Journal of the American Society of Cytopathology (2016) 5, 227–234

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jascyto.org/

ORIGINAL ARTICLE

Voided urine cytology and low-grade urothelial neoplasia of the bladder: factors that influence the sensitivity

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Received 30 October 2015; received in revised form 16 February 2016; accepted 23 February 2016

KEYWORDS
Voided urine;
Urinary bladder;
Low-grade;
Sensitivity;
LBC;
CytoRich Red

Introduction The aims of this study were to show the 10-year results of voided urine cytology (VUC) performed using liquid-based cytology (LBC) with CytoRich Red and to discuss the factors that influence the sensitivity of low-grade urothelial neoplasia (LGUN) of the urinary bladder.

Materials and method We calculated the sensitivity of VUC in 421 histologically confirmed cases included in the pathology database of Hokkaido Cancer Center in Japan and studied various factors influencing sensitivity.

Results The cumulative sensitivity of VUC was 95.8% in 143 cases of primary high-grade urothelial carcinomas, compared with 59.5% in 74 cases of LGUN. These findings were only slightly different from the previous results of Koss et al. The sensitivity in LGUN, however, showed lower values in some conditions, including in secondary cases, with a lower frequency of examinations and smaller tumor volumes. LBC preparations allowed us to observe a greater number of tumor cells and cell clusters than conventional methods in LGUN cases.

Conclusions The sensitivity of VUC can be improved by increasing the frequency of examinations and adopting a valid preparation method in order to augment the number of cells and cell clusters on individual glass slides. LBC preparations may allow cytopathologists to obtain a better sense for and understanding of the cytologic findings of LGUN.

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2213-2945/$36
http://dx.doi.org/10.1016/j.jasc.2016.02.004

Introduction

Urine cytology has a long history.[1,2] Continual efforts have been made to clarify the significance of urine cytology in the screening and/or follow-up of urinary tract cancers, and cytologists have obtained significant findings in high-grade urothelial carcinoma (HGUC).[3] There has been little success, however, in the diagnosis of low-grade urothelial neoplasia (LGUN) assessed using voided urine cytology (VUC).[4–6]

In this study, we show the 10-year results of VUC performed using liquid-based cytology (LBC) and analyze the factors influencing the sensitivity of VUC, especially in the cases of LGUN. We examined the sensitivities from several viewpoints including primary or secondary cases, histology with or without a flat lesion, frequency of cytologic examinations, tumor size, and number of atypical cells on cytology glass slides.

Materials and methods

We retrospectively analyzed the relationship between the cytology results and histologic findings entered in the pathology database of the Hokkaido Cancer Center from June 2003 to May 2013.

Case selection

Upon searching the pathology database, we selected surgically treated bladder cancer cases that were histologically diagnosed as urothelial carcinoma and were examined by VUC within a month before operation. Cases given re-transurethral resection (re-TUR) shortly after the last TUR of bladder tumor and those treated via total cystectomy were excluded from this study because such cases suffered from serious artifacts associated with final TUR and appeared to be inappropriate for evaluating the relationship between histologically proven urothelial carcinomas and VUC.

We followed the World Health Organization (WHO) histologic classification of tumors of the urinary tract[7,8] and re-evaluated invasive urothelial carcinomas from the standpoint of the histologic grade of noninvasive parts exposed to urine because the 2004 WHO classification does not demand a pathologist to divide invasive urothelial carcinomas into low- and high-grade tumors.

Urine specimens

The specimens included voided urine. Bladder urine samples obtained with a catheter for bacteriologic and cytologic examinations were included in this study because they did not differ cytologically from the voided urine samples, with the exception that the former contained normal urothelial cells freshly removed from the urethral and vesical mucosa. Bladder washing/barbotage specimens were rarely submitted to our laboratory and were thus excluded from the analysis.

Processing of urine samples

All specimens were processed according to the LBC method using CytoRich Red (BD Diagnostic Systems, Burlington, NC 27215), after which most of the specimens were also smeared with Cyto-Tek Centrifuge (Sakura Finetek, Tokyo, Japan). Cyto-Tek Centrifuge is a cell preparation instrument based on analogous principles with Cytospin (Thermo Fisher Scientific, Waltham, MA) that is very popular in the cytology laboratories of Japan.

In our laboratory, urine specimens were processed as follows. Two samples, 10 mL and 15 mL, of urine were collected from the lower part of the cup (which is submitted by the clinician). The former is used for LBC preparation following centrifugation, and the latter is smeared with Cyto-Tek Centrifuge according to the manufacturer's instructions.

In the LBC preparations, 10 mL of urine were centrifuged for 3 minutes at 800*g*, and the supernatant was decanted. Then, 0.5 to 1 mL of CytoRich Red was added to the sediment and allowed to sit for 30 minutes after stirring. Distilled water was subsequently added to the 5- or 10-mL line of the centrifuge tube, and the suspension was centrifuged for 3 minutes at 800*g*. Following decantation of the supernatant, 250 μL of distilled water was added and stirred. The specimen was then transferred with a pipette to the exclusive chamber fixed on a BD SurePath PreCoat slide (BD, Franklin Lakes, NJ) and allowed to sit for 10 minutes. In advance, a glass slide was set on a rack supplied by BD Japan for the manual procedure. A total of 1 mL of 95% ethanol was added to the chamber, and the rack was swung several times in order to adapt the specimen to the ethanol. Following decantation, the same procedure was performed again. The remaining ethanol in the chamber was drained completely, and the glass slide was immediately disconnected from the chamber and inserted into 95% ethanol in order to prevent the cells from drying. Finally, the glass slide was stained according to the Papanicolaou method. The density gradient technique adopted in the BD SurePath method for Papanicolaou tests was not used for urine samples.

Cytologic diagnosis

In all cases, specimen processing and microscopic screening were conducted by certified (Japanese Society of Clinical Cytology) cytotechnologists. Each cytodiagnosis was confirmed by a cytopathologist after discussing the results with the cytotechnologist. The cytology results were reported according to four diagnostic categories: "benign," "atypical," "suspicious," and "malignant."[9] The difference in diagnostic categories was dependent on the probability that malignant cells exist on a glass slide, regardless of cell type or grade. We typically include the description of