The Use of Pleural Fluid sCD44v6/std Ratio for Distinguishing Mesothelioma from Other Pleural Malignancies

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Objective: Differentiating metastatic adenocarcinoma from malignant pleural mesothelioma is often a challenging task. Spliced forms of CD44, such as exon v6 (CD44v6), have been implicated in tumor metastasis. We examined the diagnostic performance of soluble (s) CD44v6 and CD44 standard (sCD44std) as biomarkers for nonmesothelioma pleural malignancies in a retrospective series.

Methods: The pleural fluid from 161 patients with pleural effusion (33 mesotheliomas, 104 nonmesothelioma malignancies, and 24 benign conditions) was analyzed for sCD44v6 and sCD44std levels using an enzyme-linked immunosorbent assay kit. The ability of sCD44v6 and sCD44std levels and the sCD44v6/std ratio for distinguishing mesothelioma from nonmesothelioma malignancy were examined.

Results: Median pleural fluid concentrations of sCD44v6 but not sCD44std were significantly higher in patients with nonmesothelioma malignancy (101.5 ng/mL) than in those with mesothelioma (38 ng/mL, $p < 0.0001$). Fluids from metastatic squamous cell carcinomas exhibited particularly high sCD44v6 levels (388 ng/mL). A cutoff value of 100 ng/mL had the highest accuracy for distinguishing mesothelioma from nonmesothelioma malignancy (sensitivity 53% and specificity 88%) or metastatic adenocarcinoma (sensitivity 60% and specificity 88%). An sCD44v6/std ratio of more than 0.34 discriminated between adenocarcinoma and mesothelioma with a sensitivity of 60%, a specificity of 93%, a likelihood ratio positive of 9.97, and an area under the curve of 0.87 (95% confidence interval: 0.80–0.94).

Conclusions: The pleural fluid sCD44v6/std ratio may be a new diagnostic marker in the differential diagnosis between primary mesothelioma and other pleural malignancies. Values greater than 0.34 predict nonmesothelioma malignancy and may be a help in determining whether an invasive thoracoscopy is necessary.

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Epithelial mesotheliomas can be extremely difficult to distinguish cytohistologically from metastatic involvement of the pleura. The difficulties in establishing a differential diagnosis between mesothelioma and pleural metastases are illustrated by a study of 45 pleural fluid specimens, in which the interobserver agreement of nine expert pathologists in discriminating these two entities based on morphologic criteria alone was poor (kappa value of 0.343).[1] Therefore, staining of cell blocks or, preferably, of pleural tissue samples for a battery of mesothelial and carcinoma markers is often necessary to make a definitive diagnosis.[2]

The search for a reliable diagnostic biomarker of mesothelioma still continues. Among all reported candidates, soluble mesothelin, in serum or pleural fluid, has received the most attention.[3] Nevertheless, soluble mesothelin levels can be elevated in adenocarcinomas that metastasized to the pleura, particularly from an unknown primary site.[4]

Overexpression of the multifunctional adhesion molecule CD44 (hyaluronan receptor) and its isoforms has been found to play a major role in tumor invasion and metastasis.[5] The interplay of CD44 with its ligands modulates adhesiveness, motility, matrix degradation, proliferation, and cell survival; these are features that permit a tumor cell to proceed through all the steps of the metastatic cascade. Because the metastatic spread of mesothelioma constitutes a rare eventuality in the clinical setting, the measurement of spliced CD44 in the pleural fluid represents an attractive prospect for labeling a malignant effusion as primary (mesothelioma) or metastatic. The proteolytic cleavage of the extracellular domain of CD44 produces a soluble form (sCD44) that can be detected in biologic fluids.

The primary goal of this study was to test the hypothesis that the measurement of sCD44 standard (sCD44std) and the splice variant v6 (sCD44v6) in pleural fluid can reliably determine the underlying nature of a malignant pleural effusion, whether metastatic or not. We also tested the association between the levels of these markers and mortality.

