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*Case report*

# A case of leptomeningeal carcinomatosis from esophageal basaloid carcinoma diagnosed by quantitative reverse transcription-polymerase chain reaction for carcinoembryonic antigen

Hiroshi Okumura[1], Shoji Natsugoe[1], Naoya Yokomakura[1], Yuko Mataki[1], Yusei Haraguchi[1], Hironori Sakita[1], Yukio Mihara[1], Masataka Matsumoto[1], Shin-Ichi Imamura[2], Jun-Ichi Kuratsu[2], Sumiya Ishigami[1], Sonshin Takao[1], and Takashi Aikou[1]

[1]Department of Surgical Oncology, Digestive Surgery, Graduate School of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
[2]Department of Neurosurgery, Graduate School of Medicine, Kagoshima University, Kagoshima, Japan

We describe herein the case of a 68-year-old man who experienced leptomeningitis after esophagectomy from an esophageal basaloid carcinoma. Although the patient had a good operative course for the first 10 days after surgery, he suddenly had general convulsions with unconsciousness. He was placed on mechanical ventilation in an intensive care unit. Computed tomography and magnetic imaging resonance did not reveal any abnormal findings. No abnormal data in the cerebrospinal fluid were found by biochemical, virological, and cytological examination. The positive expression of carcinoembryonic antigen messenger ribonucleic acid in cerebrospinal fluid was detected by a quantitative reverse transcription-polymerase chain reaction method. Immunohistochemical staining using an anticytokeratin antibody confirmed the presence of tumor cells in the cerebrospinal fluid. Spontaneous breathing was recovered after treatment with systemic chemotherapy. Six months after surgery, computed tomography revealed multiple brain metastases. This case demonstrates that the quantitative reverse transcription-polymerase chain reaction method of analyzing carcinoembryonic antigen messenger ribonucleic acid may be a sensitive and useful method for determining leptomeningeal metastasis before the detection of tumors by cytological and imaging examinations in patients with cancer.

**Key words:** leptomeningeal carcinomatosis, esophageal cancer, RT-PCR, cytokeratin

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*Reprint requests to:* H. Okumura

## Introduction

Leptomeningeal carcinomatosis occurs in approximately 5% of patients with cancer, especially breast or lung cancer, and is a clinically important neurological complication.[1] Although advanced esophageal carcinoma is an aggressive disease with frequent lymph node, liver, lung, and bone metastases, central nervous system metastasis is rare. In most cases, the diagnosis of leptomeningeal carcinomatosis is made from the cytological examination of cerebrospinal fluid or from neuroradiologic confirmation by computed tomography (CT) and magnetic resonance imaging (MRI). Leptomeningeal carcinomatosis has been reported in cases of breast and lung cancer in which tumor cells could not be detected by cytology or imaging means.[2,3] Recently, cancer cells circulating in the blood, lymph nodes, and bone marrow of patients with solid tumors were detected by the reverse transcription-polymerase chain reaction (RT-PCR) method for quantifying the messenger ribonucleic acid (mRNA) expression of carcinoembryonic antigen (CEA) or by immunohistochemical staining for the presence of cytokeratin.[4–7] Here we present the first case report of leptomeningeal carcinomatosis from esophageal basaloid carcinoma. In this case, leptomeningeal carcinomatosis was diagnosed from quantitative RT-PCR for CEA mRNA and immunostaining with cytokeratin antibody when it was not detected by means of cytological examination and imaging.

