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Value of Additional Immunocytochemical Stain for Cytokeratin in the Diagnosis of Leptomeningeal Involvement of Metastatic Carcinoma

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Background: The purpose of this study was to describe potential pitfalls in the diagnosis of metastatic adenocarcinoma in cerebrospinal fluid (CSF) and to suggest additional work in association with cytokeratin immunocytochemistry for the proper diagnosis, especially in the specimens with low cellularity. **Methods:** We collected 267 cytologic specimens of CSF from patients, who were diagnosed over a 9-month period. Each of the individual samples were divided into half the sample size and processed via both, ThinPrep (TP) with Papanicolau stain and cytocentrifugation-based preparation (cytospin, CP) with immunocytochemical stain for cytokeratin. **Results:** Amongst the 267 cases, 45 cases from 22 patients were diagnosed to be positive for metastasis adenocarcinoma in CSF. TP with Papanicolau stain showed satisfactory cytomorphology when compared with specimen of CP preparation and cytokeratin immunocytochemical staining. All the TP processed cases belonged to satisfactory/superior categories based on the assessment of technical artifact, which potentially helps in decreasing diagnositc errors. However, in 10 out of 45 cases, diagnostic atypical cells were present only in one of the two slides. **Conclusions:** Immunocytochemical stain for cytokeratin along with TP processed specimen helps in decreasing potential diagnostic errors in the cytological diagnosis of metastatic carcinoma in CSF specimen.

Key Words: Cerebrospinal fluid; Neoplasms; Metastasis

Neoplastic meningitis has been reported to be clinically diagnosed in 4 to 15% of patients with solid tumor, 5 to 15% of patients with leukemia and lymphoma, and 1 to 2% of patients with primary brain tumors.[1] It is postulated that the condition remains underdiagnosed as postmortem estimates of the true incidence of the disease across all the cancers approaches to 5% of total solid tumors.[1] The diagnosis of leptomeningeal spread of carcinoma is crucial when a patient is presented with symptoms of chronic aseptic meningitis. Development of magnetic resonance imaging (MRI) increases the sensitivity of the detection, but it is limited to detecting neoplastic meningitis in around 40 to 60% of cases and most of the patients are both, cytologically and radiologically negative.[1] Several promising methods for diagnosing neoplastic meningitis have been suggested, but cerebrospinal fluid (CSF) cytology along with the contrast enhanced MRI are currently the most widely accepted techniques. One of the possible reasons for the high false negative rate of cytologic diagnosis may be the low cellularity of specimen, since assessment of cytomorphologic interpretation is limited in this situation. Moreover, different modalities of processing expose specimen to different degrees of physical forces and chemical influences, resulting in certain artifact that can affect cytomorphologic interpretation.[2] A thorough review of the literature leads to the conclusion that direct smear, cytocentrifugation and filtration techniques are worthy to be considered for routine use, with comparable diagnostic method for most nongynecologic specimens.[2] Several studies have assessed the diagnostic value of the ThinPrep® Pap Test™ (Cytyc Corporation, Boxborough, MA, USA) for special types of nongynecologic specimens such as, pleural fluid and urine.[3,4] However, the researchers did not consider the general intrinsic characteristics of the specimen, such as low cellularity of CSF specimen.

The main purpose of this study was to authenticate the usage of ThinPrep technology in the diagnosis of metastatic carcinoma in CSF and subsequently to describe potential pitfalls in the diagnosis of metastatic carcinoma in CSF and in suggesting cytokeratin immunocytochemistry as a way to overcome the associated limitation, based on our experience in a single institute.

MATERIALS AND METHODS

A total of 267 cytologic specimens of CSF submitted for diagnosis over a 9-month period in Severance Hospital (Seoul, Korea) were included in this retrospective study. All the specimens were submitted under a clinical impression of leptomeningeal involvement of carcinoma. We divided CSF specimens from each patient in half and processed one with ThinPrep (TP) with Papanicolau stain and the other with a cytocentrifugation-based preparation with immunocytochemocal stain for cytokeratin (AE1/AE3, Dako, Carpinteria, CA, USA), thus generating two slides per case. Immunocytochemical study for cytokeratin was manually performed for all the specimens. Specifications of specimen with positive findings are described in Table 1. Morphological differences as a result of the two processing modalities were assessed and diagnostic discrepancies between the methods were evaluated by two different cytopathologists (SH Kim and J Choi). Cases were included in the "positive" category when the diagnosis was either "atypical cells" or "positive for malignancy." We evaluated the following parameters according to the criteria previously used by Nassar *et al.*[5]: cellularity, presence of diagnostic elements, ease of finding diagnostic elements, cytomorphology, backgrounds, and technical artifacts. As proposed by Nassar *et al.*,[5] the parameters were assessed semiquantitatively as "unsatisfactory," "satisfactory," and "superior" categories. With respect to cellularity, we designated "superior" category when the number of tumor cells was higher than 5 in 200× field in the highest cellularity area, "satisfactory" category when the number of tumor cells was 1-4 in 200× field and "unsatisfactory" category when the number of tumor cells was less than "satisfactory" specimens. The percentage of cases of both groups belonged to either "satisfactory" or "superior" cases (i.e., sum of number of specimens that belonged to both categories) in total cases was compared by Pearson's Chi-squre test by using SPSS ver. 13.0 (SPSS Inc., Chicago, IL, USA).[5] Statistical significance was assumed when $p < 0.05$.

