Use of Liquid-Based Cytology (LBC) and Cell Blocks from Cell Remnants for Cytologic, Immunohistochemical, and Immunocytochemical Diagnosis of Malignancy*

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ABSTRACT

Great advances in screening have lowered the death rate from cervical cancer in the advanced countries. The major advances in cervical cancer screening include the Papanicolaou (Pap) test and liquid-based cytology (LBC). In this study, we aimed to use cell remnants from LBC specimens from uterine cervix and endometrium, aspirates from breast and thyroid tumors, and liquid samples (ascites, pleural effusion, and urine). Cell blocks made from cell remnants of LBC specimens were immunohistochemically or immunocytochemically stained for several biomarkers including certain tumor markers such together with hematoxylin and eosin staining for accurate diagnosis of malignancies in different samples. The findings from the cell blocks stained with these biomarkers combined with those from Pap stain led to easily diagnosis of the presence or absence of malignancies. Our findings suggest the utility of LBC and cell blocks from cell remnants in cytologic diagnosis in certain specimens.

Keywords: LBC; Cell Remnants; Cell Block; Immunohistohemistry; Immunocytochemistry; Biomarkers; Cancer; Accurate Diagnosis

1. Introduction

A technique, liquid-based cytology (LBC), enables cells to be suspended in a monolayer. LBC makes better cytological assessment possible with improved sensitivity and specificity, since fixation is better and nuclear details are well preserved in the technique. Preneoplastic and neoplastic cells are not obscured by other cells, such as normal epithelial and inflammatory cells [1-5]. This method involves collection of specimens directly into a liquid fixative, but in the case cervical specimens, a brush-like device, Cervex-brush (Rovers medical devices) is utilized. The brush is used to scrape the cervix. The brush head is then detached and immediately put into a vial containing a special commercial fixative solution, such as SurePath preservative fluid [1-5]. Smears are made from the sediment, stained and cytologically diagnosed. At present, two techniques, Thin Prep (Cytyc Corp.) and SurePap (Tripath imaging, Inc.), have been more widely used for screening and diagnosing for cervical lesions [1-5]. Using LBC, the rate of colposcopic examinations for repeated unsatisfactory conventional Papanicolaou (Pap) smears has fallen from almost 25% to 0.5% and the percentage of unsatisfactory PAP smears has fallen from 13.6% to 1.9% [6]. Thus, LBC has improved unsatisfactory conventional Pap smear rates and give significant benefits to colposcopic examinations. Moreover, LBC technique shortens laboratory turnaround times. The superiority of the quality of LBC in comparison with those of conventional Pap smears has been described [2,7,8]. The sensitivities of the conventional Pap smear and LBC tests are estimated to be 70% - 80% and 85% - 95%, respectively [9]. LBC is currently recommended for cervical cancer screening [10] with a major advantage of allowing ancillary techniques such as those used in immunocytochemistry and molecular biology [11-17]. Cell blocks made from cell or tissue remnants of LBC can also be used for immunocytochemistry or immunohistochemistry of specific biomarkers to accurate diagnosis of malignancy [16-19]. In addition, LBC has currently been used for non-gynecologic cytology [15,17,19-22].

The aim of the present study was to determine whether immunocytochemistry and immunohistochemistry of non-gynecologic and gynecologic LBC specimens and cell-blocks made from the remnant of cells in a brush-like device, which are meant to be discarded after LBC cytology, possesses diagnostic value for malignancies. Eva-
Evaluation was performed on the LBC specimens of urine, breast fine needle aspiration, body fluid (pleural effusions and ascites), cervix, and endometrium.

2. Materials and Methods

2.1. Sample Collection and Preparation
A total of 58 LBC samples that were suspected for proliferative lesions or malignancy were collected for this study. They included 24 gynecological samples (14 cervix and 10 endometrium) and non-gynecological specimens (4 fresh voided urines, 7 breast and 7 thyroid tumors aspirates, 8 ascites and 8 pleural fluid). These were from 58 patients with known or unknown carcinoma, which were obtained from 5 different municipal hospitals and 10 clinics in Gifu City, Japan. All the patients provided informed consent. These SurePath samples were processed using the BD PrepStain™ Slide Processor, BD SurePath™ Cell Enrichment, and BD CytoRich™ System (BD Japan, Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions, and then cytological diagnosis was made on each case.

