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Abstract

Five patients with malignant pleural mesothelioma (MPM) were studied to determine whether CYFRA 21-1 is useful for diagnosis of this disease. In pleural effusions, the median concentration of CYFRA 21-1 from 4 patients with MPM was significantly higher than for 34 patients with benign diseases. The sensitivity of serum CYFRA 21-1 for diagnosis of MPM was 40% and its concentration changed in proportion to disease activity in all cases. Immunohistochemically, anti-cytokeratin 19 antibody revealed strong staining in both epithelial and sarcomatous MPM tissues. Based on these results, we conclude that measurement of CYFRA 21-1 in pleural effusions and serum may be useful for diagnosing and monitoring MPM.

KEYWORDS: malignant pleural mesothelioma, tumor marker, CYFRA21-1

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Brief Note

The Usefulness of CYFRA 21-1 in Diagnosing and Monitoring Malignant Pleural Mesothelioma

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Five patients with malignant pleural mesothelioma (MPM) were studied to determine whether CYFRA 21-1 is useful for diagnosis of this disease. In pleural effusions, the median concentration of CYFRA 21-1 from 4 patients with MPM was significantly higher than for 34 patients with benign diseases. The sensitivity of serum CYFRA 21-1 for diagnosis of MPM was 40% and its concentration changed in proportion to disease activity in all cases. Immunohistochemically, anti-cytokeratin 19 antibody revealed strong staining in both epithelial and sarcomatous MPM tissues. Based on these results, we conclude that measurement of CYFRA 21-1 in pleural effusions and serum may be useful for diagnosing and monitoring MPM.

Key words: malignant pleural mesothelioma, tumor marker, CYFRA 21-1

Malignant pleural mesothelioma (MPM) is a rare disease which is associated with exposure to asbestos (1). The clinical diagnosis of MPM is often difficult. An elevated concentration of hyaluronic acid (HA) in pleural fluid or serum is associated with MPM (2, 3). However, many mesotheliomas do not produce HA, and there are other causes of elevated HA content (4). It has been shown that low molecular weight cytokeratins are present not only in simple epithelial tissue but in mesothelial tissue as well, and are useful tools in immunohistologic studies (5-7). CYFRA 21-1, a newly established tumor marker, recognizes a soluble fragment of cytokeratin 19, one of the low molecular weight cytokeratins (8, 9). We investigated the possible use of CYFRA 21-1 as a new tumor marker of MPM.

Patients and Methods

Five MPM patients were studied. Chest X-ray films of the patients are shown in Figs. 1-5. Three patients were men and two were women, with a mean age of 67.4 years. Four patients had pleural effusions and none had other malignancies. A diagnosis of biphasic type MPM was confirmed histologically in all of these patients. The samples for histologic study were obtained by needle biopsy in two cases, thoracoscopy in one case, and thoracotomy in two cases.

Pleural fluids were collected from 38 patients, including 4 with MPM and 34 with benign pleural diseases. Benign pleural disease included tuberculous pleurisy ($n = 14$), pneumonia ($n = 8$), heart failure ($n = 7$), benign asbestos pleurisy ($n = 3$), and liver cirrhosis ($n = 2$). CYFRA 21-1 concentrations were measured using a commercial kit (Enzymun-Test CYFRA 21-1; Boehringer Mannheim, Mannheim, Germany), as were those of carcinoembryonic antigen (CEA) (AIA-PAC CEA; Tosoh, Tokyo, Japan). CEA and HA concentrations in pleural effusions were measured in these patients and compared to those of CYFRA 21-1. The cutoff values used to define the upper normal limit in this study were 50 ng/ml for CYFRA 21-1, 5 ng/ml for CEA, and 50 μg/ml for HA in pleural effusion. These cutoff values corresponded to a specificity of 95% for the same series of samples. The cutoff value for CYFRA 21-1 in serum

