Diagnostic Value of Liquid-Based Cytology With Fine Needle Aspiration Specimens for Cervical Lymphadenopathy

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Background: *Cervical lymphadenopathy is a symptom that is frequently seen among outpatients, and it is important to differentiate malignant lesions from reactive lymphoid hyperplasia. Fine needle aspiration (FNA) cytology has been widely used for the diagnosis of cervical lymphadenopathy. However, some limitations of the diagnostic accuracy using conventional smear (CS) cytology have been pointed out. The diagnostic value of liquid-based cytology (LBC) with FNA specimens has not yet been fully proven.*
Methods: *Forty-two patients with cervical lymphadenopathy who underwent FNA with CS cytology from 2007 to 2011 and 123 patients who underwent FNA with LBC utilizing LBCPREP2™ from 2011 to 2015 were studied. Diagnostic values were compared between the CS and the LBC groups.*
Results: *Of the total 165 patients representing the combined CS and LBC groups, 81 (49.1%) were diagnosed as benign lymph node and 84 (50.9%) were malignant diseases including 37 (22.4%) of metastatic carcinoma except for thyroid carcinoma, 30 (18.2%) of metastatic thyroid carcinoma, and 17 (10.3%) of malignant lymphoma. The overall statistical values including sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the CS were 75%, 100%, 100%, 78.9%, and 87.1%, respectively, whereas those values for LBC were 91.2%, 100%, 100%, 90.7%, and 95.3%, respectively. The sensitivity of LBC for malignant diseases tended to be higher than that of CS cytology (*$p = 0.081$*).*
Conclusion: *LBC with FNA specimens from cervical lymphadenopathy is a useful and reliable method for the diagnosis of malignant diseases, especially of metastatic carcinomas, due to its increased sensitivity compared with CS cytology.* Diagn. Cytopathol. 2016;44:169–176. © 2016 Wiley Periodicals, Inc.

Key Words: cervical lymph node; conventional smear; fine needle aspiration cytology; head and neck squamous cell carcinoma; liquid-based cytology

Cervical lymph node (CLN) enlargement, which is frequently seen in outpatients, is mainly caused by nonspecific inflammation. However, head and neck squamous cell carcinoma (SCC), thyroid carcinoma (TC), and thoracoabdominal malignancies often metastasize to the CLN.[1,2] Furthermore, malignant lymphoma (ML) sometimes shows CLN enlargement as the first symptom. In cases of CLN enlargement in which clinical assessment imaging failed to find an obvious primary site, but lymph node metastasis from carcinoma is

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strongly suspected, excisional lymph node biopsy is considered. However, a biopsy is contraindicated as worsening the rate of lymph node recurrence and distal metastasis for head and neck SCC,[3] with a risk of seeding the cutaneous trajectory.[4] Conversely, a biopsy is essential for the diagnosis of ML with subtype classification.[5,6]

Ultrasound (US)-guided fine needle aspiration (FNA) cytology is easily performed on an outpatient basis without anesthesia, and is a less-invasive, cost-effective diagnostic procedure for breast, thyroid gland, salivary gland, and skin tumors as well as for lymph nodes.[7–13] In the majority of medical centers that perform FNA, conventional smear (CS) cytology with alcohol fixation is the standard method for processing FNA specimens. Liquid-based cytology (LBC) is an alternative processing method in which the aspirated material is immediately fixed in either an ethanol- or a methanol-based solution and is then placed on LBC slides. The LBC method is gaining popularity worldwide as the method of choice for not only gynecological but also for nongynecological smears.[5,8,14] Specific advantages of LBC are rapid and proper fixation, reduced incidence of air-drying artifacts, cleaner background by hemolysis, even distribution of cells over a smaller slide area, and increased cellularity.[15–17] LBC allows the preservation of samples for some time and makes residual samples available for further investigations such as immunocytochemistry and even molecular analysis.[18–21] These advantages result in more objective diagnosis and greater diagnostic accuracy.[14]

A large number of reports have shown the usefulness of FNA with CS cytology for the analysis of CLN lesions[7–13] while only a few reports, which lumped together lymph node lesions located in different anatomic sites, have shown the usefulness of LBC.[15,22,23] Therefore scanty information is available regarding the diagnostic value of LBC with FNA specimens for patients with CLN lesions as compared with CS cytology. Our aim in this study was to demonstrate the diagnostic accuracy of a newly developed and manual LBC method for processing of FNA specimens from CLN lesions.

