Comparison of Osteopontin, Megakaryocyte Potentiating Factor, and Mesothelin Proteins as Markers in the Serum of Patients with Malignant Mesothelioma

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Introduction: There is increasing interest in identifying a blood-based marker for the asbestos-related tumor, malignant mesothelioma. Three potential markers for mesothelioma are mesothelin, megakaryocyte potentiating factor (MPF) and osteopontin. The purpose of the current study was to directly compare these biomarkers in the same sample population, determining their sensitivity and specificity in establishing a diagnosis, and to determine if diagnostic accuracy for mesothelioma is improved by combining the data from all three markers.

Methods: Serum levels of mesothelin, MPF and osteopontin were determined by commercially available assays in 66 samples from patients with pleural malignant mesothelioma, 20 healthy individuals, 21 patients with asbestos-related lung or pleural disease, 30 patients presenting with benign pleural effusions and 30 patients with other malignancies.

Results: Serum levels of the three markers were elevated in mesothelioma patients. At a level of specificity of 95% relative to healthy controls and patients with benign asbestos related disease, the sensitivity for mesothelioma was 34% for MPF, 47% for osteopontin and 73% for mesothelin. Osteopontin and MPF were unable to differentiate patients with mesothelioma from patients with other malignancies or those presenting with transudative pleural effusions. Combining the data from the three biomarkers using a logistic regression model did not improve sensitivity for detecting mesothelioma above that of the mesothelin marker alone.

Conclusion: Serum mesothelin remains the most specific marker for the diagnosis of mesothelioma.

Key Words: Mesothelioma, Tumor markers, Mesothelin, SMRP, MPF, Osteopontin, Diagnosis.

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Malignant mesothelioma is caused by exposure to asbestos and is a fatal disease with limited treatment options.[1,2] In other forms of cancers, serum-based tumor markers have provided clinicians with a valuable aid in the management of patients. Markers may have different roles in diagnosis, monitoring, assessing prognosis, and in detection of early disease. There are no markers in routine clinical use for malignant mesothelioma, although the use of soluble mesothelin has received recent attention.

Mesothelin is a differentiation marker of mesothelial cells. Differential post-transcriptional and post-translational processing of the mesothelin gene produces four protein products: megakaryocyte potentiating factor (MPF),[3,4] mesothelin variant 1, mesothelin variant 2,[5] and serum mesothelin-related protein (SMRP).[6–8] MPF is a soluble ~32 kd protein with cytokine activity.[3,4] Mesothelin is ~40 kd glycosylated protein predominately anchored to the cell surface of normal mesothelial cells, but also present in solution (the physiological mechanism for mesothelin release from the cell surface is not clear).[9,10] Mesothelin variant 1 is the predominant form of the protein and differs from variant 2 by only eight amino acids. SMRP is a soluble protein with an identical N-terminal sequence to mesothelin but a unique C-terminal.[6]

Using an enzyme-linked immunosorbent assay (ELISA) which detects both mesothelin variant 1 and SMRP, we recently demonstrated increased levels of this biomarker in the serum of patients with mesothelioma,[11] findings recently confirmed using the same assay[12] and an independent[13] assay. Serum mesothelin is highly specific for mesothelioma (specificity 98%) with a sensitivity at diagnosis of 49% (57/117)[14] and 84% in advanced disease.[11] Despite very encouraging findings showing serum mesothelin as a useful marker in mesothelioma, we found that at diagnosis only half of mesothelioma patients were mesothelin positive; therefore, there is a need for additional markers to improve diagnostic sen-

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sitivity. Possible candidate markers include proteins that are related to mesothelin, such as MPF, or those that are independent of mesothelin but associated with the disease such as osteopontin.

Given the successful detection of mesothelin and SMRP in the serum of cancer patients, two groups recently, independently developed assays to measure levels of MPF.[15,16] Onda and colleagues showing 51/56 patients with advanced mesothelioma had elevated serum MPF.[15] As mesothelin must be cleaved or shed from the cell surface serum MPF may be at least as sensitive, if not more so, than serum mesothelin as a marker of mesothelioma. To clarify this, we measured and compared serum mesothelin and MPF levels in the same patient cohort using commercially available assays.