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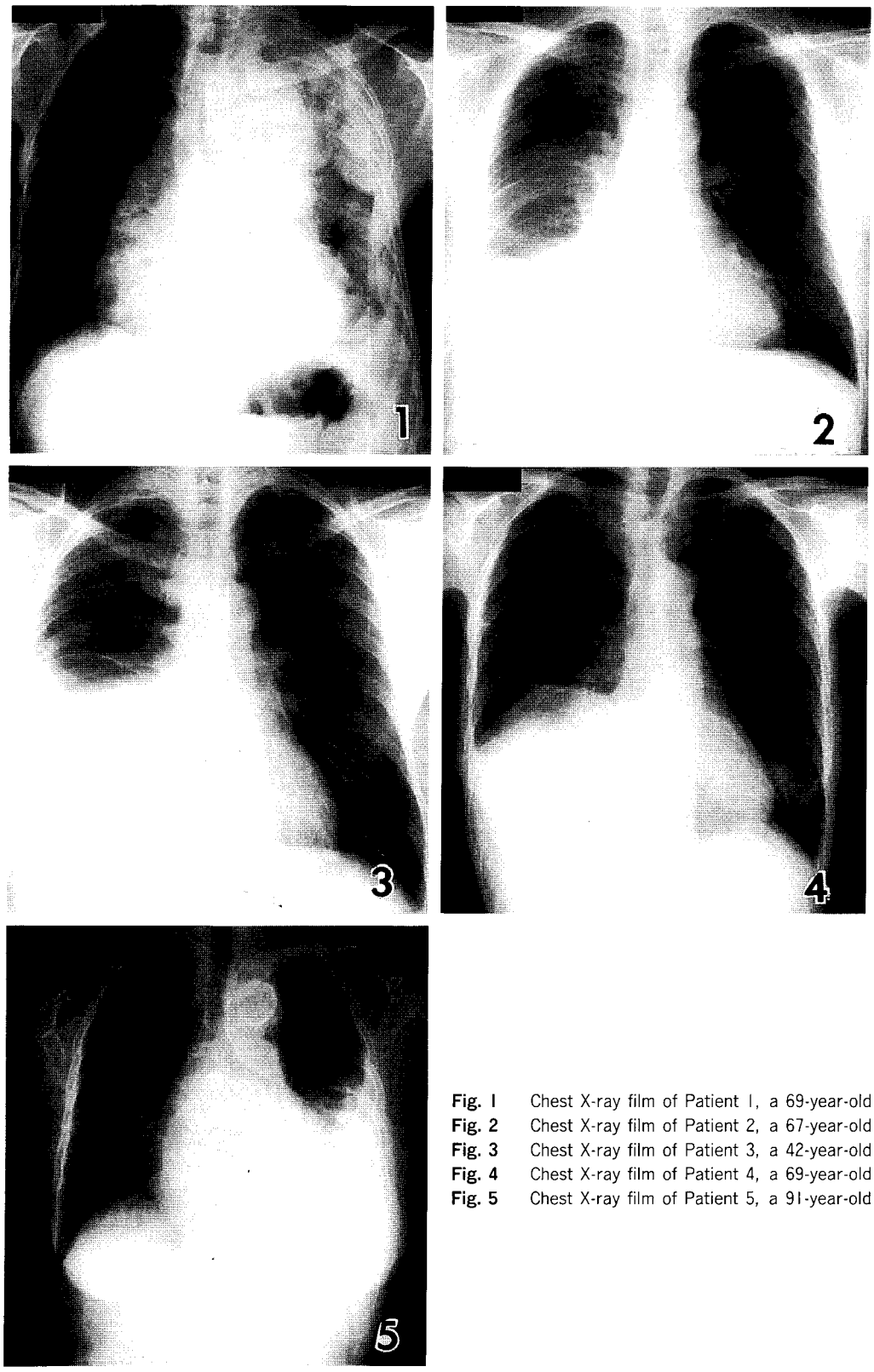

Fig. 1 Chest X-ray film of Patient 1, a 69-year-old man.
Fig. 2 Chest X-ray film of Patient 2, a 67-year-old man.
Fig. 3 Chest X-ray film of Patient 3, a 42-year-old man.
Fig. 4 Chest X-ray film of Patient 4, a 69-year-old woman.
Fig. 5 Chest X-ray film of Patient 5, a 91-year-old woman.

was set at 3.3 ng/ml as previously recommended (10).

The distribution of cytokeratin 19 in five specimens of MPM tissue was determined by the indirect immunoperoxidase method, with anti-cytokeratin 19 antibody (Cat. No. 1238825, Boehringer Mannheim) used as the first antibody (11, 12).

The Mann-Whitney U test was used for two-group comparisons, with a P value of < 0.05 considered significant.

