Diagnostic Accuracy of Cerebrospinal Fluid (CSF) Cytology in Metastatic Tumors: An Analysis of Consecutive CSF Samples

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Background: Cerebrospinal fluid (CSF) examination can be used to verify the presence of primary malignancies as well as cases of central nervous system (CNS) metastasis. Because of its importance, there have been several studies concerning the sensitivity of CSF cytology. To determine the practical use and reproducibility of diagnoses based on CSF cytology, we evaluated this test by analyzing cytology results from consecutive CSF samples.

Methods: Between July 2010 and June 2013, 385 CSF cytology samples from 42 patients were collected. The samples were gathered using a ventricular catheter and reservoir. CSF cytology of all patients was examined more than two times with immunocytochemistry for cytokeratin.

Results: Primary neoplastic sites and histologic types of patients' metastatic cancer were diverse. The overall sensitivity for detecting malignancy was 41.3%. Even within short-term intervals, diagnoses frequently changed. The diagnostic rate of CSF cytology results should not be ignored, and continuous CSF follow-up is essential for following the clinical course of patients with metastatic cancer involving the CNS.

Key Words: Central nervous system; Neoplasm metastasis; Cerebrospinal fluid

Materials and Methods

Patient selection

Between July 2010 and June 2013, 385 CSF cytology samples were collected from 42 patients with the presence of a metastatic tumor confirmed by at least two histologic or cytological studies. Cytology samples obtained before adjuvant therapies were excluded to evaluate the diagnostic rate of CSF cytology more consistently. The breakdown of the patient population was as follows: 25 males and 17 females, with a median age of 55 years (range, 29 to 77 years). The mean observation period was 5 months (range, 1 to 22 months), and the mean number of CSF examinations was 9 (range, 2 to 34).

Cerebrospinal fluid specimen collection and cytology slide preparation

All patients underwent an operation for the placement of a ventricular catheter and reservoir as well as consecutive CSF collections using the reservoir (Fig. 1). To minimize dry artifacts...
and prevent cell degeneration, samples were delivered to the Department of Pathology immediately upon collection. Samples were then processed using liquid-based cytology (LBC) (ThinPrep, Cytyc Co., Boxborough, MA, USA), an automated method of preparation and smearing of cells in a monolayer. Slides were stained and evaluated with the Papanicolaou stain-

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**Fig. 1.** Operation for ventricular catheter and reservoir placement. A localized area of the skull is removed (A), and the reservoir is placed in the defect area (B).

**Fig. 2.** Low (A) and high (B) power views of cerebrospinal fluid in a patient with metastatic adenocarcinoma of the lung. Separate case showing an atypical cell (C) confirmed as metastatic carcinoma using immunocytochemistry for cytokeratin (D).
metastatic tumor in cerebrospinal fluid cytology. of the 385 CSF samples, 54 were processed by a
conventional smear method rather than the liquid-based meth-
od because those samples were obtained when the liquid-based
method was not available for CSF cytology.

immunocytochemistry

immunocytochemistry (ICC) was performed using a Ventana
XT automated stainer (Ventana Co., Tucson, AZ, USA) with an
antibody to cytokeratin (1:300, AE1/AE3, Dako, Carpinteria,
CA, USA). Slides were incubated with primary antibody for 32
minutes at 37°C followed by a universal secondary antibody for
8 minutes at 37°C. Slides were incubated in streptavidin-horse-
radish peroxidase D for 16 minutes at 37°C, and then the sub-
strate, 3,3′-diaminobenzidine tetrahydrochloride (DAB) H2O2,
was added for 8 minutes, followed by hematoxylin and bluing
reagent counterstains at 37°C.

Table 1. Clinicopathologic features of 42 cases and diagnostic rates of cerebrospinal fluid cytology