## Case report

A stage III esophageal basaloid carcinoma according to the TNM classification[8] was found in a 68-year-old man, and he was admitted to our hospital on September 11, 2002. Physical examination findings were normal. Blood

**Table 1.** Laboratory data on admission

| | |
|---|---|
| Peripheral blood | |
| WBC ($\times 10^2/\mu l$) | 97 |
| RBC ($\times 10^4/\mu l$) | 350 |
| Hemoglobin (g/dl) | 11.9 |
| Hematocrit (%) | 34.6 |
| Platelet ($\times 10^4/\mu l$) | 37.3 |
| Coagulation system | |
| Prothrombin time (%) | 90 |
| APTT (s) | 34.7 |
| Fibrinogen (mg/dl) | 525 |
| Hepaplastin test (%) | 78 |
| Tumor marker | |
| CEA (ng/ml) | 6.2 |
| SCC (ng/ml) | 0.0 |
| Biochemistry | |
| Aspirate aminotransferase (IU/l) | 19 |
| Alanine aminotransferase (IU/l) | 13 |
| Lactate dehydrogenase (IU/l) | 395 |
| Cholinesterase (IU/l) | 115 |
| Total protein (g/dl) | 6.6 |
| Total bilirubin (mg/dl) | 0.5 |
| Alkaline phosphatase (IU/l) | 328 |
| γ-Glutamyl transpeptidase (IU/l) | 51 |
| Indocyanine green test—15 min (%) | 12.8 |
| Blood urea nitrogen (mg/dl) | 5.1 |
| Creatinine (mg/dl) | 0.4 |
| Fasting blood sugar (mg/dl) | 130 |
| C-reactive protein (mg/dl) | 3.3 |

WBC, white blood cell count; RBC, red blood cell count; APTT, activated partial thromboplastin time; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma antigen

tests showed slight elevations of white blood cell count (9700/μl) and C-reactive protein concentration (3.3 mg/dl). Examination of tumor parameters revealed an elevated serum CEA level (6.2 ng/ml) and no detectable squamous cell carcinoma antigen (Table 1). A barium study revealed a large, irregularly shaped tumor with a largest diameter of 16 cm (Fig. 1a). Endoscopy also showed a large tumor with ulceration in the esophageal wall, 25–39 cm from the patient's incisors (Fig. 1b). No invasion into the adjacent mediastinal structures and no lymph node metastasis were apparent by CT examination. On October 8, the patient underwent an esophagectomy with lymphadenectomy through a right thoracoabdominal approach. Gross examination revealed that the tumor, 10.8 × 8.5 cm in size, had an ulcerated and infiltrative appearance (Fig. 1c). Microscopically, the tumor was composed of a proliferation of severely atypical basaloid cell solid nest formations and trabeculae separated by fibrocollagenous tissue with hyaline material depositions (Fig. 2). Lymphatic and venous invasion was prominent, and metastases were confirmed in two paraesophageal nodes.

Although the patient recovered well for the first 10 days after surgery and began to eat meals, he suddenly had a systemic convulsion with unconsciousness on October 18. He was placed on mechanical ventilation in the intensive care unit. A contrast-enhanced brain CT scan was immediately performed, but no evidence of cerebrovascular disease or tumor was found (Fig. 3a). Gadolinium-enhanced MRI was also performed, but revealed no abnormal findings. Examination of the cerebrospinal fluid showed normal biochemical, virological, and cytological findings (Table 2). The electroencephalogram (EEG) showed periodic lateralized epileptiform discharges (PLEDs), particular EEG patterns consisting of unilateral, focal spike or sharp wave complexes with a periodic appearance, usually at a rate of 1–2 s (Fig. 4). This PLEDs finding was considered to be a manifestation of an increased neuronal excitability caused by different etiologies such as encephalitis, ischemic stroke, toxic metabolic cause, and chronic cerebral disorder.[9] When we checked for general metabolic diseases (vitamin deficiency, hypoglycemia, hypomagnesemia, hypothyroidism, and adrenal insufficiency), we did not find any abnormal data. Although the cytological results of the cerebrospinal fluid were negative, leptomeningeal metastasis was strongly suspected from the patient's clinical symptoms. The presence of tumor cells in the cerebrospinal fluid was sought by quantitative RT-PCR for CEA mRNA expression. Simultaneously, cells obtained from the cerebrospinal fluid were immunohistochemically stained using anti-cytokeratin antibody. Figure 5 demonstrates the result of CEA mRNA expression by real-time RT-PCR in cerebrospinal fluid, pleural effusion, peripheral blood, and bone marrow. Expression of CEA mRNA was strongly detected in cerebrospinal fluid and slightly detected in pleural effusion. A few cytokeratin-positive cells were found in the cerebrospinal fluid by immunohistochemistry (Fig. 6). These results led to the diagnosis of leptomeningeal metastasis from esophageal basaloid carcinoma.