RESULTS

Amongst the total of 267 CSF specimens, 45 cases from 22 patients belonged to "positive" category (Table 1, Fig. 1). The

Table 1. Summary of diagnosis of CSF cytology specimens

Categories	Diagnosis	n
Atypical cells (n=11)	A few scattered atypical cells showing CK positivity, consistent with metastatic carcinoma	3
	A few scattered atypical cells showing CK positivity, suspicious of metastatic carcinoma	2
	A few atypical cells showing CK positivity, highly suspicious of metastatic carcinoma	5
	A few atypical cells	1
Positive for malignancy, metastatic carcinoma		34
Total		45

CSF, cerebrospinal fluid; CK, cytokeratin.

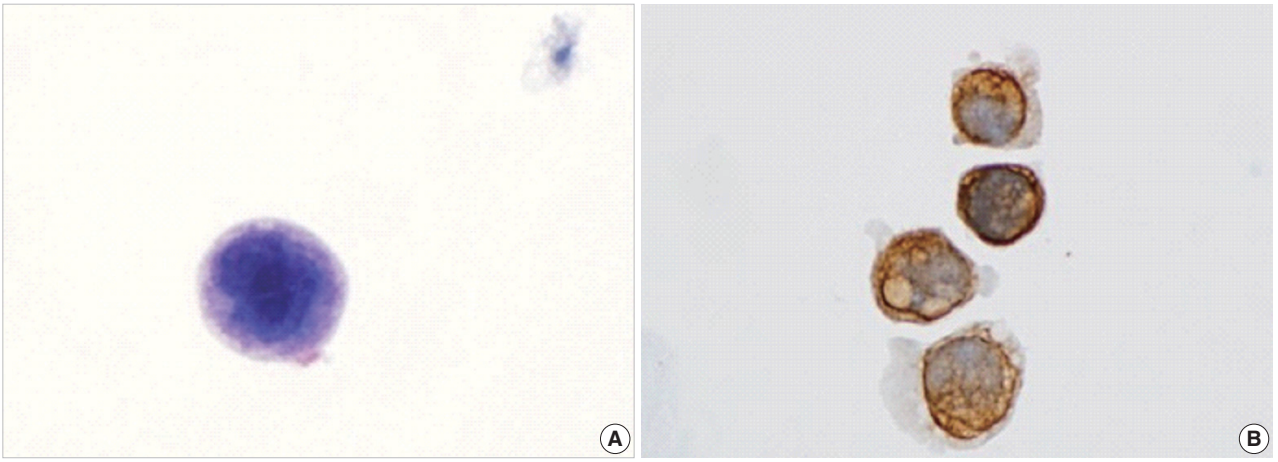

Fig. 1. Metastatic adenocarcinoma in cerebrospinal fluid specimen processed with ThinPrep (A, adenocarcinoma from stomach). Immunocytochemistry for cytokeratin staining is performed in specimen prepared by cytospin (B).

Table 2. Primary tumor sites of metastatic carcinoma

Site	No. of cases
Lung	30
Stomach	4
Breast	3
Ovary	3
Peritoneum	2
Gallbladder	2
Unknown	1
Total	45

Table 3. Comparison of cytological parameters of ThinPrep and Cytospin preparation in the evaluation of metastatic carcinoma in cerebrospinal fluid

	ThinPrep	Cytospin with immunocytochemical staining	p-value
	No. of sat/sup[a] cases (%)	No. of sat/sup[a] cases (%)	
Cellularity	30 (66.7)	27 (60)	0.662
Presence of diagnostic cells	29 (64.4)	22 (48.9)	0.202
Ease to find	25 (55.6)	24 (53.3)	1.000
Cytomorphology	35 (77.8)	3 (6.7)	0.000
Background	43 (95.6)	40 (88.9)	0.302
Technical artifacts	45 (100)	35 (77.8)	0.000

The number of cases, which belonged to "satisfactory" or "superior" category are depicted.
[a]Sat/sup = number of cases in the 'satisfactory' category or number of cases in the 'superior' category.

frequencies of primary sites of metastatic neoplasm were in the following order; lung, stomach, breast, ovary, peritoneum, gallbladder and unknown (Table 2).[6] The histological type of carcinoma was represented as adenocarcinoma in all the positive cases. The cytological features of the metastatic carcinoma were in good correlation with the previously described features like; cells with increased nuclear/cytoplasm ratio, eccentric nuclei with prominent nucleoli and signet ring features.[7] However, on the sole basis of cytologic findings, it was not possible to define primary site of metastatic carcinoma.

