Differential Diagnosis of Pleural Effusions*

Clinical Usefulness of Cell Marker Quantitation

Adrian O. Vladutiu, M.D., Ph.D.;† F. Wells Brason, M.D.;‡
and Richard H. Adler, M.D., F.C.C.P.§

Carciinoembryonic antigen (CEA), orosomucoid beta2 microglobulin, and alpha fetoprotein were quantified in the pleural fluid and serum of 58 hospitalized consecutive patients in order to differentiate malignant from nonmalignant effusions. Cytologic examination of the effusions was also performed. The orosomucoid assay was the most helpful in identifying malignant effusions; higher values (>100 mg/100 ml) were found in 74 percent (26/35) of malignant effusions. Quantitation of carciinoembryonic antigen had a high specificity (95 percent) but a low sensitivity (36 percent) for the detection of malignant pleural effusions. The parallel quantitation of CEA and orosomucoid had a higher sensitivity (86 percent) which increased when associated with cytologic examination. The sensitivity of cytology was only 46 percent. The concentrations of orosomucoid in pleural effusions correlated well with serum concentrations. Alpha fetoprotein and beta2 microglobulin quantitations were of no clinical value for diagnosing malignant effusions. It is suggested that cytologic examination combined with orosomucoid and CEA quantitation in pleural fluid have a considerable clinical value for the diagnosis of pleural effusions.

Pleural effusion is an important and common clinical finding. In some diseases, this represents the initial or the only symptom and its presence can alter the prognosis and the treatment of the concomitant disease. Pleural effusions can accompany many diseases at some stages of their evolution. However, the etiology of the effusions is often obscure and various diagnostic procedures may be required in order to find their cause. Cases in which an etiology could not be found are not uncommon.

Several primary tumors can metastasize to the pleura, and pleural effusions are often associated with carcinoma of breast, lung, or stomach. On the other hand, pleural effusion is a common sign of decompensation in heart failure, and this latter syndrome can occur superimposed on various malignancies. Other nonmalignant conditions causing pleural effusions can exist in patients with unrelated malignancy present. Although it is not yet certain that earlier diagnosis of a malignant effusion can alter the course of the disease, it is undoubtedly important to discriminate between benign and malignant effusions. This differentiation is particularly important for prognosis since malignant effusions are considered to have an ominous prognosis. Pleural effusions are readily obtained for analysis, and the examination of the cells found in effusions is considered one of the most important diagnostic tools presently available to differentiate between malignant and nonmalignant effusions.

The discriminative power of cytologic examination of pleural effusions for malignancy varies from 38 to 82 percent, averaging about 50 percent. If a pleural biopsy is also performed, the diagnostic value of the combined methods increases only slightly. Therefore, biochemical assays in the effusions were investigated.

Lately, several tumor markers such as carciinoembryonic antigen (CEA) and alpha fetoprotein have been described. Although their quantitation in serum was initially considered to be very helpful for diagnosing specific tumors, it was soon realized that their specificity for particular tumors was rather low, and their increase in serum can signify tumors of various origins. Furthermore, elevated values were found in nonmalignant diseases and even in healthy individuals. Previously, we tried to find biochemical markers which could be of value in the differential diagnosis of pleural effusions. In a retrospective study, we found that measurement of CEA could be helpful in this respect.

In the present prospective study, we measured the concentrations of CEA both in pleural fluid and in serum. Additionally, beta2 microglobulin, which was found increased in some malignant pleural effusions, as well as orosomucoid, which could be increased in malignant effusions, were measured in pleural fluid and in serum, whereas beta2 microglobulin concentrations were also measured in urine. Alpha fetoprotein was also assayed in pleural effu-
sions. An attempt was made to evaluate the clinical value of combined assays of several markers in pleural fluids.

METHODS AND MATERIALS

Sampling

Pleural effusions were obtained from 58 consecutive hospitalized patients, 24 men and 34 women, 29 to 91 years of age. The effusions were considered malignant if one of the following criteria was met: (1) demonstration of malignant cells at cytologic examination or in a biopsy specimen; (2) histologically proven primary malignancy with exclusion of any other cause known to be associated with pleural effusions (e.g., tuberculosis, lung infection, pancreatitis, congestive heart failure, connective tissue disease, etc.). Cytology and histologic examination of pleural biopsy specimens was performed according to standard techniques. Cytologic findings were considered positive or negative for malignancy. Equivocal results (so-called class III cytology) were not found in our present series. The patients with proven primary malignancy but negative effusion cytology were observed for several months, and the effusions were considered malignant only if a nonmalignant cause of the effusion was not found. Only the first pleural fluid sample was included in the study. The diagnosis of malignancy was made without knowledge of the results of the biochemical assays.