Systemic chemotherapy combined with low-dose cisplatin (5 mg/body) and 5-fluorouracil (350 mg/body) was administered for 2 weeks per month, starting November 1. After 1 month, the chemotherapy was so effective that the patient began breathing spontaneously, moving his extremities and eyes, and tossing about in bed, although he could not breathe or move his extremities and eyes at all before chemotherapy. Furthermore, the value of CEA mRNA score in the cerebrospinal fluid decreased from 3380 to 1903 (see Fig. 5). This chemotherapy was performed for three courses. However, central nervous system (CNS) functions such as moving the extremities and eyes and the respiratory function of the patient became worse on March 14, 2003. Although CT of the chest and abdomen showed no metastatic mass, a brain CT showed nine distinct metastases in the brain (Fig. 3b). On March 17, the patient died of respiratory failure.

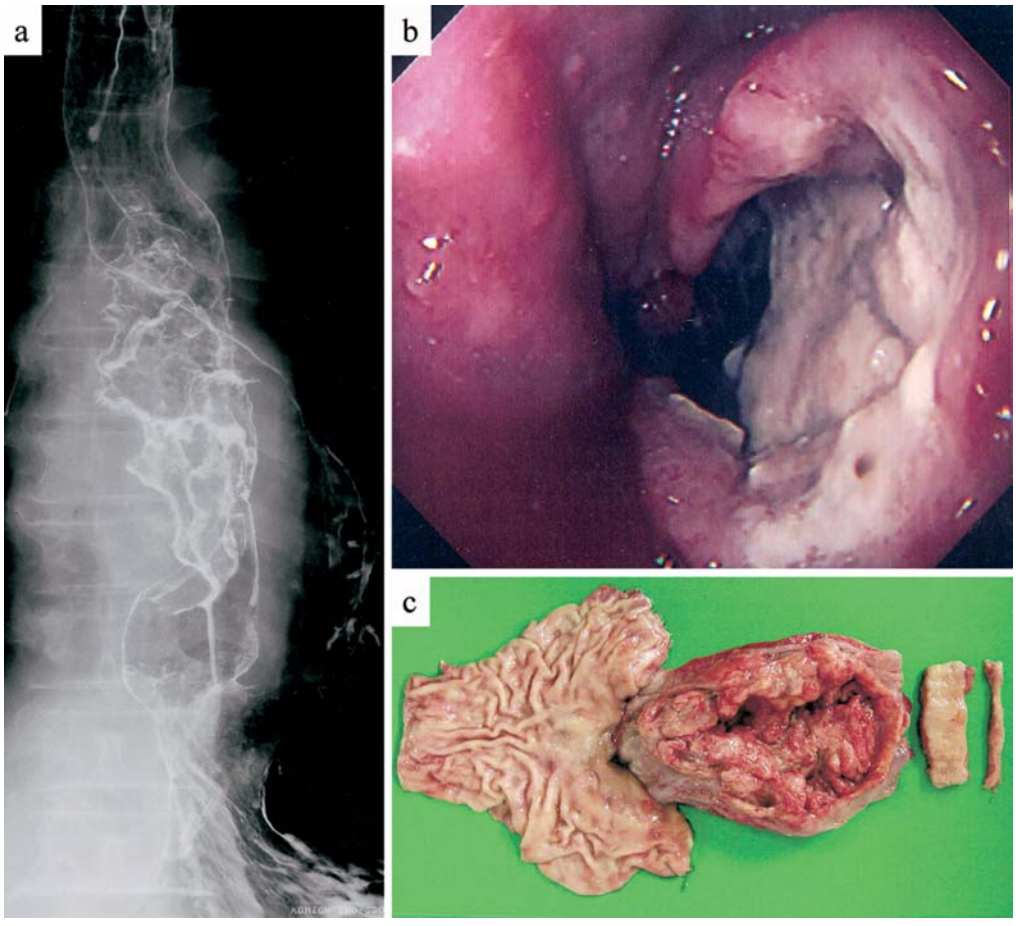



**Fig. 1. a** Esophagogram shows a 16-cm filling defect of the middle thoracic esophagus. **b** Esophagoscopy shows a huge tumor with ulceration. **c** Macroscopic findings of the resected specimen, 10.8 × 8.5 cm, with its ulcerated and infiltrative appearance