| Case No. | Sex | Age (yr) | Primary site | Histologic type | Period (mo) | Total | Negative rate | Positive rate |
|----------|-----|----------|--------------|----------------|-------------|-------|---------------|---------------|
| 1        | M   | 48       | Lung         | Adenocarcinoma | 1           | 6     | 3 (50.0)      | 3 (50.0)      |
| 2        | M   | 63       | Lung         | Adenocarcinoma | 1           | 4     | 2 (50.0)      | 2 (50.0)      |
| 3        | F   | 60       | Ovary        | Serous papillary carcinoma | 8  | 13     | 10 (76.9) | 3 (23.1) |
| 4        | M   | 64       | Lung         | Adenocarcinoma | 2           | 13    | 7 (53.8)      | 6 (46.2)      |
| 5        | M   | 56       | Rectum       | Adenocarcinoma | 7           | 12    | 12 (100.0)    | 0 (0)         |
| 6        | F   | 56       | Breast       | Invasive ductal carcinoma | 5  | 11     | 10 (90.9) | 1 (9.1)   |
| 7        | F   | 43       | Lung         | Adenocarcinoma | 3           | 13    | 8 (61.5)      | 5 (38.5)      |
| 8        | M   | 58       | Lung         | Adenocarcinoma | 11          | 22    | 19 (86.0)     | 3 (14.0)      |
| 9        | M   | 52       | Kidney       | Renal cell carcinoma | 5  | 9      | 9 (100.0) | 0 (0.0)   |
| 10       | F   | 66       | Lung         | Adenocarcinoma | 22          | 22    | 21 (95.5)     | 1 (4.5)       |
| 11       | M   | 66       | Lung         | Adenocarcinoma | 1           | 2     | 0 (0.0)       | 2 (100.0)     |
| 12       | M   | 58       | Lung         | Adenocarcinoma | 2           | 15    | 5 (33.3)      | 10 (66.7)     |
| 13       | M   | 62       | Lung         | Adenocarcinoma | 21          | 34    | 18 (52.9)     | 16 (47.1)     |
| 14       | M   | 65       | Stomach      | Adenocarcinoma | 1           | 7     | 2 (28.6)      | 5 (71.4)      |
| 15       | M   | 66       | Lung         | Adenocarcinoma | 6           | 15    | 12 (80.0)     | 3 (20.0)      |
| 16       | F   | 58       | Lung         | Adenocarcinoma | 4           | 17    | 11 (64.7)     | 6 (35.3)      |
| 17       | M   | 29       | Stomach      | Signet ring cell carcinoma | 3  | 9      | 4 (44.4)  | 5 (55.6) |
| 18       | M   | 51       | Lung         | Adenocarcinoma | 1           | 3     | 0 (0.0)       | 3 (100.0)     |
| 19       | F   | 71       | Lung         | Adenocarcinoma | 1           | 4     | 1 (25.0)      | 3 (75.0)      |
| 20       | F   | 74       | Ovary        | Serous papillary carcinoma | 3  | 8      | 0 (0.0)   | 8 (100.0) |
| 21       | M   | 64       | Stomach      | Adenocarcinoma | 1           | 4     | 0 (0.0)       | 4 (100.0)     |
| 22       | M   | 52       | Lung         | Adenocarcinoma | 1           | 8     | 1 (12.5)      | 7 (87.5)      |
| 23       | F   | 58       | Stomach      | Adenocarcinoma | 1           | 3     | 0 (0.0)       | 3 (100.0)     |
| 24       | M   | 65       | Lung         | Adenocarcinoma | 9           | 14    | 12 (85.7)     | 2 (14.3)      |
| 25       | F   | 47       | Breast       | Invasive ductal carcinoma | 1  | 2      | 0 (0.0)   | 2 (100.0) |
| 26       | F   | 40       | Breast       | Invasive ductal carcinoma | 11  | 7     | 2 (28.6) | 5 (71.4) |
| 27       | F   | 56       | Breast       | Invasive ductal carcinoma | 1  | 2     | 0 (0.0)   | 2 (100.0) |
| 28       | M   | 65       | Lung         | Small cell carcinoma | 4  | 4      | 3 (75.0) | 1 (25.0) |
| 29       | M   | 51       | Skin         | Malignant melanoma | 1  | 2     | 0 (0.0)   | 2 (100.0) |
| 30       | F   | 46       | Breast       | Invasive ductal carcinoma | 5  | 18     | 17 (94.4) | 1 (5.6)   |
| 31       | M   | 55       | Urinary bladder | Urothelial carcinoma | 1  | 2     | 1 (50.0) | 1 (50.0) |
| 32       | M   | 41       | Lung         | Adenocarcinoma | 2           | 11    | 3 (27.3)      | 8 (72.7)      |
| 33       | M   | 53       | Lung         | Adenocarcinoma | 2           | 8     | 7 (87.5)      | 1 (12.5)      |
| 34       | M   | 55       | Lung         | Adenocarcinoma | 8           | 7     | 1 (14.3)      | 7 (85.7)      |
| 35       | F   | 46       | Breast       | Invasive ductal carcinoma | 5  | 3     | 3 (100.0) | 0 (0.0)   |
| 36       | M   | 39       | Pancreas     | Adenocarcinoma | 6           | 7     | 5 (71.4)      | 2 (28.6)      |
| 37       | F   | 65       | Breast       | Invasive ductal carcinoma | 5  | 13     | 6 (46.2) | 7 (53.8) |
| 38       | M   | 31       | Skin         | Malignant melanoma | 2  | 4     | 3 (75.0) | 1 (25.0) |
| 39       | F   | 46       | Breast       | Invasive ductal carcinoma | 1  | 4     | 2 (50.0) | 2 (50.0) |
| 40       | F   | 49       | Lung         | Adenocarcinoma | 10          | 8     | 3 (37.5)      | 7 (62.5)      |
| 41       | M   | 49       | Lung         | Adenocarcinoma | 1           | 9     | 0 (0.0)       | 9 (100.0)     |
| 42       | F   | 77       | Lung         | Adenocarcinoma | 1           | 6     | 3 (50.0)      | 3 (50.0)      |
| Mean     |     | 5        | 385          |                 |             | 226   | 226 (58.7)    | 159 (41.3)    |