Patients and Methods

A retrospective study was conducted on 42 patients who underwent FNA with CS cytology for cervical lymphadenopathy from April 2007 to May 2011 (20 males and 22 females; aged 15–85 years old, median age 62 years), and on 123 patients who underwent FNA with LBC from June 2011 to March 2015 (67 males and 56 females; aged 21–96 years old, median age 63 years). For all the enrolled patients, CLN enlargement was the first clinical manifestation.

FNA cytology criteria for selection included a CLN with either a round shape, over 10 mm thick, loss of fatty hilum, invasion into the adjacent structure, calcification, or internal necrosis.[24] CLN enlargements that were more than 3 cm in the longest diameter with benign US findings were also added to criteria. Cervical cyst or abscess was excluded. US guided FNA with a 21-G needle attached to a 20-mL disposable plastic syringe and aspirator that was developed by Chiba University was performed by two experienced head and neck surgeons. Aspirates for CS cytology were immediately processed on a slide and fixed in 95% ethanol. Aspirates for LBC were processed according to the manufacturer's instructions. Briefly, aspirates were washed out into a vial (LBCPREP2^{TM}: Muto Pure Chemicals, Tokyo, Japan) that contained 15 mL of a methanol-based fixation solution. The cells were fixed for 30 min, and then centrifuged at 1660*g* for 5 min, and collected. After discarding the supernatant, 5 mL of distilled water was added to the vial, and then a coated LBC slide was inserted into the top of the vial. The vial was reversed for 10 min, and as a consequence cells adhered to the central area of the slide (measuring 31.4 mm^2) by spontaneous sedimentation. The CS and LBC slides were stained using Papanicolaou staining. The cytological diagnosis was classified into 4 categories including nondiagnostic or unsatisfactory, negative, indeterminate, and positive. Differences in cytological diagnosis for each malignancy were taken into consideration as follows[25–29]: malignant, malignancy suspicious, class IV, class V, and presence of malignant cells were regarded as positive; indeterminate and class III were regarded as indeterminate; negative, benign, class I, class II, and presence of reactive lymphoid cells were regarded as negative.

In cases of inconclusive cytological diagnosis in patients whose FNA specimens were processed using LBC, immunocytochemistry with several markers was applied to stored liquid-based prepared cells. Antigens were retrieved by boiling in the Immunosaver (diluted 1:200; Nissin EM Corporation, Tokyo, Japan) in a kitchen electric kettle for 5 min. The following antibodies were used: (a) anti-AE1,AE3 monoclonal antibody (mAb) (clone AE1/AE3; Nichirei Bioscience Inc., Tokyo, Japan); (b) anti-p16^{INK4a} mAb (clone E6H4, Roche, Basel, Switzerland); (c) anti-cytokeratin mAb (clone CAM 5.2; BD Biosciences, San Jose, CA); (d) anti-thyroid transcription factor (TTF)−1 mAb (clone SPT24; Nichirei Bioscience Inc.); (e) anti-CD20 mAb (clone L26; Nichirei Bioscience Inc.); and (f) anti-Bcl-2 mAb (clone 124; Dako, Glostrup, Denmark). The sections were sequentially incubated with mAbs for 30 min at room temperature (RT) and then with universal immune-peroxidase polymer (Histfine SAB-PO(R) kit; Nichirei Bioscience Inc.) for 30 min at RT. The signals were visualized by immersing the slides in freshly prepared 0.02% diaminobenzidine (DAB) solution for 10 min. The sections were finally counterstained with hematoxylin and mounted. The cytological diagnosis was assessed by three cytotechnologists and a cytopathologist.

All the patients diagnosed as cytologically positive then underwent an excisional biopsy for pathological diagnosis or neck dissection for treatment. The surgical

specimens were fixed in 10% buffered formalin, embedded in paraffin and 5-μm-thick sections were cut and stained with hematoxylin and eosin. Pathological diagnosis was assessed by two experienced pathologists without knowledge of the cytological diagnosis. For statistical analyses, patients diagnosed cytologically as indeterminate and as nondiagnostic or unsatisfactory were excluded. Comparison of categorical variables was performed by $\chi^2 d$ statistic, using Fisher's exact test when appropriate. A P value of <0.05 was considered to be significant. Informed consent was obtained from all the patients at the time of enrollment in this study.