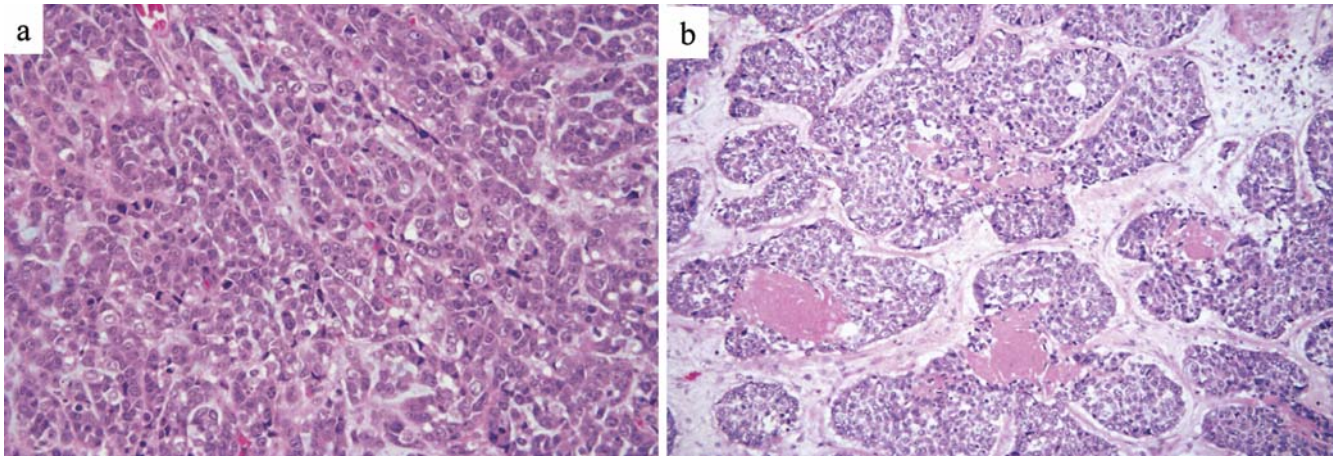



**Fig. 2a,b.** Microscopic findings of a basaloid carcinoma of the esophagus with hematoxylin and eosin. **a** The tumor was composed of a proliferation of severely atypical basaloid cells in solid nest formations and trabeculae. **b** The cells were separated by fibrocollagenous tissue with hyaline material depositions. ×200

*Quantitive RT-PCR method*

Total ribonucleic acid (RNA) was extracted from the cells found in the 5-ml aliquot of cerebrospinal fluid by a guanidinium-isothiocyanate-phenol-chloroform-based method. The complementary deoxyribonucleic acid (cDNA) was synthesized using a commercial kit (All Advantage RT for PCR Kit; Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. A CEA-specific oligonucleotide primer was designed

