Concentration of Hyaluronic Acid in Pleural Fluid as a Diagnostic Aid for Malignant Mesothelioma

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Hyaluronic acid (HA) was determined with a radiometric assay in the serum and pleural fluid of 85 patients with pleural effusions, including 15 with malignant mesothelioma, 32 with other cancer, 31 with nonmalignant inflammatory diseases, and seven with congestive heart failure. With a cutoff level at 100 μg/L, the pleural fluid concentration of HA was raised in 73 percent of patients (11 of 15) with malignant mesothelioma and in 23 percent with nonmalignant inflammatory diseases, but in none with other cancer and in none with congestive heart failure. The median concentration of pleural fluid HA was significantly higher in patients with mesothelioma than in those with other cancer (p<0.005). Determination of carcinoembryonic antigen (CEA) in pleural fluid further helped to differentiate between mesothelioma and other types of cancer; concentrations of CEA above 10 μg/L were found in four of 15 (27 percent) patients with mesothelioma, but in 38 percent of the patients with other cancer. We concluded that in the differential diagnosis of pleural effusions associated with malignant tumors a high concentration of HA in pleural fluid combined with a low concentration of CEA suggests malignant mesothelioma as opposed to other types of cancer. (Chest 1988;94:1037-39)

The diagnosis of malignant mesothelioma of the pleura is based on consistent gross, microscopic, and histochemical pathologic findings. The early diagnosis of mesothelioma is difficult, and a delay of several months often occurs between the onset of symptoms and the final diagnosis. A pleural effusion is a common presentation of mesothelioma and is the clinical observation that initiates diagnostic procedures.

Malignant transformation of cells often results in the synthesis and subsequent secretion of abnormal amounts of endogenous cellular constituents and/or tumor markers, such as carcinoembryonic antigen (CEA). Mesothelial cells synthesize collagen, laminin, elastin, and proteoglycans including hyaluronic acid (HA). Histochemical demonstration of large quantities of HA in tumor tissue is regarded as a reliable aid in the diagnosis of mesothelioma, and a high concentration of HA in pleural fluid has been considered a useful marker for the diagnosis of malignant mesothelioma. With the development of a radiometric assay principle for HA determination, its accurate measurement in body fluids has become possible. The aim of the current study was to determine the value of this new HA assay combined with determination of CEA in pleural fluid in the diagnosis of malignant mesothelioma.

Material and Methods

The series consisted of 85 patients with pleural effusion, including 77 consecutively admitted patients and eight previously diagnosed as having malignant mesothelioma. Based on the final diagnosis the patients were divided into the following four groups: (1) 15 patients with malignant mesothelioma: in 11 patients the diagnosis was based on open pleural biopsy findings obtained at thoracoscopy, thoracotomy, or autopsy; in two patients the diagnosis relied on percutaneous needle biopsy of the pleura or biopsy of a chest wall tumor; in two on cytologic examination of pleural fluid or fine needle aspiration biopsy of the lung. None of the 15 patients had any evidence of a primary extrapleural tumor. In all patients clinical follow-up disclosed a malignant course; (2) 32 patients with cancer other than mesothelioma diagnosed by cytologic and/or histologic examination. This group included ten patients with adenocarcinoma of the lung, six with squamous cell carcinoma of the lung, seven with small cell carcinoma of the lung, five with breast carcinoma, one with renal carcinoma, one with prostatic carcinoma, one with carcinoma of the uterus, and one with ovarian carcinoma; (3) 31 patients with nonmalignant inflammatory disease, including 13 with tuberculous pleurisy, six with rheumatoid arthritis, six with systemic lupus erythematosus, and six with pneumonia; and (4) seven patients with transudative pleural effusion due to congestive heart failure. Patients with empyema and patients with undetermined diagnosis were excluded from the study.

Blood and pleural fluid were collected on the same day, centrifuged, and stored at −20°C until assayed. HA was determined using a radiometric assay. Briefly, a 125I-labeled specific HA-binding protein (HABP) isolated from bovine cartilage is incubated with varying amounts of free HA of the sample, and the unbound 125I-HABP is quantified by its binding to HA covalently coupled to Sepharose particles. The amount of radioactivity pelleted with the particles is inversely proportional to the concentration of HA in the sample.

Standard curves for HA were obtained with the use of sodium hyaluronate (Healon, Pharmacia). The reference values for normal human serum according to the manufacturer are 12 to 83 μg/L (mean ± SD), 95 percent of the values being below 125 μg/L. CEA was determined with a radioimmunoassay (Pharmacia).

Statistical analyses were performed with Wilcoxon's rank sum test.
other than mesothelioma than in pleural fluid from patients with nonmalignant inflammatory diseases (p<0.001). With the cutoff level at 100 mg/L, the pleural fluid concentration of HA was raised in 11 of 15 patients with mesothelioma (73 percent) and in seven of 31 with nonmalignant inflammatory diseases (23 percent), but in none of the patients with cancer other than mesothelioma and in none of the patients with congestive heart failure.

The concentrations of HA in serum were significantly higher in patients with malignant mesothelioma than in patients with nonmalignant inflammatory diseases (p<0.05; Table 1). In these respects there were no differences between patients with mesothelioma and patients with cancer other than mesothelioma.

