ORIGINAL ARTICLE

Plasma Biomarker Enrichment of Clinical Prognostic Indices in Malignant Pleural Mesothelioma

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ABSTRACT

Objectives: Prognostic models for malignant pleural mesothelioma (MPM) are needed to prevent potentially futile outcomes. We combined MPM plasma biomarkers with validated clinical prognostic indices to determine whether stratification of risk for death in 194 patients with MPM improved.

Methods: Individuals were recruited from three different centers: a discovery cohort (83 patients with MPM) created by combining patients from two U.S. centers and a separate, independent cohort from Canada (111 patients with MPM). Univariable and multivariable analyses were performed on the initial discovery and independent cohorts separately. In the multivariable analyses, prognostic factors were adjusted for the European Organisation for Research and Treatment of Cancer (EORTC) prognostic index (PI) of mesothelioma. The prognostic significance of adding plasma biomarker data to the PI was determined by using the likelihood ratio test, comparing models with and without the addition of biomarker to the clinical PI. The predictive ability of the biomarker was then assessed formally using Harrell's C-index by applying the fitted model variables of the discovery cohort to the second, independent cohort, including and not including the biomarker with the PI.

Results: Higher levels of osteopontin and mesothelin were individually associated with worse prognosis after adjusting for the PI. In the independent cohort, incorporating either plasma osteopontin or mesothelin into the baseline predictive PI model substantively and statistically significantly improved Harrell's C-statistic. In the final prognostic model, log-osteopontin, EORTC clinical prognostic index, and hemoglobin remained as independently significant predictors and the entire prognostic model improved the optimism-corrected Harrell's C-index significantly, from 0.718 (0.67–0.77) to 0.801 (0.77–0.84).

Conclusions: These data suggest a possible role for preoperative plasma biomarkers to improve the prognostic capability of the EORTC PI of MPM.

Keywords: Mesothelioma; Prognosis; Osteopontin; Mesothelin; Biomarkers

Despite growing reports describing improvement in median survival time for malignant pleural mesothelioma (MPM), current prognostic stratification methods remain suboptimal. Multiple single-institution series have attempted to correlate clinical factors, standard laboratory parameters, and pathologic features in an

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attempt to better define MPM prognosis. The European Organisation for Research and Treatment of Cancer (EORTC) Prognostic Index[1] has been one standard for such prognostic quantification. Additionally, a surgery-based registry identified "best" clinical or pathologic stage, sex, age, histologic subtype, and curative intent surgery as associated with survival.[2] These factors were supplemented by white blood cell (WBC) count, hemoglobin (Hb) level, and platelet count.[3] This registry serves as an excellent reference source for future studies; however, it does not have an embedded, prospective uniform biospecimen collection component.

Our laboratory has long had an interest in osteopontin (OPN) as a potential biomarker in MPM. However, we recognized the importance of using plasma OPN (pOPN) instead of serum OPN and the existence of reproducibility issues depending on the enzyme-linked immunosorbent assay (ELISA) platform used.[4] Reports of the prognostic value of OPN in other malignancies have been based on chemotherapy-treated patients,[5–8] in whom (in some cases) the ELISA results were associated with poor coefficients of variation. Moreover, studies in the literature analyzing serum, plasma mesothelin, or mesothelin-related peptide (MRP)[9–11] have reported possible prognostic capabilities, but independent validations have been lacking. Fibulin-3 (FBLN3)[12,13] is a new plasma marker of MPM with no prognostic evaluations published to date.

Using the highest-quality ELISAs available, we therefore designed a trial investigating OPN, MRP, and FBLN3 as prognostic factors among other variables, including EORTC Prognostic Index, stage, and other reported laboratory biomarkers, such as the absolute neutrophil-to-absolute lymphocyte ratios (NLRs)[14] in both cytoreduced and nonsurgical patients with MPM. We report that pretherapy pOPN levels were significantly associated with overall survival in mixed populations of patients with MPM in an initial discovery set, and this finding was confirmed in a second independent and blinded data set. Moreover, plasma OPN significantly improved the concordance index (C-index) when added to the EORTC Prognostic Index. These patient cohorts were used to describe for future validation a prognostic model for MPM combining plasma biomarker data with clinical variables.