Effusion samples were collected in evacuated glass tubes with ethylenediaminetetraacetate (for CEA assay) or without anticoagulant (for beta₂ microglobulin, alpha fetoprotein, and orosomucoid assays), or in large vacuum bottles (for cytological examination). Blood samples were collected in similar tubes the same day as the effusion and a random urine sample was also obtained for beta₂ microglobulin examination. The samples of pleural fluid and urine were centrifuged at 1500 × g for 15 minutes soon after collection, and the cell pellet was discarded. The assays were performed within a week of storage at −20° C. Grossly hemorrhagic samples were discarded if they were contaminated with blood because of puncturing blood vessels. Empyema fluids were not included in this study since bacterial enzymes could alter various protein markers in the fluid. Pleural biopsy was performed by standard technique with an Abrams needle.

Assays

Carcinoembryonic antigen was measured in pleural fluid and plasma by RIA as previously described. Samples with values above 25 ng/ml were retested after dilution with pleural fluid which did not contain measurable amounts of CEA. Beta₂ microglobulin was quantitated in effusion, serum, and urine by a solid phase RIA. Alpha fetoprotein was measured by RIA using standards obtained from human amniotic fluid and 125I-labeled alpha fetoprotein. Orosomucoid (alpha₁-acid glycoprotein) was quantitated in serum and pleural fluid by radial immunodiffusion.

RESULTS

Thirty-seven malignant (64 percent) and 21 nonmalignant effusions were studied. The cytologic examination for malignant cells was positive in 46 percent, and the biopsy examination showed neoplastic changes in 34 percent of patients with malignant effusions tested. The combined cytology and biopsy examination detected 51 percent of malignant effusions. Sixteen patients (43 percent) had lung carcinoma, 14 (38 percent) had breast carcinoma, 3 had ovarian carcinoma with pleural metastases, and 4 had lymphoma. In the nonmalignant effusions group, there were 12 patients with congestive heart failure, 4 with cirrhosis, 3 with pneumonia, and 2 with acute pancreatitis.

Orosomucoid

The concentrations of orosomucoid in benign effusions ranged from 28 to 239 mg/100 ml with a mean of 85 ± 13 mg/100 ml (SE) (Table 1). In general, orosomucoid concentrations were lower in the pleural fluid than in the serum. There was a good correlation between the orosomucoid concentration in the nonmalignant effusions and in the corresponding sera (r = 0.88; P < 0.001).

Although in malignant effusions the orosomucoid concentration was in general higher than in the nonmalignant ones, averaging 140 ± 10 mg/100 ml (range 48 to 330 mg/100 ml), there was some overlap between the values found in the nonmalignant and the malignant effusions. A good correlation between the fluid and the serum values of orosomucoid in patients with malignant effusions (r = 0.74; p < 0.001) was found. When the cut-off value of 100 mg/100 ml was taken to differentiate between benign (< 100 mg/100 ml) and malignant effusions

<table>
<thead>
<tr>
<th>Table 1—Orosomucoid, CEA, and Beta₂ Microglobulin Concentrations* in Pleural Effusion and Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orosomucoid, mg/100 ml</strong></td>
</tr>
<tr>
<td><strong>Carcinoembryonic antigen, ng/ml</strong></td>
</tr>
<tr>
<td><strong>Beta₂ microglobulin, μg/100 ml</strong></td>
</tr>
</tbody>
</table>

*Average ± SE.
(> 100 mg/ml), the diagnostic sensitivity of the orosomucoid assay was 74 percent, the specificity 65 percent, the predictive value of positive results 79 percent, the predictive value of negative results 59 percent, and the efficiency of the assay 71 percent (Table 2). By the assay alone, 71 percent of malignant effusions with negative cytology could be diagnosed. There was no correlation between the fluid levels of CEA and orosomucoid.

Carcinoembryonic antigen

Only one patient (4.7 percent) with nonmalignant effusion (ie, liver cirrhosis) had CEA concentration in the pleural fluid above 10 ng/ml; (fluid, 18.1 ng/ml; plasma, 7.5 ng/ml). The mean CEA value for the other 20 patients was 3.8 ± 1.1 ng/ml (SE). In general, the CEA values in the effusions were similar to or lower than those in the plasma (r = 0.58; P < 0.001).

In malignant effusions, only 39 percent of patients had values above 10 ng/ml (in two additional patients, CEA concentration in the effusions was 9.9 ng/ml). The average CEA concentration was 248 ± 104 ng/ml (range 0.5 to 2132). In patients with malignant effusions and negative cytology (as defined above), the CEA concentration was above 10 ng/ml in 33 percent of cases.