Table I. Final and/or Pathological Diagnosis of a Primary and/or Lymph Node Lesion in Patients who Underwent FNA Cytology from Cervical Lymph Node

Diagnosis		*CS*	*LBC*	*CS+LBC*
Malignant: 84 (50.9%)				
Metastatic ca.: 67 (40.6%)				
Carcinomas except for thyroid: 37 (22.4%)				
Head and neck: 24(14.5%)				
Oropharynx	SCC	3	6	9
	Lymphoepithelial ca.		2	2
	Mucoepidermoid ca.		1	1
Larynx	SCC		4	4
Hypopharynx	SCC	1	1	2
Oral	SCC		2	2
Sinonasal	SCC		2	2
	Small cell ca.		1	1
Salivary gland	SCC	1		1
Except for head and neck: 13 (7.9%)				
Esophagus	SCC	1	2	3
Lung	Small cell ca.	1	1	2
	SCC		1	1
Prostate	Adenoca.		2	2
Skin	SCC		2	2
Pancreas	Invasive ductal ca.		1	1
Colon	Adenoca.	1		1
Primary unknown	SCC		1	1
Thyroid: 30 (18.2%)				
	Papillary ca.	10	19	29
	Undiffemtiated ca.		1	1
Malignant lymphoma: 17 (10.3%)				
	DLBCL	4	8	12
	Follicular lymphoma		3	3
	Hodgkin lymphoma		1	1
	T cell lymphoma		1	1
Benign: 81 (49.1%)				
Reactive lymphadenitis		20	58	78
Lymph node tuberculosis			3	3
Total		42	123	165

CS: conventional smear (2007–2011); LBC: liquid-based cytology (2011–2015); SCC: squamous cell carcinoma; DLBCL: diffuse large B-cell lymphoma.

Results

The final and/or pathological diagnosis of the primary and/or lymph node lesion in patients who underwent FNA from a CLN with both CS cytology and LBC are listed in Table I. Out of the total 165 patients that were analyzed from 2007 to 2015, which represents the combined CS and LBC groups, 23 types of malignant diseases formed lesions in the CLN including 37 (22.4%) patients with metastatic carcinoma except for TC, 30 (18.2%) patients with metastatic TC, and 17 (10.3%) patients with ML. Metastasis of head and neck SCC to a CLN was found in 20 (12%) of 165 patients. Diffuse large B-cell lymphoma (DLBCL) was the most common subtype in 12 (70.6%) of 17 ML patients.

Representative cytological features of FNA specimens from CLNs that were processed with LBC were shown including metastatic oropharyngeal SCC (Fig. C-1), metastatic prostate carcinoma (Fig. C-2), metastatic TC (Fig. C-3), and DLBCL (Fig. C-4). Immunocytochemical analyses on sequential cytological preparations of LBC were performed in 10 (34.5%) of 29 patients with carcinomas except for TC for either AE1,AE3 (Figs. C-1b and C-2b), p16 (Fig. C-1c) or CAM5.2 (Fig. C-2c), in 3 (15%) of 20 patients with metastatic TC for either AE1,AE3 (Fig. C-3b) or TTF-1 (Fig. C-3c), and in 6 (46%) of 13 patients with ML for both CD20 (Fig. C-4b) and Bcl-2 (Fig. C-4c). Oropharyngeal SCC metastasizing to the CLN was strongly suspected in a patient without an obvious primary site by physical examination because the carcinoma cells prepared by liquid-based preparation with FNA specimens from CLN were p16 positive as shown in Fig. C-1. Ultimately

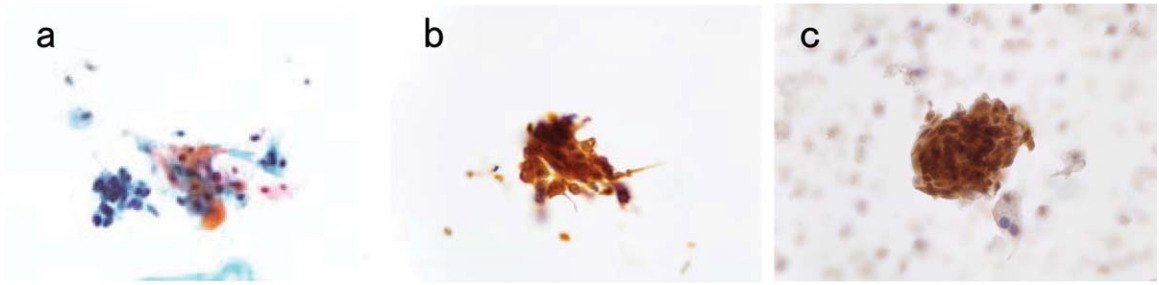

Fig. C-1. Cytological features of LBC with FNA specimens from a metastatic cervical lymph node from oropharyngeal squamous cell carcinoma. (a) Clusters with moderate anaplasia, hyperchromatic nuclei, scanty dense cytoplasm, and orangeophilic cells were observed (Papanicolaou stain). (b) Positive staining was observed for AE1/AE3. (c) Positive staining was observed for p16 (40×).