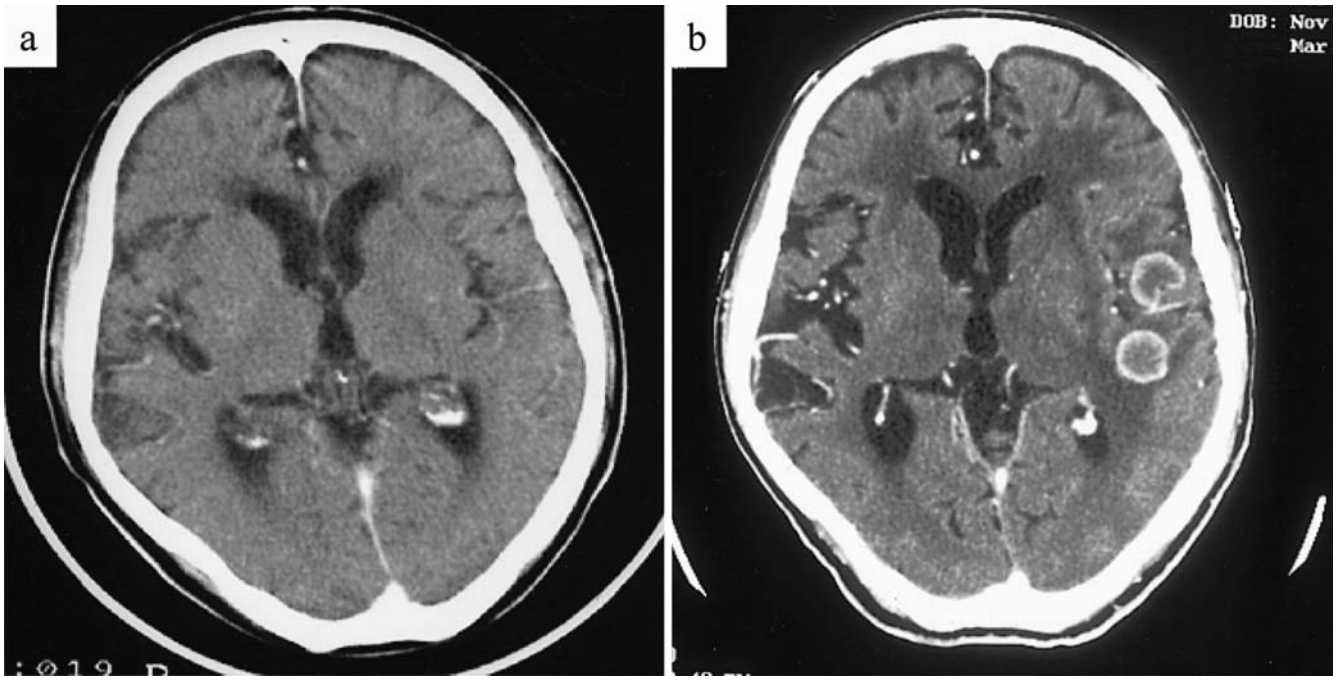



**Fig. 3.** **a** An enhanced computed tomography brain scan was performed on October 18, 2002. No evidence of cerebrovascular disease or tumor was found. **b** In an enhanced computed tomography brain scan was performed on March 14, 2003, metastatic tumors in the brain were confirmed

**Table 2.** Laboratory data of cerebrospinal fluid

| | |
|---|---|
| Glucose (mg/dl) | 103 |
| Sodium (mEq/l) | 138 |
| Chloride (mEq/l) | 114 |
| Total protein (mg/dl) | 87.5 |
| Cell number (/ml) | 10 |
| Neutrophil (%) | 63 |
| Lymphocyte (%) | 5 |
| Monocyte (%) | 32 |
| Immunoglobulin G (mg/dl) | 13.4 |
| CEA (ng/ml) | 0.4 |
| SCC (ng/ml) | 0.0 |
| Cytology | Negative |
| Herpesvirus type 1, 2 | Negative |

