The Application of the Johns Hopkins Template on Liquid-Based (SurePath™) Urine Cytology Samples

Running Title: Hopkins Template for Urine Cytology

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Abstract

Background: To evaluate the utility of the Johns Hopkins Hospital Template in detection of high-grade urothelial carcinoma. **Methods:** A computerized search of our laboratory information system was performed for 2009 through 2011 for all urine cytology cases processed by the SurePath™. We included only cases with correlating surgical pathology within 6 months after the urinary samples were obtained. The original cytologic diagnoses were reclassified according to the Johns Hopkins Template and these cytologic diagnoses were then correlated with the followup surgical pathology diagnoses. **Results:** A total of 273 urine samples with histopathologic followup were identified. The reclassified cytologic diagnoses included negative for urothelial atypia or malignancy (NUAM) 110; atypical urothelial cells of undetermined significance (AUC-US) 83; atypical urothelial cells, cannot exclude high-grade urothelial carcinoma (AUC-H) 49; high-grade urothelial carcinoma (HGUC) 29; and low-grade urothelial carcinoma (LGUC) 2. More than one-half of patients (58%) who had biopsy-confirmed high-grade urothelial lesions had a preceding cytologic diagnosis of AUC-H or HGUC. AUC-H and HGUC are associated with high-grade urothelial lesions in 80% and 90% of the cases and show statistical significance when compared with AUC-US or NUAM ($P<0.05$). **Conclusion:** The Hopkins Template is useful and effective in identifying patients with high-grade urothelial lesions who need to undergo cystoscopy.

Keywords: urine; cytology; Johns Hopkins Template; urothelial carcinoma; SurePath

Introduction

Given its high sensitivity and specificity for the detection of high-grade urothelial tumors, urinary cytology continues to play an important role in the management of patients with urothelial carcinoma. Urinary cytology nicely compliments cystoscopic examination, a method that detects most low-grade urothelial tumors. Over the decades, several classification schemes for reporting urine cytology have been proposed and the nomenclature has evolved in accordance with changes in the histologic classification of the bladder cancers.[1-5] However, unlike cervical cytology, there has not been widespread acceptance and use of any particular reporting scheme for urine cytology studies. The Johns Hopkins Hospital Template for Reporting Urine Cytology is the foundation for the newly proposed "Paris classification for reporting urine cytology" which has proposed 7 diagnostic categories for urine cytology samples, including negative for urothelial atypia or malignancy (NUAM); atypical urothelial cells of undetermined significance (AUC-US); atypical urothelial cells, cannot exclude high-grade urothelial carcinoma (AUC-H); high-grade urothelial carcinoma (HGUC); low-grade urothelial carcinoma (LGUC); other (squamous carcinoma, adenocarcinoma, etc.); and inadequate (**Table 1**).[6,7] The aim of this study is to examine the applicability of this recently published diagnostic nomenclature.

Material and Methods

This study was approved by Indiana University Institutional Review Board (protocol number 1401456334). A computerized search of our laboratory information system was performed for 2009 through 2011 for all urine cytology cases processed by the SurePath (BD-TriPath Imaging, Burlington, NC) liquid-based preparation technique. Only cases with correlating surgical pathology samples (biopsies and/or resection specimens) obtained within 6 months after the

urine cytology samples were included in the study. If there were multiple urine samples from the same patient before the biopsy, only the worst urine cytology diagnosis was included for analysis. In our institution, urinary cytology was reported using the descriptive term such as "no atypical cells identified," "atypical urothelial cells," "suspicious for urothelial carcinoma," "low-grade urothelial carcinoma," "high-grade urothelial carcinoma," etc. The original reports were transcribed into the Johns Hopkins Template. For example, "no atypical cells identified" cases were assigned to NUAM category, "atypical urothelial cells" were assigned to AUC-US category, and "suspicious for urothelial carcinoma" were assigned to "AUC-H." The original cytologic slides were then re-reviewed by first author HHW and, based on the proposed cytologic criteria; they were reclassified according to the Hopkins Template.[7] The urothelial cells demonstrating nuclear features of hyperchromasia, irregular nuclear membrane, high nucleus-to-cytoplasm ratio, and anisonucleosis were classified as either AUC-H or HGUC depending on the quantity or quality of the cells. When the atypical cells did not show cytologic features of high-grade urothelial carcinoma and were not consistent with normal or reactive urinary tract elements they were classified as AUC-US. These cytologic diagnoses were then correlated with the followup surgical pathology diagnoses. This study was blinded to the patients' clinical history and outcome.