Results

CYFRA 21-1 concentrations in pleural effusions from patients with MPM (median: 787.5 ng/ml; interquartile range [IR]: 275 to 1350 ng/ml) were significantly higher than in those from patients with benign diseases (median: 19.6 ng/ml; IR: 6.8 to 35.4 ng/ml; Mann-Whitney U test: $P < 0.0012$). The median (and IR) pleural fluid CEA concentrations for MPM and benign pleural diseases were 1.0 ng/ml (0.6 to 1.5 ng/ml) and 0.55 ng/ml (0.5 to 1.3 ng/ml), respectively. The median (and IR) pleural fluid HA concentrations were 170.5 μg/ml (36.5 to 775.0 μg/ml) and 28.0 μg/ml (25.75 to 31.25 μg/ml). There were no significant differences between the two groups with respect to HA and CEA ($P = 0.0869$, $P = 0.5681$, respectively).

In pleural effusions, using a cutoff value of 50 ng/ml for CYFRA 21-1 (defined at the 95 % specificity level for distinguishing malignant and benign disease), all of the effusions from MPM patients were positive. No patient with MPM was positive for CEA when the cutoff value for CEA was set at 5 μg/ml. When the cutoff value for HA was set at 50 μg/ml, 50 % of MPM patients were positive for HA.

The median (and IR) concentrations of serum CYFRA 21-1 for the five MPM patients were 3.2 ng/ml (1.3 to 121.5 ng/ml) at the time of diagnosis. With a serum cutoff value of 3.3 ng/ml, the sensitivity of CYFRA 21-1 for MPM was 40 %. Furthermore, we monitored serum concentrations of CYFRA 21-1 in five patients for more than 2 months and compared these with their clinical course. The CYFRA 21-1 concentration was less than the cutoff value in one patient who was in remission after chemotherapy. However, in the remaining patients, the serum CYFRA 21-1 concentration was elevated in proportion to the disease progression. In particular, in one patient with adrenal gland metastasis, the serum concentration of CYFRA 21-1 was more than

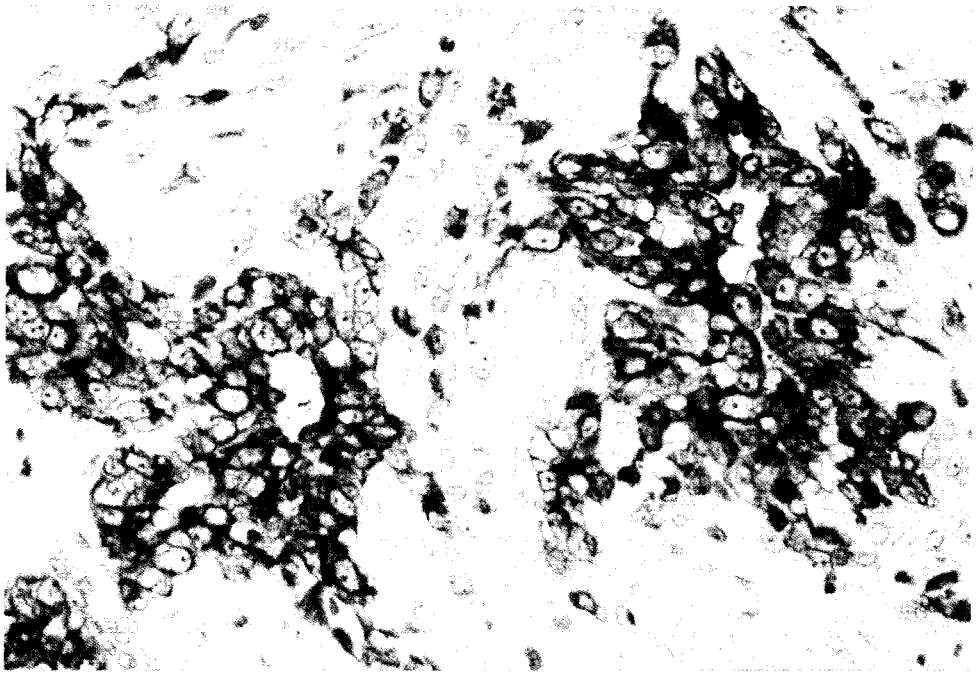

Fig. 6 Immunohistochemical findings of the tumor in Patient 2 showing a positive reaction for cytokeratin 19 (original magnification × 100, alkaline phosphatase-labelled avidin and new fuchsin). The X-ray film of the Patient 2 is shown in Fig. 2.