CEA, carcinoembryonic antigen; SCC squamous cell carcinoma antigen

on the basis of the report by Gerhard et al.[4] The sequences were 5′-TGTCGGCATCATGATTGG-3′ for the sense strand and 5′-GCAAATGCTTTAAGGA AGAAGC-3′ for the antisense strand. The fluorescent and LC-Red probe sequences used for CEA identification were 5′-CCTGAAATGAAGAAACTACAC CAGGGC-fluorescein and 5′-LC-Red640-GCTATAT CAGAGCAACCCCAACCAGC-phosphorylation. Amplifications of CEA by the PCR method using a quantitative fluorescence LightCycler instrument (Roche Diagnostics, Mannheim, Germany) was carried out in 20 μl reaction mixture containing 2 μl LightCycler FastStart DNA Master Hybridization Probes (Roche) as a master mixture, 3.0 mM $MgCl_2$, 0.5 μM of each sense and antisense primer, 0.4 μM fluorescent probe, 0.2 μM LC-Red probe, and 5 μl template cDNA in a LightCycler capillary. Before amplification, 0.32 μl anti-Taq DNA polymerase antibody (TaqStart antibody; Clontech) was added to the reaction mixture, which was then incubated at room temperature for 5 min to avoid primer prolongation. The temperature profile was used for amplification: denaturation for 1 cycle at 95°C for 10 min and 40 cycles at 95°C for 10 s, 60°C for 15 s, and 72°C for 5 s. Real-time PCR monitoring was achieved by measuring the fluorescent signal at the end of each cycle's annealing phase. To prove the integrity of isolated RNA, a PCR assay with primers and probes specific for the gene glyceraldehyde phosphate dehyrogenase (GAPDH) mRNA was carried out under the same conditions as described above. The primer sequences used for GAPDH amplification were 5′-TGAACGGGAAGCTCACTGG-3′ for the sense strand and TCCACCACCCTGTTGCTGTA-3′ for the antisense strand. The probe sequences used for GAPDH identification were 5′-TCAACAGCGACA CCCACTCCT-fluorescein and 5′-LC-Red640-CACC TTTGACGCTGGGGCT-phosphorylation. The optimal reagent concentrations and PCR cycling conditions were established by Nihon Gene Research Laboratories (Sendai, Japan). External standards for CEA and

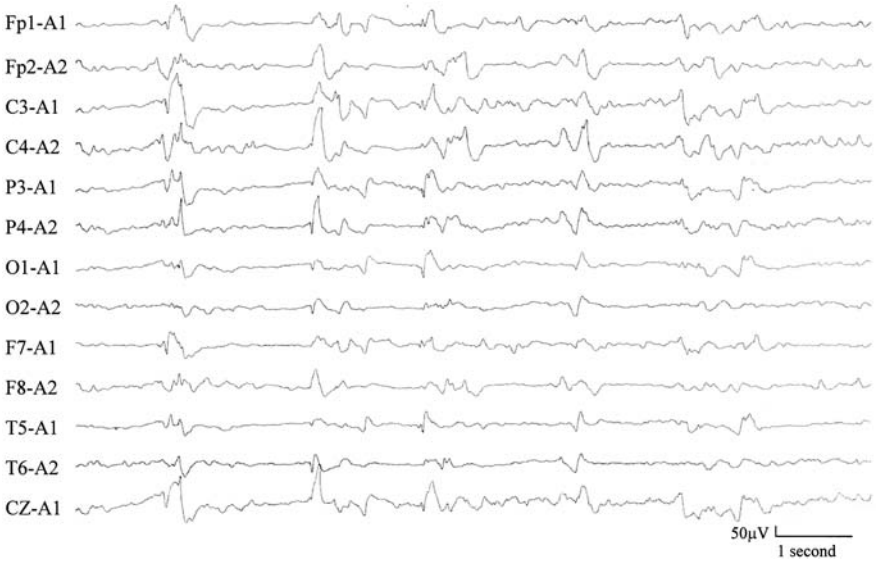



**Fig. 4.** Electroencephalogram (EEG) showed periodic lateralized epileptiform discharges (PLEDs)

GAPDH mRNA were prepared by 10-fold serial dilutions of cDNA synthesized by MKN45. The CEA mRNA values were adjusted with GAPDH mRNA values and presented as relative CEA mRNA scores (CEA mRNA/GAPDH mRNA $\times 10^5$; cutoff, 2.78).