Another strategy to improve diagnostic sensitivity is to incorporate the information from multiple markers. Pathologists use suites of immunohistochemical markers in tissue to improve diagnostic accuracy of the histopathologic findings[17,18] and multiple serum markers have achieved greater sensitivity and specificity in the diagnosis of some cancers, i.e., ovarian[19,20] and prostate cancer.[21]

Recently, we tested the hypothesis that combining data from two markers (mesothelin and CA125) would improve the sensitivity of the mesothelin marker used alone to distinguish mesothelioma patients from other asbestos-exposed individuals; however, we found that this combination was not significantly different to the results for mesothelin alone.[14]

Osteopontin is a secreted 44 kd glycoprotein with roles in cell-matrix interactions, cell migration, and other diverse functions. Serum osteopontin levels are elevated in breast, ovarian, lung, and prostrate cancer.[22] Osteopontin has been shown to be a useful adjunct to CA125 in the detection of recurrent ovarian cancer.[19] In mesothelioma serum, osteopontin has a sensitivity of 78% at a specificity of 85% relative to subjects exposed to asbestos.[23]

The purpose of the current study was to directly compare each of these biomarkers in the same sample population and to determine if diagnostic accuracy for mesothelioma is improved by combining these three markers.

PATIENTS AND METHODS

Patients and Controls

Serum samples were collected from consecutive patients presenting at the respiratory clinics of either Sir Charles Gairdner Hospital or the Hollywood Specialist Centre in Perth, Western Australia and form part of the Australian Mesothelioma Tissue Bank. We obtained written and oral informed consent from participants. This study was approved by the human ethics committees of Sir Charles Gairdner and Hollywood Hospitals. The final diagnosis in all patients was confirmed by pathologists experienced in the diagnosis of mesothelioma and effusions and included clinical follow-up of all cases until death or for an average of 6.8 months (range, 1–42 months) to confirm that the clinical pattern matched the diagnosis. Effusions were classified as being malignant or nonmalignant on the basis of cytologic and immunohistochemical features. Nonmalignant effusions were classified as exudates or transudates by Light's criteria.[24]

Blood samples were collected by routine venepuncture into clotted blood tubes. Samples were allowed to clot for at least 2 hours at room temperature, or alternatively were stored at 4°C before processing. Samples were centrifuged at 1200 rpm for 10 minutes, then the supernatant was removed, aliquoted, and stored at −80°C until use.

Serum Mesothelin

Soluble mesothelin concentrations were determined in duplicate following the manufacturer's instructions using a double-determinant ELISA assay, the MESOMARK kit, supplied by Fujirebio Diagnostics (Malvern, PA). Mesothelin concentrations were determined from a standard curve performed on each plate and expressed as nanomolar. Dilution of samples was carried out, if necessary, using the diluent supplied by the manufacturer. All assays were performed on coded samples by technical staff unaware of the patient's diagnosis.

MPF Assay

The Human N-ERC/Mesothelin Assay kit was purchased from Immuno-Biologic Laboratories (Gunma, Japan) and the manufacturer's recommended protocol was followed. Serum samples were assayed undiluted and concentrations of MPF were determined from a standard curve performed in parallel. The manufacturer's reported assay sensitivity was 0.024 ng/ml; the concentrations for samples from 0.024 ng/ml to 0.011 ng/ml were extrapolated from the standard curve, for the purposes of data analysis concentrations of samples below 0.011 ng/ml were assigned a value of 0.005 ng/ml.

Osteopontin Assay

The human osteopontin assay, a double-determinant ELISA, was purchased from Immuno-Biologic Laboratories. The manufacturer's recommended protocol was followed except serum was used as the assay substrate instead of plasma. Serum samples were diluted 1:3 in buffer supplied with the kit. The manufacturer's reported assay sensitivity was 3.33 ng/ml, the concentrations for samples from 3.3 ng/ml to 0.5 ng/ml were extrapolated from the standard curve, and for the purposes of data analysis concentrations of samples below 0.5 ng/ml were assigned a value of 0.1 ng/ml.

Statistical Analysis

Differences between groups of patients were assessed by Student t test after transforming the biomarker values to the log scale for which the distributions were closer to normality. For the same reason, median biomarker values were estimated from the mean on the log scale and exponentiated to provide the estimate of the median on the original scale. All reported p values are two sided. A level of $p < 0.05$ was accepted as significant. Receiver operating characteristic (ROC) curves display the trade-off between sensitivity and specificity for biomarkers differentiating between groups of patients. Area under the curve (AUC) for two biomarkers, including "markers" formed by combining multiple biomarkers, was compared using the method of DeLong et al.,[25] which accounts for the correlation because of the markers being measured on the same set of serum samples. To combine biomarkers, we first transformed data with the natural logarithm, and then standardized the markers relative