Methods

Patient Populations

We retrospectively analyzed patients with MPM who were prospectively recruited at the time of diagnosis from three different centers; they all provided signed informed consent to obtain plasma for biomarker studies. An initial cohort (n = 83) was created by combining patients from two centers: the New York University (NYU) Langone Medical Center (44 patients with MPM who were treated between 2007 and 2012) and the Barbara Ann Karmanos Cancer Institute (KCI) (39 patients with MPM who were treated between 1998 and 2006). A separate, independent cohort came from the Princess Margaret Cancer Centre (PMCC) (111 patients with MPM who were treated between 2004 and 2012); their levels of biomarkers were determined at NYU without advanced knowledge of their clinical and survival information. The sequencing of the component therapies for patients receiving multimodality therapy varied according to the individual institutions' protocols (Supplementary Table 1). When performed, surgery included maximal cytoreduction by pleurectomy decortication, extended pleurectomy, or extrapleural pneumonectomy along with nodal sampling/dissection.[15] The EORTC clinical prognostic index (CPI) defined patients as having a good (<1.27) or poor prognosis (≥ 1.27) using a weighting score of Eastern Cooperative Oncology Group performance status, histologic diagnosis, sex, and pretreatment WBC counts.[1] The Cancer and Leukemia Group B (CALGB) index used regression trees to examine prognostic variables in 337 patients treated in seven phase II clinical trials. Six prognostic groups were identified on the basis of age, performance status, Hb level, WBC count, and presence or absence of chest pain and weight loss.[16]

Specimen Characteristics and Plasma Biomarker Analyses

Ethylenediaminetetraacetic acid (EDTA)-treated plasma samples were collected before therapy, within a few weeks of the initial histologic diagnosis of mesothelioma, at all three centers and stored locally at −80°C until use. ELISAs, in duplicate, were performed in the NYU Thoracic Surgical Laboratory for initial discovery, and second, independent cohorts were tested for OPN (R&D Systems, Minneapolis, MN), mesothelin (R&D Systems), and FBLN3 (USCN Life Sciences, Wuhan, Hubei, People's Republic China). All plasma biomarker analyses were performed blinded to patient information. The OPN ELISA from R&D Systems was chosen because it was shown by Anborgh[4] to be the most consistent of the OPN ELISAs available. The MRP ELISA was used because the soluble MRP (SMRP) assay was commercialized and unavailable for research purposes in the United States and because data from our laboratory has demonstrated significant correlation between SMRP and MRP ($r = 0.7314$, $p < 0.0001$, 95% confidence interval [CI] for $r = 0.5040$–0.8640). Only one ELISA is commercially available for FBLN3.

Statistical Analysis

Clinicopathologic prognostic index, laboratory prognostic index, treatment prognostic index, CPI, and

biomarker variables were compared across the initial discovery and independent cohorts using the chi-square and Kruskal-Wallis tests. Survival times were measured from start of first treatment (surgery, chemotherapy, or radiation) to death or last follow-up time; for patients receiving solely supportive care, survival times were measured from time of diagnosis to death or last follow-up. The primary analysis performed was to evaluate the role of the three plasma biomarkers as independent predictors of outcome using association analyses in the discovery data set and to confirm the significant factors in the second, independent data set. The discovery data were analyzed initially at NYU, whereas the PMCC data were analyzed at PMCC, independently and blinded to what was found at NYU. Final analyses for the manuscript were performed at PMCC. For this, the Kaplan–Meier method and log-rank tests were used to assess differences in overall survival curves. Cox proportional hazard models generated hazard ratios (HRs) and 95% CIs in univariable and multivariable analyses. The nonlinear effect of the clinical factors and biomarkers on overall survival was assessed using multiple fractional polynomials models.[17–20] The need for nonlinear transformations was observed for only mesothelin and OPN: the logarithmic transformation log(biomarker value/100) was used for all analyses, denoted by log-mesothelin and log-OPN, respectively.

Univariable and multivariable analyses were performed on the discovery and independent cohorts separately. In separate multivariable analyses, prognostic factors were adjusted primarily for either the EORTC or CALGB CPIs. Because of the complexity of the CALGB CPI, its limited clinical use, and its conventional reclassification in subsequent use and because the original derivation using a partitioning approach had some categories based on very small numbers of patients, it was dichotomized into groups 4 to 6 versus 1 to 3. In contrast, the EORTC CPI was treated as a continuous variable. For Cox proportional hazard models, the proportionality of hazards assumption was assessed for each of the models in two ways: graphically by using Schoenfeld residuals and by formal testing.[17]