2.2. Preparation of Cell Blocks
Residual specimens collected in PreservCyt solution from gynecological samples used for thrombin cell block preparation. Cell blocks were prepared primarily according to the method described by Keyhani-Rofagha and Vesey-Schecket [23] and Yang et al., [24]. In brief, the residual SurePath sample was placed in a 50 mL Falcon tube and spun in Beckman Coulter Allegra 6 Centrifuge (Beckman Coulter, Fullerton, Calif) for 10 minutes at 2000 rpm. The supernatant was poured off, which left the small pellet/button in the bottom of the tube. Approximately 5 drops of plasma were added to the cell button to resuspend it. Then approximately 5 drops of bovine thrombin (Fisher Scientific Item # 23-306291; Thermo Fisher Scientific, Waltham, Mass) were added, and the mixture was allowed to stand for 10 minutes. The cell pellet was fixed in 10% formalin for 10 minutes. The cell block materials were transferred to biopsy bags by pouring the contents of the Falcon tube into a biopsy bag over a funnel and beaker. The cell block materials in the biopsy bags were placed into a histology cassettes. The cassettes were processed as routine surgical specimens.

Fresh effusions, including voided urines, ascites, and pleural fluids, were centrifuged and sediments were used to make cell blocks by plasma-thrombin method. Briefly, after decanting supernatant, several drops of plasma and thrombin were added to the sediments to mix by gentle vortex and the mixture was then allowed to clot, followed by fixation with 10% buffered formalin solution for at least 1 hour before being processed for embedding in paraffin blocks.

2.3. Immunohistochemical and Immunocytochemical Stainings
Slides made from cell blocks and LBC specimens were treated in the BenchMark XT Automated Slide Preparation System (Roche Diagnostics Japan, K.K., Tokyo) using a 3,3’-diaminobenzidine kit, and were slightly counterstained with Harris haematoxylin, according to the manufacturer’s instructions. Negative controls were prepared by use of CONFIRM Negative Control Mouse Ig (MOPC-21, Ventana Japan, Co., Ltd., Yokohama City, Japan) and CONFIRM Negative Control Rabbit Ig (Polyclonal, Ventana Japan, Co., Ltd.) exclusion of the primary antibody. Known positive controls were also included. Antibodies for immunocytochemistry and immunohistochemistry used in this study included p16, p53, p63, CK20, Ki-67, CD10, CD45, CD56, estrogen receptor (ER), thyroid transcription factor 1 (TTF-1), carcinoembryonic antigen (CEA), β-catenin, carbohydrate antigen 19-9 (CA19-9), calretinin, and human epidermal growth factor receptor 2 (Her2). Their sources are listed in Table 1.

3. Results
Uterine cervix LBC samples were cytologically diagnosed as 2 ASC-H, 2 HSIL (severe dysplasia), 3 carcinoma in situ (CIS), 5 squamous cell carcinomas (SCCs) or 2 adenocarcinomas. The remnants were obtained from

<table>
<thead>
<tr>
<th>Antibodies</th>
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<td>P16</td>
<td>BD</td>
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<td>P53</td>
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*Diluted by the supplier.*
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the samples suspected of CIS, SCC, and adenocarcinoma. We confirmed their diagnoses on the histologic slides stained with hematoxylin and eosin (H & E) from cell blocks. Endometrial LBC samples were diagnosed atypical 2 endometrial hyperplasias and 8 endometrioid adenocarcinomas. They also were confirmed on their H & E-stained histologic slides from cell blocks. Seven breast tumor aspirates and 7 thyroid tumor aspirates were cytologically diagnosed as breast cancers (6 papillotubular carcinoma and 1 intracystic papillary carcinoma) and papillary carcinomas, respectively on their LBC slides, and the diagnoses were confirmed by histological examination using the respective blocks. All of 4 voided urine LBC samples were cytologically urothelial carcinomas and this diagnosis was confirmed histologically on H & E-stained sections made from the respective blocks. Cytological diagnoses of eight ascites were all adenocarcinoma from malignancies of digestive tissues (5 of colorectal and 3 of stomach cancers). These diagnoses were histopathologically confirmed on H & E-stained histologic sections made from the respective blocks. Eight pleural fluids LBC specimens were cytologically diagnosed as 7 adenocarcinomas from lung cancers and one malignant mesothelioma. The diagnosis of these cases was confirmed by histological investigation on H & E-stained histologic sections made from the respective blocks.