The median concentration of CEA in pleural fluid of patients with mesothelioma was 2.2 μg/L (range 0.9 to 5037.0) and in pleural fluid of patients with cancer other than mesothelioma 6.9 μg/L (range 1.4 to 1166.7). With a cutoff value of 10 μg/L, the concentration of CEA in pleural fluid was raised in four (two with HA levels below 100 mg/L) of 15 patients with mesothelioma (27 percent) and in 12 of 32 patients with cancer other than mesothelioma (38 percent). Considering only patients with adenocarcinoma, CEA concentrations were above 10 μg/L in ten of 18 patients (56 percent). In none of the patients with nonmalignant diseases (20 patients studied) was a pleural fluid CEA concentration above 10 μg/L observed.

**RESULTS**

The concentrations of HA in pleural fluid are shown in Figure 1. Table 1 also shows the concentrations of HA in serum and the pleural fluid/serum HA indices. The median concentration of HA was significantly higher in exudative than in transudative pleural effusions (p<0.001). Pleural fluid from patients with malignant mesothelioma contained significantly higher concentrations of HA than pleural fluid from patients with cancer other than mesothelioma (p<0.005). There were significantly lower concentrations of HA in pleural fluid from patients with cancer

**DISCUSSION**

The development of a specific radiometric assay for HA offers several advantages compared with earlier methods of analysis: it is easier to perform, more rapid, and more accurate. Determination of HA seems to give important aid both for diagnostic purposes and for the understanding of pathophysiologic events in connective tissue metabolism.

HA is widely distributed in connective tissue and mesenchymal cells including the mesothelial cells of

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**Table 1—Concentration of Hyaluronic Acid (Median and Range) in Serum and Pleural Fluid of Patients with Pleural Effusions from Various Causes**

<table>
<thead>
<tr>
<th>Diagnosis (No.)</th>
<th>Serum</th>
<th>Pleural Fluid</th>
<th>Pleural Fluid/Serum Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range), μg/L</td>
<td>Median (range), mg/L</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Malignant mesothelioma (15)</td>
<td>118 (5-6,006)*</td>
<td>125.0 (9.5-378.4)†</td>
<td>220 (12-466)†</td>
</tr>
<tr>
<td>Cancer other than mesothelioma (32)</td>
<td>88 (17-6,400)</td>
<td>24.5 (0.6-63.0)§</td>
<td>270 (10-1,160)</td>
</tr>
<tr>
<td>Nonmalignant inflammatory disease (31)</td>
<td>57 (24-3,400)</td>
<td>69.4 (8.8-280.0)</td>
<td>117 (20-488)</td>
</tr>
<tr>
<td>Congestive heart failure (7)</td>
<td>145 (82-622)</td>
<td>6.8 (3.7-15.7)</td>
<td>50 (10-160)</td>
</tr>
</tbody>
</table>

*Mesothelioma vs nonmalignant inflammatory disease (p<0.05).
†N = 11.
‡Mesothelioma vs cancer (p<0.005).
§Cancer vs nonmalignant inflammatory disease (p<0.001).
the pleura and peritoneum. Malignant mesotheliomas produce HA, occasionally in such large quantities that the pleural fluid takes on a viscous appearance. Our results show that determination of HA in pleural fluid can be used to distinguish between mesothelioma and other types of cancer, including adenocarcinomas. This differential diagnosis is of particular concern, since there may be difficulties in distinguishing between adenocarcinoma and malignant mesothelioma on cytologic and histologic grounds. Although the presence of CEA generally has been regarded as indicating adenocarcinoma, the presence or absence of CEA-related antigens in mesotheliomas has been a matter of controversy. We observed high pleural fluid CEA levels in four patients who were diagnosed as having mesothelioma on clinical and morphologic bases. Two of them had extremely high HA levels in combination with a probably nonspecific moderate increase in CEA. In two the very high CEA levels (445 and 5037 μg/L) occurred together with low HA levels. The possibility that this pattern reflects a nonmesothelial cell origin of the tumor cannot be ruled out, and this emphasizes the need for future studies to correlate expression of HA to morphologic characteristics of mesothelial cells.

Interestingly, the concentration of HA in pleural fluid was lower in patients with cancer other than mesothelioma than in patients with nonmalignant inflammatory diseases. Some patients with nonmalignant inflammatory disease had pleural fluid HA concentrations comparable to those of patients with mesothelioma. There were no significant differences in pleural fluid HA concentrations between the various groups of nonmalignant inflammatory diseases, but high values were observed in patients with rheumatoid arthritis. The mechanisms regulating the in vivo synthesis of HA are unknown, but it has been observed that some inflammatory mediators may induce an increased production of this polysaccharide. The high concentrations of HA in rheumatoid pleural effusions may be related to the inflammatory rheumatoid nodules of the pleural membranes sometimes seen in rheumatoid pleurisy. The differences in HA concentration between pleural effusions associated with malignant and benign diseases may result from a deficient local inflammation and/or weaker mesothelial cell reaction in cancer patients than in patients with benign diseases (tuberculosis, pneumonia, connective tissue diseases).

From a diagnostic point of view, the pleural effusions associated with benign diseases are well characterized by using other biochemical analyses, e.g., glucose, lactate, lysozyme, β2-microglobulin, adenosine deaminase, and complement components of pleural fluid. Although the influence of any of the biochemical measures of pleural fluid on clinical management has been questioned, our results show that in the diagnosis of pleural effusions associated with malignant tumors, high levels of HA in pleural fluid combined with low levels of CEA should raise the suspicion of mesothelioma. In this clinical setting one should consider even strongly more invasive diagnostic procedures such as thoracoscopy or thoracotomy for histologic and histochemical confirmation of mesothelioma.

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REFERENCES