The prognostic significance of adding plasma biomarker data to different CPIs was determined with the likelihood ratio test by comparing models with and without the addition of biomarker to the EORTC or CALGB CPIs. When a significant association ($p < 0.05$) was found between a plasma biomarker and survival in Cox models, the predictive ability of such a biomarker was then assessed formally using Harrell's C-index.[18,21] This was done by applying the fitted model variables of the initial cohort to the second, independent cohort. Furthermore, to measure the improvement of the discriminative power, Harrell's C-indices were calculated for each of the model cohorts, including the biomarker and the model with the clinical prognostic index alone. A bootstrap resampling algorithm was applied to estimate the CIs of the difference between the C-indices (CPI alone versus CPI plus biomarker) using 200 bootstrap replicates each time.

Finally, a prognostic model was constructed using pooled data; it included significant plasma biomarkers and clinical prognostic factors selected on the basis of clinical rationale. Pooling was necessary to ensure that there were adequate events per predictor variable; however, using pooled data meant that no external validation was available. Thus, bias-corrected C-indices and CIs for the difference in C-indices between final prognostic models with or without plasma biomarkers were calculated through a bootstrap algorithm to account for optimism.[22] This difference in the C-indices reflects the improvement in the predictive ability attributed to the prognostic effect of log-OPN and log-mesothelin. Additionally, goodness of fit survival curves of the final models were assessed through visual inspection of observed versus predicted survival curves after grouping the risk scores derived from the prognostic model into tertiles.

All analyses were performed in R, v.3.0.2 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). Parametric and Cox proportional hazards models were fitted using the survival package,[23] C-indices were calculated using Hmisc package.[24]

Results

Baseline information for the discovery (NYU/KCI) and second, independent (PMCC) cohorts is presented in Table 1. Compared with the NYU/KCI cohort, the PMCC cohort consisted of patients who had a more advanced stage of disease at diagnosis, worse performance status, more significant weight loss, and worse prognosis by the EORTC CPI. However, patients in the validation cohort also had higher baseline Hb levels and were more likely to have received aggressive cytoreductive surgery combined with radiation ($p < 0.05$, each comparison). The discovery and validation cohorts were also different in terms of the therapies used (Supplementary Table 1). In contrast, within the discovery cohort, patients of the subcohorts from KCI and NYU had similar baseline characteristics (Supplementary Table 2).

Although the distributions of plasma mesothelin and OPN levels were similar between the KCI/NYU and PMCC cohorts, the validation cohort had lower baseline levels of FBLN3 (Table 1 and Supplementary Fig. 1). More advanced stage of disease was associated with higher levels of plasma OPN and mesothelin (but not FBLN3) in the KCI/NYU cohort (Supplementary Table 3). In the PMCC cohort, more advanced stage of disease was

Table 1. Baseline Information for the Initial Discovery (NYU/KCI) and Independent (PMCC) Data Sets

Covariate	Units	NYU/KCI (n = 83)	PMCC (n = 111)	*p* Value[a]
Clinicopathologic Variables				
Age	Median (range), y	63 (34-86)	65 (36-83)	0.54
Sex				0.04
Female	n (%)	21 (25%)	14 (13%)	
Male	n (%)	62 (75%)	97 (87%)	
Performance status (ECOG)				0.007
0	n (%)	32 (39%)	20 (18%)	
1	n (%)	37 (45%)	66 (59%)	
2 or higher	n (%)	14 (17%)	25 (23%)	
Chest pain				0.56
No	n (%)	37 (47%)	57 (51%)	
Yes	n (%)	42 (53%)	54 (49%)	
Missing	n	4	0	
Weight loss				<0.001
No	n (%)	67 (85%)	63 (57%)	
Yes	n (%)	12 (15%)	48 (43%)	
Missing	n	4	0	
Histologic diagnosis				1.00
Epithelial	n (%)	58 (70%)	77 (69%)	
Other	n (%)	25 (30%)	34 (31%)	
Stage				<0.001
I/II	n (%)	26 (31%)	9 (8%)	
III/IV	n (%)	57 (69%)	102 (92%)	
Laboratory Data				
Hemoglobin level	Median (range), g/dL	12.0 (8-15)	13.3 (7-17)	<0.001
Missing	n	4	2	
White blood cell count	Median (range) $\times 10^9$/L	8.0 (4-33)	8.0 (4-23)	0.54
Missing	n	4	1	
NLR	Median (range)	3.2 (1.4-19)	3.7 (1.3-15)	0.31
Missing	n	39	1	
Platelet count	Median (range) $\times 10^9$/L	302 (41-895)	326 (136-880)	0.15
Missing	n	4	1	
Treatments				
Cytoreductive surgery				0.02
No	n (%)	32 (39%)	62 (56%)	
Yes	n (%)	51 (61%)	49 (44%)	
Chemotherapy				0.26
No	n (%)	19 (23%)	34 (31%)	
Yes	n (%)	64 (77%)	77 (69%)	
Radiation				<0.001
No	n (%)	79 (95%)	47 (42%)	
Yes	n (%)	4 (5%)	64 (58%)	
Clinical Prognostic Indices				
EORTC prognosis				0.03
Good prognosis	n (%)	26 (33%)	20 (18%)	
Poor prognosis	n (%)	53 (67%)	90 (82%)	
Missing	n	4	1	
CALGB[b]				
Groups 1-3	n (%)	53 (67%)	64 (58%)	0.23
Groups 4-6	n (%)	26 (33%)	46 (42%)	
Missing	n	4	1	
Plasma Biomarkers				
Fibulin-3 level	Median (range), ng/mL	101 (40-316)	54 (2-204)	<0.001
Missing	n	1	0	