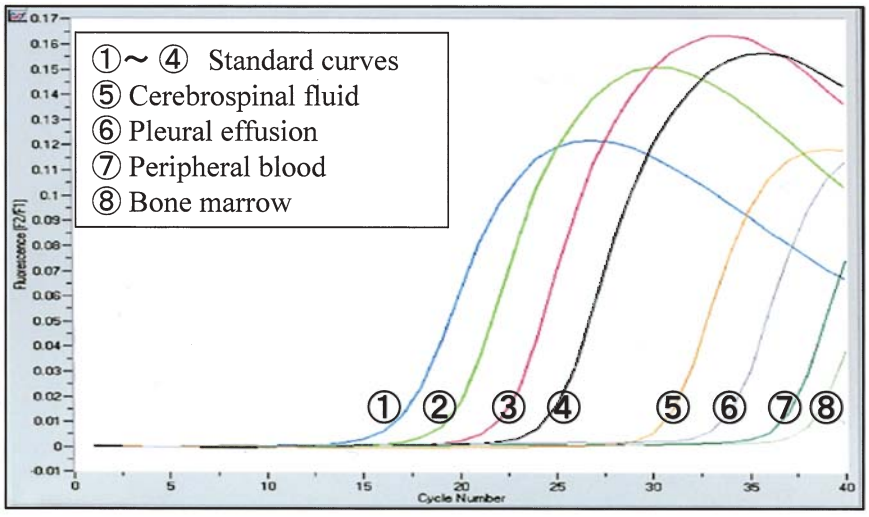
*Immunohistochemical staining*

A 5-ml aliquot of cerebrospinal fluid was centrifuged at 1500 rpm for 10 min and a cell pellet was formed. The pellet was mounted on a glass slide with Cytospine fixative, centrifuged at 1000 rpm for 3 min, and dried for 1 h in a dryer. After the slide was washed in Tris-buffered saline (TBS), it was incubated for 60 min at 37°C with the monoclonal antibody cocktail AE1/AE3 (antihuman cytokeratin; Dako, Carpinteria, CA, USA), which is reactive with a broad spectrum of human cytokeratins.[6] After the slide was washed in TBS, it was incubated with peroxidase-labeled polymer conjugated to primary antibody for 30 min. After the slide was again washed in TBS, it was incubated with a substrate chromogen (Fast Red Substrate System; Dako) for 10 min, washed in water, counterstained with hematoxylin, and placed under a coverslip with glycerin gelatin (Glycegel; Dako).

## Discussion

Metastasis from esophageal cancer into the central nervous system is uncommon and leptomeningeal carcinomatosis is extremely rare. To our knowledge, only two patients with leptomeningeal carcinomatosis from esophageal carcinoma have been described in previous reports.[10,11] One patient had a postoperative recurrence of adenocarcinoma and the other patient had squamous cell carcinoma treated by chemotherapy. Leptomeningeal carcinomatosis was diagnosed in these patients only by radiologic findings. This article is the first reported case of leptomeningeal carcinomatosis from esophageal carcinoma after surgery, in which the existence of CEA mRNA was demonstrated by real-time RT-PCR and cytokeratin-positive cells were demonstrated in cerebrospinal fluid by immunohistochemical staining.

The diagnosis of leptomeningeal carcinomatosis has been most commonly made by cytological examination of the cerebrospinal fluid. Positive cytology results were obtained in 50% of patients with leptomeningeal carcinomatosis after an initial lumbar puncture and in about 85% of patients who had undergone three lumbar punctures.[1] Although radiologic studies, contrast-enhanced CT, or gadolinium-enhanced MRI have been used to reveal subarachnoid masses, diffuse contrast enhancement of the meninges, and hydrocephalus without a mass lesion, their success rate in diagnosing leptomeningeal carcinomatosis has been about 50%–60%.[1,12,13] Recently, immunohistochemical assays for epithelial-specific antigens such as cytokeratins and genetic diagnostic procedures such as the RT-PCR method have been used to detect micrometastasis in the lymph nodes, blood, and bone marrow.[4–7] Only one report of leptomeningeal metastasis from lung adenocarcinoma diagnosed by RT-PCR with CEA mRNA has been previously published.[14] In the present case, accurate diagnosis of leptomeningeal carcinomatosis was not made by the cytological examination of the cerebrospinal fluid or by imaging techniques. Examination of CEA mRNA expression in the cerebrospinal fluid was thought to be more sensitive than these other methods.