The morphological comparison of cytological parameters between TP and cytospin (CP) processed specimen was performed according to previously proposed categories.[5] As expected, TP with Papanicolau staining showed satisfactory cytomorphology when compared with specimens prepared by cytospin preparation and cytokeratin staining. In contrast to previous findings, there was no significant difference in the cellularities of both the groups.[8] Especially, all the TP processed cases belonged to satisfactory/superior categories in the assessment of technical artifact (Table 3).

Table 4. Distribution of diagnostic cells

Category	No. of cases
Diagnostic cells in both	35
Only in ThinPrep	3
Only in Cytospin/Immunocytochemistry	7

Even though TP with Papanicolau stain gave satisfactory results for diagnosis, there was also significant diagnostic caveat in 10 out of 45 cases; diagnostic atypical cells were present in only one slide out of the two (Table 4). Specially, when the number of diagnostic cells was limited in TP slides, a single atypical cell with cytokeratin immunopositivity was critical for diagnostic decision.

DISCUSSION

Limited literature is available regarding the usage of TP for diagnosing leptomeningeal involvment of carcinoma. We analyzed a total of 267 CSF specimens processed by the TP, with clinical impression of leptomeningeal involvement of primary tumor outside the central nervous system. Even though almost all the cases exhibited positive findings on imaging studies, only 45 cases showed positive findings during the cytologic examination. One of the possible causes for the observed false negatives could be low cellularity of the CSF specimen. Regarding the origin of the primary sites, lung, breast, and stomach were the most common sites for the orgin of metastatic neoplasm as also described previously.[2] The most common histologic type of carcinoma was adenocarcinoma as had been reported previously.[2] However, since each and every individual insititutes have their own areas of specialty and clinical practice is closely related to the actual number of specimens, our results may not reflect the true cases of incidence.

Although liquid-based monolayer technology is a widely used technique that has achieved acceptance for processing a majority of cytological specimens, limited literature is available on the suitabilty of technique for metastatic carcinoma. Recently, Sioutopoulou *et al.*[6] descibed their experience about TP liquid-based cytology with focus on the diagnosis of metastatic tumors in CSF. They validated this form of cytology as an everyday routine diagnostic method for metastatic tumors in the CSF. Liquid-based monolayer technology has also been considered as a valuable tool for further management and planning of treatment owing to associated advantages like, better cytomorphology, higher cellularity per slide, clear background and repro-

ducibility; which enables the use of immunocytochemistry on the same sample.[6] We confirmed these ideas based on our research experience. However, in our opinion, application of the TP does not overcome the drawback of low cellularity of the CSF specimen, since we did not observe better cellularity of specimen in the TP when compared to CP. Sioutopoulou *et al.*[6] did not provide appropriate information on the assessment of the cellularity of a given specimen when compared with other methods. However, in the present case, generally low cellularity of specimens could be one of the possible reasons for the observed discrepancy.

Since TP and CP processed specimens were stained with different staining methods, the results from the retrospective comparative analysis require to be understood with limitation inevitably; but we believe that fewer technical artifacts in TP during the process enables satisfactory cytomorphology for assessment of individual cells when the cellularity of the speicmen is guaranteed. However, in the case of specimens with limited cellularity, like CSF, distorted cytomorphological features by the artifacts during processing with any methods may potentially increase the rate of diagnostic errors.

We believe that the intrinsic chracteristic of low cellularity of specimen can be compensated by generating two slides for side-by-side preservation of cytomorphology (TP) and application of immunocytochemical studies. It is noteworthy that 10 out of 45 cases were diagnosed with malignant cells, which was apparent only in the cytokeratin immunocytochemical staining slide or TP slides, and the differential diagnosis was feasible when we confirmed cytokeratin-positive atypical cells in cytospin prepared slides in association with the TP slides. Thus, TP with routine cytokeratin immunocytochemical staining might be of help in making a confirmatory diagnosis. As mentioned, a single atypial cell with cytokeratin positivity may help in making the diagnostic decision. Regarding the potential presence of normal cytokeratin positive cells (i.e., choroid plexus cells) within the CSF, previous study by de Reuck and Vanderdonckt[9] revealed that choroid plexus and ependymal cells were found only in 10 out of 2,660 CSF cases. Thus, a small number of cytokeratin positive atypical cells with an impression of metastatic carcinoma may be conisidered to be a significant diagnostic clue. Generating additional slides may increase the cost of the examination, but we believe that there will be a reduction in the number of inconvinient repetitive examinations.

In summary, using additional cytokeratin immunostaining slides along with the TP processed slide is a reasonable solution to overcome the drawbacks associated with cellularity of a CSF specimen, in terms of preservation of cytomorphology and increase in diagnostic sensitivity.

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