The CEA concentration in the fluid did not correlate well with the plasma level (r = 0.31; P < 0.1). The ratio between the level in the fluid and in the plasma was greater than 2 in some patients with malignancy (eg, 824 vs 4; 492 vs 8; 2020 vs 12 ng/ml), but in others, it was lower than 1 (eg, 15 vs 141 ng/ml in a patient with bronchogenic carcinoma). In patients with malignant effusions and low CEA concentrations (< 10 ng/ml), the pleural fluid level was equal to or lower than the plasma level. Thus, it appeared that the ratio CEA in fluid/CEA in plasma had no diagnostic value.

When 10 ng/ml was taken as the cut-off, the diagnostic sensitivity of the CEA assay for malignancy was 39 percent, the specificity 95 percent, the predictive value of positive results 93 percent, the predictive value of negative results 50 percent, and the efficiency of the assay 61 percent (Table 2).

There was no correlation between the type of the tumor and the values of CEA or orosomucoid in the pleural effusions, ie, neither assay showed more positive results in one particular type of tumor (eg, lung or breast).

The parallel assays for CEA and orosomucoid, together with the cytologic analysis of pleural effusions, had a higher sensitivity for diagnosing malignant effusions than the individual assays for CEA or orosomucoid (Table 3).

Alpha Fetoprotein

The concentrations of alpha fetoprotein in 25 effusions of malignant and nonmalignant etiologies were

<table>
<thead>
<tr>
<th>Table 2—Accuracy of CEA and Orosomucoid Quantitation for Diagnosis of Malignant Pleural Effusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.</strong></td>
</tr>
<tr>
<td>Prevalence of malignant effusions</td>
</tr>
<tr>
<td>Sensitivity‡</td>
</tr>
<tr>
<td>Specificity</td>
</tr>
<tr>
<td>Predictive value of a positive test</td>
</tr>
<tr>
<td>Predictive value of a negative test</td>
</tr>
</tbody>
</table>

*Positive CEA assay was considered when the concentration was > 10 ng/ml; for orosomucoid a positive test was considered when the concentration was > 100 mg/ml. 
‡Either one or both tests were positive. 
§Sensitivity, frequency of positive results in malignant effusions; specificity, frequency of negative results in nonmalignant effusions; predictive value of positive test, frequency of malignant effusions in patients with positive results; predictive value of negative test, frequency of nonmalignant effusions in patients with negative results; and efficiency, percentage of effusions correctly classified (malignant and nonmalignant) by the test.

<table>
<thead>
<tr>
<th>Table 3—Accuracy of Combined Assays for CEA, Orosomucoid and Cytology for Diagnosis of Malignant Pleural Effusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.</strong></td>
</tr>
<tr>
<td>Sensitivity*</td>
</tr>
<tr>
<td>Specificity</td>
</tr>
<tr>
<td>Predictive value of a positive test</td>
</tr>
<tr>
<td>Predictive value of a negative test</td>
</tr>
<tr>
<td>Efficiency</td>
</tr>
</tbody>
</table>

*See Table 2 for definition of terms.
below 15 ng/ml, ie, below the sensitivity of our assay and similar to levels found in the serum of many normal individuals.

**Beta₂ Microglobulin**

A wide range in the concentrations of beta₂ microglobulin was found in both malignant and nonmalignant effusions; in nonmalignant effusions, the average value was 8.6 ± 2.9 µg/100 ml (range 0.4 to 69.2), while in malignant effusions, it was 4.9 ± 0.5 µg/100 ml (range 1.3 to 15.3). A very good correlation between the fluid and serum values was observed in both nonmalignant (r = 0.99; P < 0.001) and malignant effusions (r = 0.81; P < 0.001). The highest concentration (69.2 µg/100 ml) was found in a patient with uremia and congestive heart failure who had a very low urinary excretion of beta₂ microglobulin. The urinary levels of beta₂ microglobulin were often indirectly correlated with the serum and pleural fluid levels, ie, in patients where the fluid and serum concentrations were high the urinary concentrations were generally low.

Although all four patients with malignant lymphomas had increased beta₂ microglobulin concentration in both pleural fluid and serum when compared to patients with other malignancies, the number of patients was too low to draw any definite conclusions regarding the clinical value of beta₂ microglobulin assay of pleural effusions.

**Discussion**

The present findings showed that cytology alone can diagnose only about one-half of the malignant pleural effusions (sensitivity 46 percent), and this finding is similar to those of other reports. However, the prevalence of malignancy in our series was higher than reported by others. The prevalence influences the predictive value of a test and this might explain the high predictive values for CEA and orosomucoid found in this study (Table 2).