PATIENTS AND METHODS

Patients

In two Spanish university hospitals (Arnau de Vilanova, Lleida, and Virgen del Rocio, Sevilla), a pleural fluid databank on all patients who undergo diagnostic thoracentesis has been prospectively maintained for the last 15 and 25 years, respectively. All pleural fluid specimens are immediately processed, coded, and stored in aliquots at −80°C until assayed. For this study, we used a computer-generated stratified randomization of effusions to select 94 samples from one biobank and 67 from the other, all collected between 1995 and 2009. This was done to guarantee a proportionate number of patients among the different etiologies. Nevertheless, 75% of the mesothelioma samples were provided by one center due to the higher prevalence of this neoplasm in the corresponding geographical area. The hospital ethics committees of the participant centers approved this study, and patients signed consent forms for their specimens to be stored for analysis and future research.

Clinical Diagnosis

Effusions were classified as being malignant on the basis of cytologic and/or immunohistological features, whereas the diagnosis of benign effusions depended on well-established clinical criteria. In particular, the diagnostic method in all mesothelioma cases was a thoracoscopic pleural biopsy.

Pleural Fluid Assays

Pleural fluid levels of human sCD44std and sCD44v6 were measured at one center by an enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (Bender MedSystems, Vienna, Austria). These ELISAs detect all circulating CD44 isoforms comprising the standard and the sCD44v6 protein sequences, respectively. All samples were analyzed in duplicate, and each data point represented the mean of both measurements. The laboratory personnel who performed the tests were blinded of the clinical diagnoses.

Statistical Analysis

Values are reported as the median with interquartile range because they were not normally distributed. Mann-Whitney *U* test (two groups) or Kruskal-Wallis test (more than two groups) with a post hoc Bonferroni correction was used to compare continuous variables. Receiver operating characteristic (ROC) curves were generated to define the optimal cutoff values of sCD44v6, sCD44std, and their ratio to discriminate mesothelioma from either nonmesothelioma malignancy in general or metastatic adenocarcinoma in particular, searching for specificity greater than 85%. Likelihood ratios (LRs) with 95% confidence intervals (CIs) were calculated for the binary cutoff values identified by ROC analysis.

A Kaplan-Meier analysis evaluated the correlation of sCD44v6, sCD44std, and their ratio and mortality. Two-sided p values less than 0.05 were considered statistically significant. All statistical analyses were conducted with a statistical software package (SPSS, version 16; SPSS Inc.; Chicago, IL).

RESULTS

Patients Characteristics

The study population comprised 93 men and 68 women with an overall median (interquartile range) age of 65 years (51–77 years). Patients were classified into five groups according to the cause of the pleural effusion: mesotheliomas (33 patients), metastatic adenocarcinomas (48 patients), lymphomas (39 patients), metastatic squamous cell carcinomas (17 patients), and benign effusions (24 patients). Table 1 details the underlying etiologies of the patients.

Pleural Fluid Levels of sCD44std and sCD44v6

There were no significant differences of pleural fluid sCD44std levels among the groups (Table 2). Nevertheless, the median concentrations of pleural sCD44v6 were significantly lower in patients with mesothelioma (38 ng/mL) than in those with nonmesothelioma malignancies (101.5 ng/mL, $p < 0.0001$) or benign conditions (64.5 ng/mL, $p = 0.023$). Similarly, the ratio of pleural fluid sCD44v6 to sCD44std was lower and reached statistical significance in the mesothelioma group (0.18), when compared with patients with nonhematologic metastatic tumors (0.5, $p < 0.0001$) (Figure 1). Notably, squamous cell carcinomas exhibited higher pleural fluid levels of sCD44v6 (388 ng/mL) and higher sCD44v6 to sCD44std ratios (0.64) than any other cause of pleural effusion (83 ng/mL and 0.25, respectively; all $p < 0.0001$). The concentrations of sCD44std and sCD44v6 were similar among the three histologic types of mesothelioma (data not shown).

TABLE 1. Causes of Pleural Effusion in the Study Population

Diagnosis	No. (%)
Mesothelioma	33 (20.5)
Epithelioid	29
Sarcomatoid	2
Biphasic	2
Adenocarcinoma	48 (30)
Lung	18
Breast	17
Unknown primary	8
Miscellaneous	5
Lymphoma	39 (24)
B-cell neoplasms	29
T-cell neoplasms	7
Hodgkin lymphoma	3
Squamous cell carcinoma	17 (10.5)
Lung	13
Miscellaneous	4
Benign effusions	24 (15)
Heart failure	8
Parapneumonics	6
Tuberculosis	3
Miscellaneous	7