(continued)

Table 1. Continued

Covariate	Units	NYU/KCI (n = 83)	PMCC (n = 111)	*p* Value[a]
Mesothelin level	Median (range), ng/mL	54 (4-272)	43 (5-910)	0.41
Missing	n	1	0	
Osteopontin level	Median (range), ng/mL	120 (23-849)	108 (19-588)	0.24

[a]*p* Value comparing the NYU/KCI and PMCC data sets using nonparametric Kruskal-Wallis tests.
[b]The EORTC clinical prognostic index was divided into good and poor prognoses on the basis of a regression value cutpoint of 2.7. Weight loss was defined as a weight loss of 10% or more.
NYU, New York University; KCI, Karmanos Cancer Institute; PMCC, Princess Margaret Cancer Centre; ECOG, Eastern Cooperative Oncology Group; NLR, neutrophil-to-lymphocyte ratio; EORTC, European Organisation for Research and Treatment of Cancer; CALGB, Cancer and Leukemia Group B.

associated with higher levels of plasma mesothelin only, but only 8% of this cohort had early Stage I/II disease.

Despite their having more aggressive disease, with more aggressive management, patients in the validation cohort had better survival than did those in the discovery cohort ($p = 0.04$ by the log-rank test; Supplementary Fig. 2 and Table 2). Survival was similar in the NYU and KCI subcohorts that constituted the discovery cohort ($p = 0.68$). All deaths were attributable to MPM.

In all survival analyses, no violations of the assumption of proportionality were observed. Nonlinear effects of mesothelin and OPN (but not FBLN3) on OS were observed (Supplementary Fig. 3); log-transformation was found to best address this issue, and log-mesothelin and log-OPN were used for all prognostic analyses. The relationships between individual clinicopathologic, laboratory, and treatment variables with survival in the discovery and validation data sets are presented in Table 2. Many of the non–treatment-related variables have been incorporated into the EORTC[1] and CALGB[16] CPIs, which were both significantly prognostic in both the initial discovery and second, independent cohorts (Table 2). Of the plasma biomarkers evaluated in the KCI/NYU cohort, higher levels of log-OPN and log-mesothelin (but not FBLN3) were each individually associated with worse prognosis after adjusting for CPIs (Table 3) and were therefore further evaluated for their predictive ability in the independent PMCC cohort. In the PMCC cohort, incorporating either plasma log-OPN or log-mesothelin into the baseline predictive CPI models substantively and significantly improved Harrell's C-statistic as the CIs of the difference in C-statistics did not cross zero (Table 3).

In developing a prognostic model that includes log-OPN and log-mesothelin, we utilized the entire data set of individuals with complete data on all key variables (n = 154); missing individuals had clinicodemographic and outcomes data similar to those of the discovery cohort. Predictors included in this model were chosen on the basis of clinical factors: in addition to log-OPN and log-mesothelin, we included CPI, clinical stage, and three laboratory values associated with outcome in our data sets (Hb level, NLR, and platelet counts). The EORTC CPI was included in the model over the CALGB CPI because it had better performance in our population (Table 3), is the more commonly used CPI in clinical practice, and was generated from a regression model as a continuous variable. Within this prognostic model, log-OPN, EORTC CPI, and Hb level continued to remain as independently significant predictors, and the entire prognostic model improved the optimism-corrected Harrell's C-index significantly from 0.718 (0.67–0.77) to 0.801 (0.77–0.84) (Table 4). Model calibration curves (Fig. 1) visually confirm good fit between predicted and observed survival curves when these prognostic scores are used.