Cytological, histological, immunocytochemical, immunohistochemical findings on some of these cases are shown in Figures 1-6. A case of cervical LBC sample that was not clinically suspected of malignancy showed HSIL, possible CIS (Figure 1(a)). Histopathological examination in the cell block specimen suggested CIS (Figure 1(b)). Cancer cells were immunohistochemically positive for Ki-67 (Figure 1(c)) in their nuclei, β-catenin (Figure 1(d)) in their cell membrane and cytoplasm, and p16 (Figure 1(d)) in their nuclei as well as cytoplasm. An endometrial LBC sample showing atypical endometrial hyperplasia (Figure 2(a)) was finally diagnosed as moderately-differentiated adenocarcinoma (Figure 2(b)) with positive reactivity for ER antibody (Figure 2(c)) in the cell block specimen. An aspirate from the thyroid tumor was cytologically suspected follicular tumor (Figure 3(a)) and subsequent histological examination in the cell block specimen revealed papillary carcinoma (Figure 3(b)) that was positive for TTF-1 (Figure 3(c)). A breast tumor aspirate was cytologically suspicious of papillotubular carcinoma (Figure 4(a)) and this was confirmed by histological examination (Figure 4(b)) and Her2 immunohistochemistry (Figure 4(c)) in the cell block specimen. A LBC specimen from a patient with ascites showed a cluster of atypical glandular cells containing signet-ring cells (Figure 5(a)). In the cell block specimen from this LBC specimen, there were numerous adenocarcinoma cells containing signet-ring cells (Figure 5(b)) with positive reaction for Ki-67 antibody

Figure 1. (a) LBC showed a cluster of atypical squamous cells, suggesting CIS (Pap stain); (b) Histopathology of the cell block tissue from remnant of LBC indicated CIS (H & E stain); Immunohistochemistry of (c) Ki-67; (d) β-catenin; and (e) p16 showed positive reaction for these antibodies. Bars are 10 μm.
Figure 2. (a) Endometrial LBC showed a papillary cluster containing a tubular pattern (arrow), suggesting atypical endometrial hyperplasia (Pap stain, bar = 10 μm); (b) Histopathology of the cell block section from LBC sample indicated moderately-differentiated adenocarcinoma (H & E stain, bar = 50 μm); (c) Endometrial cancer cells on a cell block section were immunohistochemically positive for ER antibody (ER immunohistochemistry, bar = 10 μm).

Figure 3. (a) LBC of thyroid tumor aspirates showed a cluster containing atypical follicular cells, suggesting follicular tumor (Pap stain, bar = 10 μm); (b) Histopathology of the cell block section from the aspirate indicated papillary carcinoma (H & E stain, bar = 50 μm); (c) Thyroid cancer cells on a cell block section were immunohistochemically positive for TTF-1 antibody (TTF-1 immunohistochemistry, bar = 10 μm).

Figure 4. (a) LBC of breast tumor aspirates showed a papillary cluster containing atypical ductal cells, suggesting papillotubular carcinoma (Pap stain, bar = 10 μm); (b) Histopathology of the cell block section from the aspirate confirmed the cytological diagnosis (H & E stain, bar = 50 μm); (c) Breast cancer cells on a cell block section were immunohistochemically positive for Her2 antibody (Her2 immunohistochemistry, bar = 10 μm).

Figure 5. LBC of ascites showed presence of adenocarcinoma with signet-ring cells in the fluids (Pap stain, bar = 10 μm); (b) Histopathology of the cell block section from the LBC sample indicated signet-ring cell carcinoma (H & E stain, bar = 50 μm); (c) Cancer cells on a cell block section were immunohistochemically positive for Ki-67 antibody (Ki-67 immunohistochemistry, bar = 10 μm).
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In conclusion, LBC with immunocytochemistry and cell block sections with immunohistochemistry result in enhanced specimen quality, and accurate diagnosis, and diminished false negative cases. LBC has potential as a screening tool for cancer and precancerous lesions in several tissues other than gynecologic organs. Cell block tissues made from remnants and residual LBC samples, aspirates, and fluid samples may also have applications for practice in the field of cytopathology.

5. Acknowledgements

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