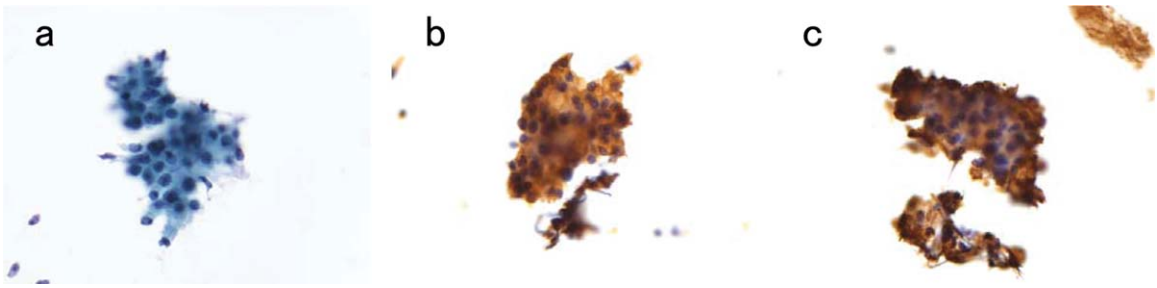

Fig. C-2. Cytological features of LBC with FNA specimens from a metastatic cervical lymph node from prostate carcinoma. (a) Clusters of atypical cells with a polygonal shape and foamy cytoplasm were present (Papanicolaou stain). (b) Positive staining was observed for AE1/AE3. (c) Positive staining was observed for CAM5.2 (40×).

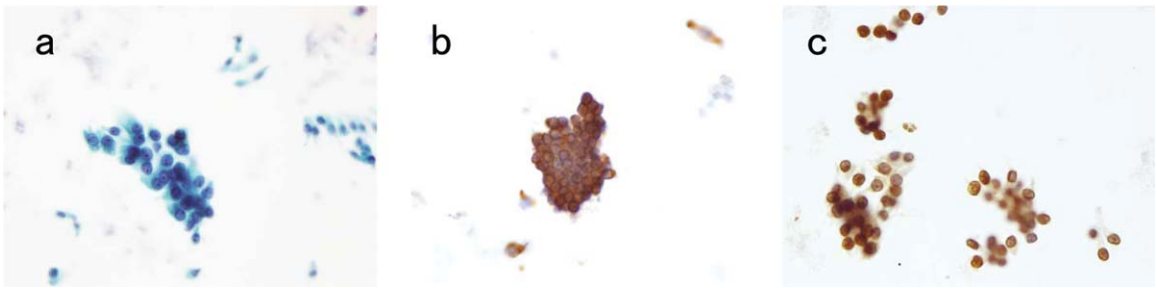

Fig. C-3. Cytological features of LBC with FNA specimens from metastatic papillary thyroid carcinoma. (a) Sheets and papillary structures with irregular and hyperchromatic nuclei, intranuclear grooves, and pseudoinclusion were observed (Papanicolaou stain). (b) Positive staining was observed for AE1/AE3. (c) Positive staining was observed for TTF-1 (40×).