Discussion

Prognostic biomarkers, including clinicodemographic information, standard laboratory parameters, and novel plasma- or tissue-based tests have tremendous potential to improve care by assisting clinicians in appropriately tailoring therapies to individual patients' needs. The low disease incidence and diverse treatment strategies have hindered the discovery of prognostic biomarkers in MPM. Nonetheless, studies attempting to define novel prognostic markers in MPM have been reported, chiefly in patients receiving supportive care or chemotherapy only. Both the EORTC prognostic score and the CALGB index, which includes a combination of clinical and laboratory variables, have been validated prognostically in studies of noncytoreduced MPMs, and recently were again validated by Meniawy et al.[26] in 274 patients with MPM; of the two prognostic indices, the EORTC prognostic index has been the more commonly used.

Novel MPM prognostic biomarkers include tissue-based assays, including a four-gene expression test,[26] expression arrays,[27] P16/CDKN2A homozygous deletion,[28] high nuclear grade,[29] and mir-29c*,[30] all of which have been reported to have prognostic significance; however, few have been blindly validated. The most extensively published candidate blood-based prognostic biomarkers include sMRP[10,31–34] and OPN, along with NLR and fibrinogen.[35] At least three single reports point to OPN as a possible prognostic factor in MPM in nonsurgical MPM cohorts.[34,36,37] The study by Hollevoet et al. involved 48 patients, all of whom received only

Table 2. Univariable Analysis of Baseline Characteristics on Overall Survival for the Initial Discovery (NYU/KCI) Data Set and for the Second, Independent (PMCC) Data Set

Survival Characteristic	NYU/KCI			PMCC		
Median follow-up time	11 mo			16 mo		
Median overall survival time	11 (95% CI: 8-14) mo			18 (95% CI: 14-22) mo		
Percentage alive at 6 mo	70%			86%		
Percentage alive at 12 mo	41%			67%		
Percentage alive at 24 mo	23%			26%		
Characteristic	HR (95% CI)	*p* Value	Global *p* Value[1]	HR (95% CI)	*p* Value	Global *p* Value[a]
Clinicopathologic Variables						
Age per 10 year increase	1.18 (0.9-1.5)		0.17	1.34 (1.02-1.8)		0.04
Sex			0.24			0.24
Male vs. female	1.40 (0.8-2.5)			1.52 (0.8-3.1)		
Performance status (ECOG)			<0.001			0.011
1 or higher vs. 0	2.99 (1.8-5.1)			2.49 (1.2-5.0)		
Chest pain			0.08			0.053
Yes vs. no	1.58 (1.0-2.6)			1.54 (0.99-2.4)		
Weight loss			<0.001			<0.001
Yes vs. no	3.32 (1.7-6.4)			2.8 (1.8-4.4)		
Histologic diagnosis			0.14			<0.001
Other vs. epithelial	1.48 (0.0-2.5)			2.65 (1.6-4.3)		
Stage			<0.001			0.92
III/IV vs. I/II	3.93 (2.2-7.2)			1.05 (0.5-2.4)		
Laboratory Data						
Hemoglobin level per 1g/dL increase	0.83 (0.7,0.9)		0.003	0.73 (0.6-0.9)		<0.001
White blood cell count per 10^9/L increase	2.08 (1.2-3.6)		0.01	2.69 (1.5-4.8)		0.001
NLR per unit increase	1.13 (1.0-1.2)		0.007	1.09 (1.0-1.2)		<0.05
Platelet count per 50 x 10^9/L increase	1.15 (1.1-1.3)		<0.001	1.22 (1.1-1.3)		<0.001
Treatments						
Cytoreductive surgery			<0.001			<0.001
Yes vs. no	0.30 (0.2-0.5)			0.37 (0.2-0.6)		
Chemotherapy			0.001			0.30
Yes vs. no	0.41 (0.2-0.7)			0.78 (0.5-1.3)		
Radiation			0.66			<0.001
Yes vs. no	1.26 (0.5-3.5)			0.42 (0.3-0.7)		
Clinical Prognostic Indices						
EORTC (per 1.0 unit increase in value)	2.68 (1.8-4.1)		<0.001	3.07 (2.0-4.7)		<0.001
CALGB (Groups 4-6 vs. groups 1-3)	4.07 (2.3-7.1)		<0.001	1.78 (1.1-2.8)		0.01
Plasma Biomarkers[b]						
Fibulin-3 per 50 ng/mL increase	0.87 (0.7-1.1)		0.23	1.32 (1.0-1.7)[c]		0.06
Log-mesothelin per log(ng/100 mL) increase	2.16 (1.5-3.1)		<0.001	1.28 (1.1-1.6)		0.22
Log-osteopontin per log(ng/100 mL) increase	3.31 (2.3-4.8)		<0.001	3.93 (2.9-5.4)		<0.001