| | CEA-mRNA score (Cut off>2.78) |
|---|---|
| Cerebrospinal fluid | 3380 (after chemotherapy: 1903) |
| Pleural effusion | 6.74 |
| Peripheral blood | 0.12 |
| Bone marrow | 0.015 |

**Fig. 5.** The results of determining carcinoembryonic antigen (*CEA*) *mRNA* expression in cerebrospinal fluid, pleural effusion, peripheral blood, and bone marrow following RT-PCR. CEA mRNA is strongly expressed in cerebrospinal fluid and slightly expressed in pleural effusion. After chemotherapy, the CEA mRNA score in cerebrospinal fluid decreased from 3380 to 1903. mRNA score, CEA mRNA/GAPDH mRNA $\times 10^5$; cutoff, 2.78

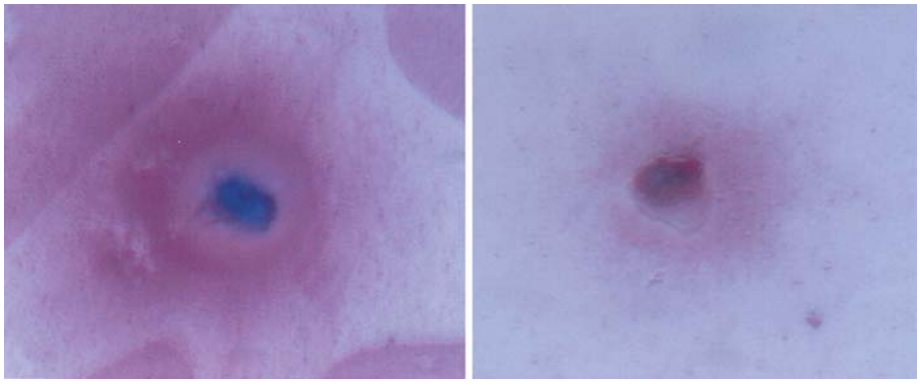


**Fig. 6.** Cytokeratin-positive tumor cells in cerebrospinal fluid were found by immunohistochemistry. $\times 400$

We thought the criteria of the indication to perform this method were as follows: (1) advanced cancer, (2) no obvious findings of brain metastasis by imaging means, (3) negative cytological results of cerebrospinal fluid, and (4) leptomeningeal symptoms such as paralytic symptoms in the limbs, headache, vomiting, or systemic convulsion.

The mechanisms of the cause of systemic convulsion and unconsciousness under the condition without any radiologic evidence in this case might be diffuse brain

damage due to meningitis carcinomatosis. The damage might diffusely extend to not only the cerebral cortex but also to the medulla oblongata, causing systemic convulsion, unconsciousness, and respiratory failure. Those mechanisms were supported by the PLEDs finding of the EEG.[9]

The purposes of treatment in patients with leptomeningeal carcinomatosis are to improve the neurological disorder and to prolong survival. The median survival time of patients with leptomeningeal carcinomatosis was 4–6 weeks and such patients commonly died of progressive neurological dysfunction.[3] Many reports have addressed the treatment of leptomeningeal carcinomatosis from leukemia, but few have addressed that from solid tumors. Cooper et al. reported a case of complete remission of cerebral metastasis in a patient with ovarian cancer after the administration of cisplatin.[15] In our case, after systemic chemotherapy, the clinical symptoms decreased in proportion to the CEA mRNA value. Thus, once a diagnosis of leptomeningeal carcinomatosis has been made, systemic chemotherapy should be started as soon as possible.

In conclusion, in the present case, quantitative RT-PCR with CEA mRNA was useful for diagnosing leptomeningeal carcinomatosis. Therefore, the examination of CEA mRNA expression by RT-PCR should be performed whenever an unknown brain disorder undetected by cytological examination of cerebrospinal fluid and by imaging occurs in patients with cancer.

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