700 μg/ml just before death.

An immunohistochemical study of five cases of MPM was performed with anti-cytokeratin 19 antibody. This study showed strong staining in all five cases in the sarcomatous as well as the epithelial portions of MPM tissues. One of the MPM tissues in immunohistochemical study is shown in Fig. 6.

Discussion

Cytokeratins and other intermediate filaments of the cell are well known to be present in various normal and pathologic tissues (9, 13). The expression of cellular cytokeratins is specific for particular tissues. Cytokeratin 19, one of the low molecular weight cytokeratins, is expressed in simple epithelium and mesothelium along with other intermediate filament proteins (9, 14, 15). Low molecular weight cytokeratins have previously been used to distinguish between reactive benign pleural disease, MPM, and other malignant diseases by immunopathologic staining (5-7). CYFRA 21-1 has been established as a new tumor marker which detects the soluble fragment of cytokeratin 19 (8). For these reasons, we investigated the usefulness of CYFRA 21-1 in the diagnosis of MPM.

In general, CYFRA 21-1 is thought to be specific for lung cancer (16). However, in this study, the concentration of CYFRA 21-1 in pleural effusions was much more useful for diagnosis of MPM than was HA. In addition, no MPM patients were positive for CEA. It is true that one cannot make a definitive diagnosis of MPM nor rule out other malignancies, including lung cancer, by simply measuring the pleural fluid concentration of CYFRA 21-1. In addition, the possibility of malignancy cannot be excluded even if all tumor markers in a pleural effusion are negative. However, by using HA, CEA and CYFRA 21-1 in combination, we should be able to accurately predict the presence of MPM. In practice, a pleural or peritoneal effusion is often the first sign of mesothelioma and may be present for several months before diagnosis. Concentrations of CYFRA 21-1, CEA and HA can be measured easily in pleural effusions and if positive for CYFRA 21-1 or HA and negative for CEA, would alert the physician to a high probability of mesothelioma, prompting the physician to undertake the invasive procedures (thoracotomy, thoracoscopy and laparotomy) necessary for pathologic confirmation of the diagnosis of mesothelioma. This would greatly aid in making a diagnosis at a much earlier stage than is often now the case. Otherwise, this study included 3 pleural effusions from benign asbestos pleursy, which was diagnosed by thoracotomy in two cases. Although pleural tissues were histologically accompanied with mesothelial hyperplasia, none were positive for CYFRA 21-1.

In turn, when a patient with MPM has undergone surgical intervention or chemotherapy, an important clinical problem is distinguishing between stabilized disease and tumor recurrence, especially when no measurable lesions are present. Although in this study the sensitivity of serum CYFRA 21-1 for MPM was only 40 % at the time of diagnosis, the CYFRA 21-1 concentration changed in proportion to disease course in all cases. These results suggest that the concentration of CYFRA 21-1 in serum reflects the MPM tumor burden and that measurement of CYFRA 21-1 may be useful for monitoring this malignancy.

In the immunohistochemical studies presented here, cells in both the epithelial and sarcomatous regions of MPMs possessed abundant cytokeratin 19, the soluble fragment of which may be shed into pleural fluid and the bloodstream.

Subsequent to this study, we encountered another case of sarscomatous type MPM case. Although the tumor cells were immunohistochemically positive for cytokeratin 19, CYFRA 21-1 in pleural effusions was under the cut off value in this case. So we speculate that the elevation of CYFRA 21-1 concentration is mainly due to the epithelial component of the MPM tissue in addition to the tumor volume.

We conclude from these results that CYFRA 21-1 may be a useful tumor marker of MPM for both diagnostic and therapeutic purposes. However, in this study we did not collect any data concerning CYFRA 21-1 concentrations for other malignancies, so comparison between MPM and other malignancies was not performed. In addition, the mechanism by which CYFRA 21-1 is shed from mesothelioma cells still remains unclear. Thus, further investigation, including studies of patients with lung cancer, is needed to confirm the significance of CYFRA 21-1 in MPM and to clarify the mechanism of its release from tumor cells.

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