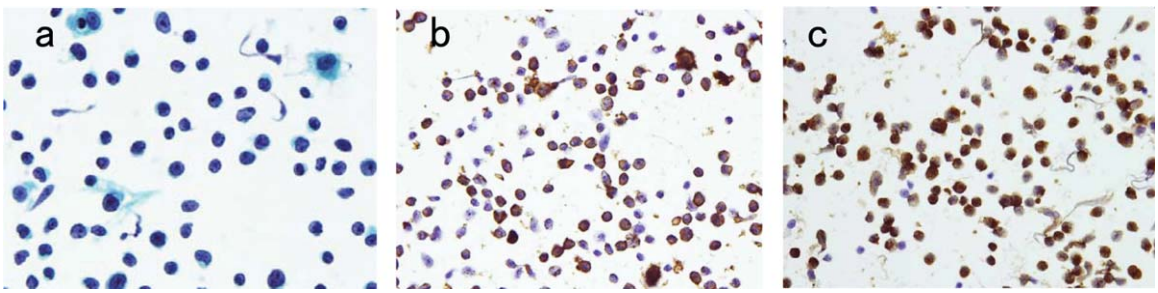

Fig. C-4. Cytological features of LBC with FNA specimens from diffuse large B cell lymphoma. (a) Large size lymphoma cells were present (Papanicolaou stain). (b) Positive staining was observed for CD20. (c) Positive staining was observed for Bcl-2 (40×).

the primary site was pathologically proven from tonsillectomy specimens.

The diagnostic rate of pathological malignancy using CS cytology was 52.4% (Table II), and that of LBC was 50.4% (Table III). In the cytological diagnosis, 11.9% of the CS samples and 7.3% of the LBC samples were considered as nondiagnostic or unsatisfactory samples, and 14.3% of the CS samples and 6.5% of the LBC samples were considered as indeterminate. Conversely, 28.6% of the CS samples and 42.3% of the LBC samples were considered as positive diagnosis. There were no statistically significant differences between the indeterminate and the positive rates for CS cytology and LBC. The overall statistical values including sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of CS cytology were 75%, 100%, 100%, 78.9%, and 87.1%, respectively, whereas the values for LBC were 91.2%, 100%, 100%, 90.7%, and 95.3%, respectively (Table IV). The sensitivity of LBC for malignant diseases tended to be higher than that of CS cytology ($p = 0.081$). The sensitivity of LBC for metastatic TC was significantly higher than that of CS cytology ($p = 0.015$).

Discussion

The CLN is a common and main target of FNA cytology for proper and rapid diagnosis in patients with variable

Table II. Comparison of Histological and Cytological Diagnosis in Patients who Underwent CS Cytology from Cervical Lymph Nodes

		Pathological diagnosis			
		Malignant 22 (52.4%)			*Benign 20 (47.6%)*
Cytotological diagnosis		*Metastatic carcinomas except for TC*	*Metastatic TC*	*ML*	*Reactive*
Nondiagnostic or unsatisfactory	5 (11.9%)				5 [5]
Negative	19 (45.2%)	1	*2*	1	15 [10]
Indeterminate	6 (14.3%)	1	3	2	0
Positive	12 (28.6%)	6	5	1	0
Total		8 (19%)	10 (23.8%)	4 (9.5%)	20 (47.6%)

CS: conventional smear; TC: thyroid carcinoma; ML: malignant lymphoma; []: number of patients diagnosed with clinical findings and imaging studies.

Table III. Comparison of Histological and Cytological Diagnosis in Patients who Underwent LBC from Cervical Lymph Nodes

		Pathological diagnosis				
		Malignant 62 (50.4%)			*Benign 61 (49.6%)*	
Cytological diagnosis		*Metastatic carcinomas except for TC*	*Metastatic TC*	*ML*	*Reactive*	*Tb*
Nondiagnostic or unsatisfactory	9 (7.3%)				9 [9]	
Negative	54 (43.9%)	0	0	5	46 [44]	3 [2]
Indeterminate	8 (6.5%)	1	1	3	3	0
Positive	52 (42.3%)	28	19	5	0	0
Total		29 (23.6%)	20 (16.3%)	13 (10.6%)	58 (47.2%)	3 (2.4%)

LBC: liquid-based cytology; TC: thyroid carcinoma; ML: malignant lymphoma; Tb: lymph node tuberculosis; []: number of patients diagnosed with clinical findings and imaging studies.