Various substances (enzymes, metals, specific proteins) were measured in pleural effusions, but their discriminative value for separating malignant from nonmalignant effusions was not proven. Orosomucoid is the main component of the seromucoid fraction of human serum. It is an acute phase protein which increases in inflammation, pregnancy, and cancer. It has been considered to be synthesized in the liver, but recent observations suggested a leukocyte surface origin for orosomucoid in serum. In a previous report, we could not find a cut-off value for the orosomucoid which would separate benign from malignant effusions. However, the present study showed statistically significant (p < 0.01) higher values of orosomucoid in malignant effusions (140 ± 10 mg/100 ml SE) than in nonmalignant ones (85 ± 13 mg/100 ml). These values are similar to those of Rudman et al and confirm another report showing increased orosomucoid concentration in malignant effusions. The discrepancy between the present results and our previous findings could be due to technique since in this prospective study, the samples were assayed sooner after collection than previously. The cut-off value of 100 mg/100 ml gave a sensitivity of 74 percent which is higher than of the cytologic examination.

Carcinoembryonic antigen is a glycoprotein component of the glycoalyx of entodermal epithelium found to be elevated in the plasma of patients with various tumors. Recently, CEA was quantitated in pleural effusions to differentiate benign from malignant effusions. Arbitrary cut-off values of 10, 12,17 or even 20 ng/ml were chosen depending on the technique used, and this explained the various reported sensitivities of the CEA assay from 25 percent to 57 percent. In malignant effusions, CEA concentrations were usually higher than in the corresponding plasma samples, suggesting a cellular origin of the CEA in effusions perhaps due to an active secretion by the tumor cells. It is noteworthy that chemotherapy, radiation, or cortisone can influence the CEA concentration in the effusions.

The present results on a small number of patients showed that although the CEA assay of the pleural fluid has a high specificity (95 percent) and high predictive value for positive results (92 percent), it has a low sensitivity for detecting malignant effusions. Therefore, CEA assay cannot be relied upon alone to differentiate benign from malignant effusions. Moreover, serial measurements seemed more meaningful than a single measurement since some patients could be at an earlier stage in the course of their disease. Perhaps immunohistochemical assays of CEA in the cell sediment could be used in the future in cases of uncertain cytologic findings.

Beta₂ microglobulin is a protein with unknown function with a structure related to the histocompatibility antigens. High beta₂ microglobulin concentrations were found in serum in patients with various tumors. The serum beta₂ microglobulin concentration depends on the renal function (since beta₂ microglobulin has a low molecular weight and is readily released into the urine) and on the age. Impaired renal function might allow a “back up” of beta₂ microglobulin into the serum. However, there was no correlation between the level of beta₂ microglobulin in cerebrospinal fluid (CSF) and the level in plasma, suggesting an independent production of
beta-2 microglobulin in CSF.\textsuperscript{54} Increased beta-2 microglobulin levels in body fluids in some diseases may correspond to an increased local production, \textit{eg}, in CSF in brain tumors\textsuperscript{54} or in synovial fluid in rheumatoid arthritis.\textsuperscript{2} It has been shown that some neoplastic cells can produce more beta-2 microglobulin \textit{in vitro} than normal cells.\textsuperscript{54,55} From the present study, it appears that the measurement of beta-2 microglobulin concentration is not important for diagnosing malignant effusions. Although consistently high values are found in lymphoma, this disease is often diagnosed before pleural effusion develops.

Alpha fetoprotein concentrations are raised in serum mainly in hepatoma and some tumors of the germ cells, although high levels were found in several other conditions, \textit{eg}, ataxia telangiectasia, tyrosinemia, etc.\textsuperscript{28} From our limited study, it appeared that measurement of alpha fetoprotein concentrations in pleural fluid is of no clinical value.

The present study showed that measurement of orosomucoid in pleural fluid is valuable for diagnosis of malignant effusions. Thus, 71 percent of malignant effusions with negative cytology were diagnosed by an increased value (> 100 mg/100 ml) of orosomucoid. The quantitation of orosomucoid is inexpensive and easy to perform in any laboratory. The CEA assay, while more elaborate and expensive, seems to be important in discriminating between benign and malignant effusions only when performed in association with the orosomucoid quantitation. The combined assays could detect 86 percent of the malignant effusions with negative cytology. The parallel assays of orosomucoid and CEA could be particularly valuable in cases of doubtful cytologic findings and should replace other biochemical assays.

ACKNOWLEDGMENTS: The technical help of Mrs. Caryl Fox, Miss Suzanne Maciejewski, and Mrs. Barbara Roach and the secretarial help of Mrs. Jacqueline Teitz are gratefully acknowledged.

REFERENCES
12 Sarcione EJ: Synthesis of a1-acid glycoprotein by the isolated perfused rat liver. Arch Biochem Biophys 1983; 100:516-23

CHEST, 79: 3, MARCH, 1981

DIFERENTIAL DIAGNOSIS OF PLEURAL EFFUSIONS 301