Values are presented as number (%).
M, male; F, female.
Interpretation criteria

Cases without atypical cells suggestive of metastasis were diagnosed as negative for malignancy. A positive diagnosis of malignancy was defined as the presence of atypical cells with cytokeratin immunoreactivity, regardless of the amount (Fig. 2). There were some cases that presented with atypical cells on Papanicolaou-stained slides, but it was not possible to evaluate many of these cases using cytokeratin ICC slides because the cells disappeared during the staining process. In those cases, we termed the diagnosis suspicious for malignancy, and these cases were regarded as positive findings when calculating the diagnostic rates. All slides, including those for ICC, were independently reviewed by two pathologists (S.H.K and Y.S.B).

RESULTS

As summarized in Table 1, primary malignancy sites were diverse. Lung was the most common primary site with 22 cases, followed by breast with 6 cases. The histologic types of primary malignancies are shown in Table 1. Of the 385 specimens, 132 were diagnosed as positive for malignancy and 27 were suspicious for malignancy, for a total of 159 specimens considered to have a positive diagnosis of malignancy. Among the 42 patients, 3 were never diagnosed as malignant by cytology examination even though all were confirmed to have CNS metastasis on brain tissue biopsy. Eight patients were consistently diagnosed as positive for malignancy in serial CSF examinations. The positive malignancy diagnosis rates in the other 31 patients ranged from 4.5% to 87.5%, and the mean positive rate was 41.3%.

Table 2 demonstrates two representative cases (case nos. 4 and 12) from our series. During a period of two months, these two patients underwent 13 and 15 CSF cytology examinations, which yielded positive results in 6 and 10 of the tests, respectively. The intervals between CSF cytology examinations ranged from 0 to 13 days and were short enough to enable more meticulous analysis of consecutive diagnostic rates. However, the results appeared random, without a consistent trend. Even within the same day, the diagnoses made from two specimens were different.

DISCUSSION

Rapid transport is important for optimal cellular preservation in CSF materials, which can be cytolysed quickly. In order to reduce the number of nondiagnostic cases, CSF materials should be examined as soon as possible after collection. From a routine diagnostic viewpoint, degeneration is one of the most problematic artifacts when diagnosing cytology slides, as CSF specimens tend to degenerate more readily than other cytology specimens. These artifacts can affect the diagnostic accuracy and reduce specimen adequacy. We were able to control artificial factors by reminding clinicians of the importance of rapid processing and encouraging them to submit the samples immediately.

