIS (Representative Data)

SUBJECT 22: WEEKS IN STUDY VERSUS MESOMARK (nM)



A mesothelioma patient underwent extrapleural pneumonectomy at time 0, followed adjuvantly by an investigational oral antiangiogenic therapy. Post-operative CTs revealed no progression for 30 weeks, at which time intra-thoracic and subcutaneous recurrences were documented. Note, however, that the MESOMARK levels were rising as early as 15 weeks post surgery.

LONGITUDINAL ANALYSIS

The following table provides the per patient distribution with respect to longitudinal change in SMRP values. A total of 85% (17/20) of the patients with documented recurrence of disease on-study, showed significant increases in the MESOMARK assay values, while 27% (3/11) of the patients with no documented recurrence of disease while on-study, showed no significant increase in the MESOMARK assay value. The total concordance in this study was 65% (20/31).

CHANGES IN DISEASE PER PATIENT

CHANGES IN SMRP (V)	PROGRESSION	NO PROGRESSION	TOTAL
≥30%	17	8	25
<30%	3	3	6
Total	20	11	31

Antigen Stability

ANTIGEN STABILITY IN SAMPLE COLLECTION

Fifty-one (51) serum samples, 30 of which were spiked with two different levels of 569-reactive antigen were assayed fresh, within 36 hours of the time of draw. Samples were then stored at 2-8°C and samples were tested on Days 3 and 7. SMRP values were compared back to Day 0 values to determine the percent recovery following storage.

The mean percent recoveries were 92% and 94%, on Days 3 and 7, respectively.

ANTIGEN STABILITY FOLLOWING FREEZE/THAW CYCLES

Thirteen (13) serum samples were assayed fresh and following each of ten freeze/thaw cycles. Eight (8) of the serum samples were spiked with two different levels of 569-reactive antigen; one (1) of the samples was obtained from a mesothelioma patient. Percent recoveries were determined by comparing the determined SMRP value at each freeze/thaw cycle to the original, fresh value.

Average percent recoveries at each of the freeze/thaw cycles, across all 13 samples tested, ranged from 89-98%.

OVERALL PERCENT RECOVERY

FREEZE/THAW CYCLE	PERECNT RECOVERY
F/T 1	97
F/T 2	91
F/T 3	93
F/T 4	92
F/T 5	89
F/T 6	95
F/T 7	96
F/T 8	93
F/T 9	98
F/T 10	93

Sample Processing

Fifty (50) matched serum samples were collected in red top tubes and serum separator tubes, from 50 different individuals. Thirty (30) samples were spiked with two different levels of 569-reactive antigen. SMRP values were determined and percent recovery between the matched samples was calculated.

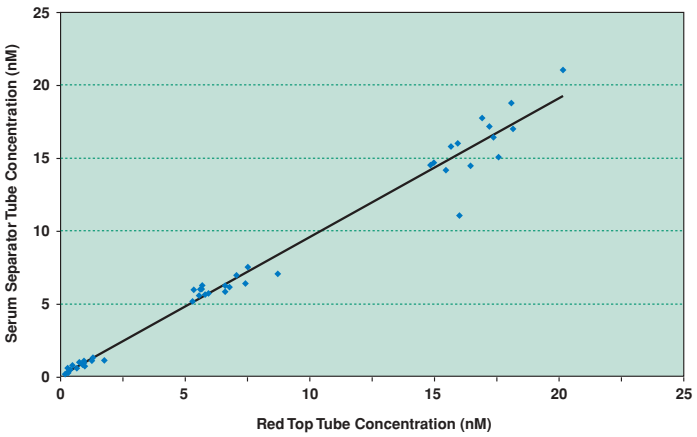
The grand percent recovery across all 50 matched samples was 100% recovery. Percent recoveries were also analyzed by analyte level and were determined to be consistent across the range of the assay (see Table below).

MEAN ANALYTE CONCENTRATION RANGE	AVERAGE PERCENT RECOVERY	MEDIAN PERCENT RECOVERY
0.3 - 1.0 nM* (n=14)	106	98
1.1 - 10.0 nM (n=18)	95	97
10.1 - 25.0 nM (n=14)	95	98

CORRELATION ANALYSIS

y = 0.9528x + 0.0292

R2 = 0.9808



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