Note: Weight loss was defined as a weight loss of 10% or more.

[a]Global *p* values compare across all levels of a single variable. Although discovery and validation analyses are provided in the same table, the analyses were performed sequentially.

[b]Each plasma biomarker was analyzed as a continuous variable. For the plasma biomarkers, the primary key findings are in the discovery cohort; univariable analysis of plasma biomarkers for the validation cohort is presented for completeness.

[c]This value is being presented for completeness; as there was no significance in the discovery cohort, fibulin-3 was not formally validated.

NYU, New York University; KCI, Karmanos Cancer Institute; PMCC, Princess Margaret Cancer Centre; HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; NLR, neutrophil-to-lymphocyte ratio; EORTC, European Organisation for Research and Treatment of Cancer; CALGB – Cancer and Leukemia Group B.

chemotherapy, and the prognostic significance of OPN was not compared with the EORTC prognostic criteria. Grigoriu et al. found both SMRP and OPN to be prognostic in 91 patients with MPM who were treated with or without surgery; but again, they did not combine the markers with each other or with clinical prognostic criteria. It is interesting that in both these studies, as in ours, stage of disease did not persist as an independent predictor of death in multivariate analysis or in the final prognostic models. Nevertheless, biomarker studies of the prognostic implications of pOPN have been largely ignored owing to the inability to reproduce its value as a *specific diagnostic* marker of MPM and because of the aforementioned performance differences across available ELISA platforms. Another recently described biomarker, FBLN3,[38] was reported to have diagnostic

Table 3. Predictive Models of Overall Survival for Log-Osteopontin and Log-Mesothelin

		Adjusted for EORTC CPI		Adjusted for CALGB CPI	
Cohort	Variable	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
NYU/KCI	log-osteopontin	2.70 (1.8-4.0)	<0.001	2.71 (1.8-4.1)	<0.001
	log-mesothelin	1.94 (1.4-2.8)	<0.001	1.63 (1.1-2.4)	0.009
PMCC	log-osteopontin	3.53 (2.6-4.9)	<0.001	4.05 (2.9-5.6)	<0.001
	log-mesothelin	1.27 (1.1-1.5)	<0.001	1.40 (1.2-1.7)	<0.001

Discovery (NYU/KCI) Cohort

Prognostic Variables	EORTC CPI	CALGB CPI
CPI alone (for log-osteopontin analysis),[a] C-index (95% CI)	0.649 (0.59-0.70)	0.641 (0.59-0.69)
CPI alone (for log-mesothelin analysis),[a] C-index (95% CI)	0.645 (0.59-0.70)	0.640 (0.59-0.69)
CPI + log-osteopontin, C-index (95% CI)	0.767 (0.71-0.82)	0.763 (0.71-0.81)
CPI + log-mesothelin, C-index (95% CI)	0.692 (0.63-0.76)	0.724 (0.66-0.79)
Improvement in Harrell's C-indices when adding log-osteopontin[b]	0.118 (0.10-0.18)	0.122 (0.11-0.18)
Improvement in Harrell's C-indices when adding log-mesothelin[b]	0.045 (0.03-0.11)	0.084 (0.06-0.13)

Validation (PMCC) Cohort

Prognostic Variables	EORTC CPI	CALGB CPI
CPI alone, C-index (95% CI)	0.596 (0.55-0.64)	0.602 (0.54-0.66)
CPI + log-osteopontin, C-index (95% CI)	0.811 (0.76-0.86)	0.781 (0.73-0.83)
CPI + log-mesothelin, C-index (95% CI)	0.650 (0.58-0.72)	0.649 (0.58-0.71)
Improvement in Harrell's C-indices when adding log-osteopontin[b]	0.216 (0.20-0.26)	0.179 (0.16-0.23)
Improvement in Harrell's C-indices when adding log-mesothelin[b]	0.054 (0.03-0.12)	0.047 (0.03-0.10)

Note: (*Top panel*) Cox proportional hazard models of association; *p* values are derived from likelihood ratios comparing the models with and without the biomarker of interest. (*Middle and bottom panels*) Prognostic model evaluation comparing Harrell's C-indices with and without the biomarker of interest in the model. Log-mesothelin and log-osteopontin are in log(ng/100mL) units.

