ORIGINAL ARTICLE

Plasma Biomarker Enrichment of Clinical Prognostic Indices in Malignant Pleural Mesothelioma

Harvey I. Pass, MD,[a,*] Chandra Goparaju, PhD,[a] Osvaldo Espin-Garcia, MMath,[b] Jessica Donington, MD,[a] Michele Carbone, MD,[c] Devalben Patel, BSc,[b] Zhuo Chen, PhD,[b] Ronald Feld, MD,[b] John Cho, MD,[b] Shirish Gadgeel, MD,[d] Antoinette Wozniak, MD,[d] Abraham Chachoua, MD,[a] Natasha Leighl, MD,[b] Ming-Sound Tsao, MD,[b] Marc de Perrot, MD,[b] Wei Xu, PhD,[b] Geoffrey Liu, MD[b]

[a]*Langone Medical Center, New York University, New York, New York*
[b]*Princess Margaret Cancer Centre, Ontario Cancer Institute, University Health Network and University of Toronto, Toronto, Ontario, Canada*
[c]*University of Hawaii Cancer Center, Honolulu, Hawaii*
[d]*Karmanos Cancer Institute, Wayne State University, Detroit, Michigan*

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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

*Corresponding author.

Disclosure: Dr. Pass and Wayne State University have filed a patent application for the use of osteopontin in the diagnosis and prognosis of pleural mesothelioma (US 20090311721 A1). The remaining authors declare no conflict of interest.

Address for correspondence: Harvey I. Pass, MD, Department of Cardiothoracic Surgery, NYU Langone Medical Center, 530 First Avenue, Suite 9V, New York, NY, 10016. E-mail: harvey.pass@med.nyu.edu

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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