carcinomas or ML.[9] CLN enlargement includes a certain frequency of various types of malignant disease. The percentages of patients with malignant disease in reports that analyzed FNA cytology of CLN ranges from 46% to 69.4%.[7,11,23] Our combined results of CS cytology and LBC showed a similar rate of 50.1% of 165 patients analyzed with malignant disease. In addition, our results showed that various malignancies that included 23 types of malignant diseases formed CLN lesions. Garbar et al.[22] reported that 20 types of malignancies formed CLN lesions in only 95 patients analyzed. It may not be well known that thoracoabdominal carcinomas as well as head and neck SCC often metastasize to the CLN.[13] Not only the diagnostic modality and treatment method, but also prognosis differs widely for each malignant disease even if CLN enlargement is the first or only symptom. The most expected role for FNA cytology in analysis of CLN enlargement is the differentiation of metastatic carcinoma and ML from reactive lymphoid hyperplasia. If metastatic carcinoma in the CLN is suspected cytologically, additional information with regard to pathological characteristics and the primary site are useful for further examination. If carcinoma cells are detected in FNA specimens from the CLN in patients who do not show an obvious primary site, then FDG-PET, but not an excisional lymph node biopsy, has to be indicated. Otherwise, if ML is suspected cytologically, an excisional lymph node biopsy and serum interleukin-2 receptor (IL-2R) measurement have to be performed immediately.[9,13] Thus, FNA cytology for analysis of the CLN plays an important role in the diagnosis of human malignant diseases.[7–9,13]

Table IV. Statistical Comparison of CS and LBC in Patients who Underwent FNA from Cervical Lymph Node

	CS (%)	*LBC (%)*	*p-value*
Sensitivity	75	91.2	0.081
Specificity	100	100	—
PPV	100	100	—
NPV	78.9	90.7	0.178
Accuracy	87.1	95.3	0.105

CS: conventional smear; LBC: liquid-based cytology; PPV: positive predictive value; NPV: negative predictive value.
Diagnoses cytologically as indeterminate and nondiagnostic or unsatisfactory were excluded for the statistical analysis.

We had previously diagnosed CLN lesions by FNA with CS cytology until 2011. However, as shown in the results, the nondiagnostic or unsatisfactory rate of CS cytology was thought to be as high as 11.9%. In the literature, the unsatisfactory rate in FNA with CS cytology of CLN ranges from 4.7% to 12%.[22,30] In some of the CS slides we prepared, cytological features such as air-drying

artifacts and unclear background of red blood cells resulted in a higher nondiagnostic or unsatisfactory rate. We have employed the LBC method as a routine examination for CLNs since 2011. Our results showed that the nondiagnostic or unsatisfactory rate for CLN was likely to be reduced from the 11.9% observed with of CS cytology to 7.3% with LBC. Previous reports have shown a diagnostic sensitivity of 83–97% and a specificity of 91–100% in CLN with metastatic carcinoma for FNA with CS cytology.[9,13] Nasuti et al.[30] demonstrated that FNA cytology of lymph nodes located at different anatomic sites could be used effectively for staging a variety of non-lymphoid malignancies, as evidenced by a 94% correlation with the surgical pathology diagnosis. In this study, the sensitivity for metastatic TC to a CLN increased from the 71.4% obtained with CS cytology to 100% with LBC. In addition, the sensitivity for metastatic carcinoma except for TC increased from 86% of the CS to 100% of the LBC. Furthermore overall sensitivity increased from the 75% obtained with CS cytology to 91.2% with LBC. These changes may be a result of the decrease in the nondiagnostic or unsatisfactory and indeterminate rate in the cytological diagnosis and the increase in the malignant (positive) rate due to the easily diagnosable cytological features of LBC in terms of a clearer background, monolayer cell preparation, and good cellular preservation. There has only been a few reports of the use of LBC for CLN.[22,23,31,32] Garbar et al.[22] reported that the accuracy of FNA cytology for general lymph node enlargement was identical between CS cytology and LBC. However, Rossi et al.[23] reported a higher diagnostic value with a sensitivity of 98.6% and a specificity of 100%, of LBC combined with immunocytological analyses in FNA specimens from general lymph nodes compared with CS cytology. Mygdakos et al.[15] showed that LBC was greatly superior to CS cytology in all cases with nongynecological lesions including CLNs.