[a]The baseline CPI-only C-indices reflect the patient samples available for the associated biomarker analyses; there was one missing log-mesothelin value (and no missing los-osteopontin values) in the discovery data set that results in minor differences in the CPI-only indices.

[b]Confidence intervals for the difference in Harrell's C-indices were calculated using 200 bootstrap replicates. Intervals that did not cross zero are interpreted as having the Harrell's C-index demonstrate significant improvement with the addition of the biomarker.

NYU, New York University; KCI, Karmanos Cancer Institute; PMCC, Princess Margaret Cancer Centre; CPI, clinical prognostic index; HR, hazard ratio; CI, confidence interval; EORTC, European Organisation for Research and Treatment of Cancer; CALGB, Cancer and Leukemia Group B.

discrimination in plasma for diagnostic purposes but was not prognostic in plasma. Two studies have reported that FBLN3 may be prognostic when measured in the pleural effusions of patients with MPM.[38,41] The NLR could not be validated as a reliable prognostic biomarker in a recent report from Meniawy et al.[25]

The novelty of the present study, therefore, was to design a study that examined whether the most-studied

Table 4. Prognostic Model Development: Pooled Analysis

	Hazard Ratio (95% Confidence Interval)	*p* Value
Log-osteopontin	3.57 (2.5-5.1)	<0.001
Log-mesothelin	0.97 (0.8-1.2)	0.80
EORTC CPI	1.99 (1.4-2.9)	<0.001
Stage III/IV vs. I/II	1.36 (0.8-2.4)	0.30
Hemoglobin level	0.10 (0.0-0.3)	<0.001
Platelet count	2.56 (0.6-10)	0.19
Neutrophil-to-lymphocyte ratio	0.96 (0.5-2.0)	0.91

Prognostic model	C-Index, Original	C-Index, Optimism-Corrected	Difference in C-Index
Without log-osteopontin, log-mesothelin	0.726 (0.68-0.78)	0.718 (0.67-0.77)	
With log-osteopontin, log-mesothelin	0.813 (0.78-0.85)	0.801 (0.77-0.84)	0.087 (0.08-0.12)

Note: Because of missing data, only 154 pooled cases were analyzed. Selection of variables for the prognostic model was based on clinical rationale. The EORTC CPI was included in the model as it was the more commonly used CPI and because its general performance was better than with the CALGB CPI (see Table 3); as this CPI did not include stage, we included that variable separately. Finally, three laboratory variables not found in the EORTC CPI were also included. The regression equation generating the risk score is RS = 1.273 * log(osteopontin[ng/mL]/100) - 0.025 * log(Mesothelin(ng/mL)/100) + 0.686 * EORTC CPI - 2.349 * Hemoglobin(g/dL) - 0.0402 * NLR/10 + 0.941 * Platelets[$\times 10^9$ /L]/1000 + 0.307 (if stage III/IV; if I/II, omit this last term). Median (range) of risk score values is 0.046 (-2.88 to 3.28).

CPI, clinical prognostic index; EORTC, European Organisation for Research and Treatment of Cancer.