Most previous studies concerning the diagnostic rates of CSF examination used the lumbar puncture as a diagnosing modality. However, lumbar puncture can be harmful to patients and difficult for clinicians because it is an invasive procedure. It is this for reason that most previous studies regarding the diagnostic accuracy of CSF performed only one CSF examination per patient. In this study, however, consecutive CSF sampling was performed using the ventricular catheter and reservoir from

Table 2. Consecutive cerebrospinal fluid cytology results of case no. 4 and case no. 12

| Case No. 4 | Cytological diagnosis | Date       | Case No. 12 | Cytological diagnosis | Date       |
|-----------|----------------------|------------|-------------|----------------------|------------|
| 1         | Negative for malignancy | Oct 20, 2010 | 1           | Positive for malignancy | Feb 5, 2013 |
| 2         | Positive for malignancy  | Oct 23, 2010 | 2           | Positive for malignancy | Feb 15, 2013 |
| 3         | Negative for malignancy  | Oct 30, 2010 | 3           | Negative for malignancy  | Feb 25, 2013 |
| 4         | Positive for malignancy  | Nov 2, 2010 | 4           | Positive for malignancy  | Feb 27, 2013 |
| 5         | Positive for malignancy  | Nov 8, 2010 | 5           | Positive for malignancy  | Mar 5, 2013 |
| 6         | Negative for malignancy  | Nov 10, 2010 | 6          | Positive for malignancy  | Mar 6, 2013 |
| 7         | Suspicious for malignancy | Nov 23, 2010 | 7           | Negative for malignancy  | Mar 12, 2013 |
| 8         | Positive for malignancy  | Nov 24, 2010 | 8           | Positive for malignancy  | Mar 14, 2013 |
| 9         | Negative for malignancy  | Nov 24, 2010 | 9           | Positive for malignancy  | Mar 18, 2013 |
| 10        | Negative for malignancy  | Dec 1, 2010 | 10          | Negative for malignancy  | Mar 21, 2013 |
| 11        | Suspicious for malignancy | Dec 3, 2010 | 11          | Positive for malignancy  | Mar 26, 2013 |
| 12        | Negative for malignancy  | Dec 9, 2010 | 12          | Negative for malignancy  | Mar 27, 2013 |
| 13        | Negative for malignancy  | Dec 16, 2010 | 13         | Positive for malignancy  | Mar 29, 2013 |
| 14        | Suspicious for malignancy | Feb 5, 2013 | 14          | Negative for malignancy  | Apr 2, 2013 |
| 15        | Positive for malignancy  | Feb 27, 2013 | 15          | Positive for malignancy  | Apr 3, 2013 |
Metastatic Tumor in Cerebrospinal Fluid Cytology

Temporary CSF cytology examinations are no longer sufficient for estimating clinical course because chemotherapy and optional radiotherapy are generally standard treatment modalities in patients with CNS metastasis. Although the total number of cases included was not remarkable, this study is noteworthy in that we tried to evaluate the diagnostic rates of CSF cytology using consecutive examination of CSF samples from each patient. Moreover, in our study, samples were obtained from patients receiving adjuvant treatments such as chemotherapy and radiation therapy, which may have affected the diagnostic rates of CSF analysis. From the viewpoint of daily practice, our results may be more practical and accurate because most patients with CNS metastasis are now managed with ancillary treatments.

We could not analyze the results of CSF examinations with statistics of sensitivity or specificity because not all cytology specimens had a concordant tissue biopsy. In other words, the negative findings could not be directly considered as false negatives because we could not completely exclude the possibility that the negative findings resulted from true tumor regression due to adjuvant therapies. However, as shown in Table 2, results were distributed unevenly although all examinations were performed within very short intervals, even within the same day in some cases. Therefore, we suggest that the negative findings are not the results of tumor regression but false negative results. If so, the value of 41.5% can be regarded as sensitivity of CSF cytology in patients being treated with adjuvant therapies due to CNS metastasis.

Several possible explanations exist for the negative results. First, several factors influencing the quality of CSF cytology specimens should be investigated. In our study, cytology specimens from positive cases were more cellular than those from negative cases. However, this cannot be the reason for the negative results because CSF is physiologically acellular. The volume of the submitted specimen can affect the diagnostic accuracy, but unfortunately this aspect was not evaluable because the amount of fluid was not promptly recorded. Dry artifact is one of the most important factors in determining the quality of CSF slides. As mentioned earlier, we could maintain the quality of CSF cytology by notifying clinicians of the importance of rapid transport. However, specimen transport could not be controlled precisely and evenly because the specimens were transported by different clinicians with different time intervals. There was no significant difference among negative results when grouped according to the sites of primary tumors (data not shown). Taken together, the negative results should be regarded as a multifactorial phenomenon without a specific reason.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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