LBC has been gaining popularity as the method of choice for analysis of nongynecological areas over the past 15 years.[14,33–35] In the LBC method, the two most commonly used systems, which are US Food and Drug Administration (FDA)-approved systems, are ThinPrep™ (Hologic, Marlborough, MA) and SurePath™ (BD Diagnostics. TriPath, Burlington, NC). In the ThinPrep™ system, cells are collected in a methanol-based solution (Cytolyt™) and are then transferred on to a positively charged slide with a gentle positive pressure.[23] In contrast, in the SurePath™, cells are collected in an ethanol-based solution (CytoRich™), centrifuged, and are then slowly sedimented onto a poly-L-lysinated slide.[36] These systems are useful for the occasional processing of a large amount of samples at large-scale medical centers. However, the initial investment cost is high for the purchase of an automated processing machine with a computer-assisted device to obtain a thin layer of cells. LBCPREP2™, which is a recently developed manual LBC system, is just a vial that contains 15 mL of a methanol-based fixation solution. A separate vial is used for each sample and only a centrifuge is required to collect the cells. Therefore, an expensive machine and a computer-assisted device are not required, and this system can be easily employed by small-scale hospitals. We had analyzed and shown that the unsatisfactory or nondiagnostic rate, the sensitivity and specificity of LBC using LBCPREP2™ in the diagnosis of TC were 9.1%, 95.2%, and 100%, respectively (unpublished data). Previous reports have shown a diagnostic sensitivity of 76.3–96.1% and a specificity of 54.9–81% in thyroid nodule with TC for FNA with LBC using either ThinPrep™ or SurePath™ system.[36–39] The unsatisfactory or nondiagnostic rate of the LBC systems ranges from 8.2% to 22.5% in the literature.[34,37,39] Therefore, the diagnostic value of LBCPREP2™ was thought to be comparable to that of other major LBC systems. Some morphological changes are noted in the LBC method using LBCPREP2™ compared with CS cytology including altered, reduced, or lost background material, smaller, and more fragmented cell clusters, smaller cell size, well-preserved nuclear detail, more prominent nucleoli, and more easily visualized cytoplasm. Therefore, cytopathologist should be cautious in interpreting LBC results if this is the only methodology employed. Several morphological differences were shown and observed among LBCPREP2™, ThinPrep™, and SurePath™ preparations which reflect different sampling devices, collection media, and processing techniques.[17,36,40] However, all the LBC methods have common and excellent findings such as reduced incidence of air-drying artifacts, cleaner background, even distribution of cells over a smaller slide area, and increased cellularity.

Immunocytochemical markers of malignant cells in the CLN may aid in delineating the epithelial nature of the malignancy. For example, although up to 80% of oropharyngeal SCC are caused by oncogenic high-risk human papilloma virus (HPV), the detection of HPV in carcinoma cells in the CLN strongly implicates the oropharynx as the primary site.[41] p16 is considered as one of the surrogate markers for high-risk HPV infection, and p16 immunochemistry is the most useful method for detection of high-risk HPV as a routine laboratory examination. We demonstrated that oropharyngeal carcinoma CLN metastasis was suspected in a patient without an obvious primary site in the oropharynx because p16 was expressed in liquid-based prepared carcinoma cells aspirated from the CLN. After the detection of p16 positivity by immunocytochemical analysis, the primary site was pathologically proven from tonsillectomy specimens. Markers for cytokeratin, including whole cytokeratin (AE1/AE3), CK5/6 (D5/16B4), CK7 (OV-TL 12/30), CK20 (Ks20.8), and

CK8/18 (CAM5.2), are useful for focusing on a primary site of epithelial origin.[41] TTF-1 is one of the known markers for TC. In this study, we were able to stain stored cells, prepared by liquid-based processing, with Bcl-2 and CD20, which was useful for the diagnosis of ML. In comparison with CS cytology, LBC is more suitable for immunocytochemical analysis of the expression of several markers because a number of slides can be made, and stored for about 6 months, from cells prepared by liquid-based processing if sufficient amounts of cells are aspirated and collected. LBC specimens were suitable for molecular biological analysis for improving the diagnostic accuracy of malignant tumors. v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations were identified on LBC material from TC with high reproducibility, feasibility, and informative results.[42] LBC specimens were also used to determine the physical status of HPV DNA by in situ hybridization (ISH) and HPV genotyping by polymerase chain reaction (PCR) for identifying patients at high risk of cervical carcinoma[43] and of epidermal growth factor receptor (EGFR) mutation for lung carcinoma.[44] To search for immunoglobulin gene arrangements, using PCR for ML was reported to be possible with LBC specimens.[45] Using LBCPREP2™ specimens, we have already analyzed mRNA expression of several genes by RT-PCR (unpublished data). In the future, the combination of these technologies with cells prepared by liquid-based processing could take the place of surgical lymph node excision.

In conclusion, LBC with FNA specimens using LBCPREP2™ was useful for the diagnosis of metastatic carcinoma to a CLN with a higher diagnostic accuracy than with CS cytology due to a decrease in the unsatisfactory or nondiagnostic and indeterminate rate. The diagnostic value of LBCPREP2™ was almost identical to that of other previously reported major LBC systems.

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