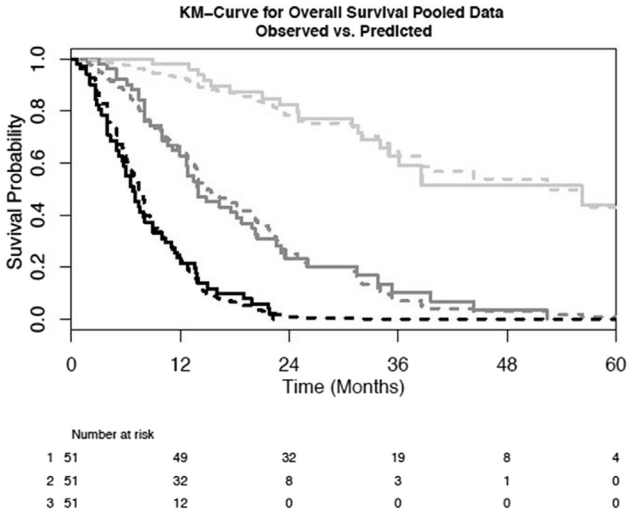

Figure 1. Visual inspection of model fit curves evaluating tertiles of the risk score generated from the pooled prognostic model. The observed (*solid*) and predicted (*dashed*) survival curves are presented. The darkest color represents the worse prognostic risk scores, with progressively lighter lines representing the higher tertiles of risk score. The tertile risk score ranges are as follows: worst prognosis category (risk scores -2.88 to -0.619), intermediate prognosis category (risk scores -0.619 to 0.710), and best prognosis (risk scores 0.71 to 3.28).

MPM plasma and serum biomarkers have added value to known clinical prognostic indices. Using a carefully constructed and blinded discovery and validation analysis that combines all the plasma-based biomarkers as well as the most reliable clinical and laboratory parameters was also one of the novel aspects of the study. We examined most of the reported biomarkers in MPM in both cytoreduced (56%) and noncytoreduced patients (44%) and used an independent cohort for validation. The number of patients in the analysis (n = 188) is small compared with that in the recently published IASLC database (n = 1494), but it compares favorably with the number of patients analyzed by Curran et al.[1] (n = 204) and most recently by Meniawy et al.[25] To maintain maximum stringency, we required that all plasma biomarker levels be measured on samples obtained before the initiation of therapy and used a discovery set followed by a blinded validation cohort. Unlike MPM, other tumor types can allow for "matching" of cohorts owing to a large number of available patients. Our patients were recruited prospectively from institutions with considerable expertise in the treatment of MPM, but the components and timing of the multimodality treatment approaches were not uniform across the cohorts. In fact, the PMCC investigators are unique in their promising reports for preoperative radiation therapy and extrapleural pneumonectomy in MPM.[39] The test set from KCI was collected before the discovery and validation sets, which were collected simultaneously from NYU and PMCC. Nevertheless, despite the study samples having been accrued over a 14-year period, the median survivals of the KCI and NYU samples were not significantly different when each site's patients with stage I/II disease (28 months versus 31 months, respectively, $p = 0.48$) and patients with stage III/IV disease (12 months versus 16 months, respectively, $p = 0.19$) were compared.

Studying mesothelioma is a special case because of its rarity compared with lung cancer. Methodologies that can be readily applied to common cancers are difficult to apply to mesothelioma. In univariable analyses, some of the variables that were significantly associated with reduced survival across all cohorts have been previously reported to be similarly prognostic, including poor performance status, weight loss, low Hb level, high WBC count, increased NLR, high platelet count, and inability to cytoreduce. The EORTC prognostic index was significant in both the discovery and validation cohorts, and it was used as a surrogate for all non–laboratory-related clinical variables.

As stated earlier, what has been lacking in previous reports on MPM biomarkers but addressed by us is whether any of the reported plasma biomarkers provide added value to the clinical CPI according to C-statistic comparison. Both log-OPN and log-mesothelin were predictive of survival when added to the EORTC CPI, and both increased the C-index for the discovery and validation sets. Only after we demonstrated that there was added value to both a discovery and validation set did we use a pooled analysis model, which should be validated in future trials. By using a bootstrap internal validation modeling approach, we attempted to compensate for overfit or "optimism," and this approach has greater power in demonstrating improvement in prognostication. In our analysis, using separate development and validation data sets may actually be inferior to pooling all the data and using bootstrap internal validation. As endorsed by Harrell et al[22] and by Steyerberg and Lingsma,[40] the pooled effect estimates in the model are more likely to be accurate than are the estimates in either group alone.

As the MPM staging system undergoes revision, supplementary prognostic factors in addition to the usual clinicopathologic demographics could add value in the selection of patients for radical and potentially morbid procedures. These supplementary factors, including plasma biomarkers, could alert clinicians that certain patients with MPM are not candidates for cytoreductive surgery because their chance of survival is limited by a more aggressive phenotype. To potentially validate the CPI for the pooled data described in this study, an international effort to collect blood elements before treatment

must be addressed. Such efforts should be limited not only to potential cytoreducible patients but also to those enrolled in ongoing novel therapeutic trials.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.02.006.

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