Statistical Methods

We used 2x2 tables to calculate sensitivity, specificity, positive predictive value, and negative predictive value. Fisher exact tests were used to evaluate statistical significance and two-tailed tests were used with P values ≤ 0.05 being considered statistically significant (IBM SPSS V.19.0, 2010).

Results

A total of 273 urine samples with histopathologic followup were identified from 72 female and 201 male patients ranging in age from 4 to 95 years with a mean age of 64. There were 220 voided urine and 53 catheterized urine specimens. All urinary samples were prepared using the liquid-based SurePath method. After converting all of our original urine cytologic diagnoses into the Johns Hopkins Template nomenclature, we re-reviewed the SurePath slides of each case and reclassified the diagnoses into the appropriate category based on the proposed cytologic criteria. We upgraded NUAM to AUC-H in 4 cases and to AUC-US in 6 cases. In addition, 7 cases of AUC-US were upgraded to AUC-H. The majority of the original diagnoses remained unchanged. The final reclassified cytologic diagnoses included NUAM 110, AUC-US 83, AUC-H 49, HGUC 29, and LGUC 2. The followup histopathologic diagnoses for NUAM included benign nonneoplastic (BNN) 90, HGUC 15, LGUC 1, and nonurothelial cancers 4 (1 small cell carcinoma, 1 squamous carcinoma of cervical origin, 1 prostatic adenocarcinoma, and 1 leiomyosarcoma). For AUS-US, surgical pathology followup included BNN 32, HGUC 33, LGUC 12, and nonurothelial cancers 6 (2 adenocarcinomas of the urinary bladder, 2 small cell carcinoma and 2 metastatic colonic adenocarcinomas). For AUC-H, the followup included BNN 7, HGUC 39, LGUC 1, and nephrogenic adenoma 2. For LGUC, 2 cases diagnosed on urine cytology were confirmed by the followup surgical pathology. For HGUC, histopathologic followup included BNN 3 and HGUC 26. The rate of malignancy for NUAM, AUC-US, AUC-H, and HGUC was 18%, 61%, 82%, and 90% respectively (**Table 2**). More than one-half of patients (58%) who had biopsy-confirmed HGUC had a preceding cytologic diagnosis of AUC-H or HGUC. When patients with AUC-US were added to the analysis, 87% of patients with HGUC had at least one abnormal urinary cytology result. If we include AUC-US as abnormal

results, the sensitivity, specificity, positive predictive value, and negative predictive value for detection of cancer is 86%, 67%, 73%, and 82% respectively (**Table 3**); but if we exclude AUC-US and then the sensitivity, specificity, positive predictive value, and negative predictive value become 49%, 91%, 85%, and 63% (**Table 4**). Using a set of well-defined cytologic criteria for high-grade urothelial lesions such as hyperchromasia, irregular nuclear membrane, increased nucleus-to-cytoplasm ratio, and anisonucleosis, we found a high degree of association of AUC-H and HGUC with a histologic diagnosis of high grade urothelial carcinoma at 80% and 90%, respectively, versus 40% for AUC-US and 14% for NUAM ($P<0.05$).

Discussion

Urine cytology is a useful, inexpensive test for screening patients with hematuria or at risk for urothelial carcinoma. It has also been used for the surveillance of patients with urothelial carcinoma. The advantages of urine cytology include ease of obtaining the specimens and high sensitivity and specificity in diagnosing high-grade urothelial lesions.[8] The most important indicator for the surgeons to have a patient undergo cystoscopy is a cytologic diagnosis of HGUC and the Johns Hopkins Hospital Template was designed for the purpose of targeting those patients who need cystoscopy.[6] In our study, there was a high degree of association of AUC-H and HGUC with a histologic diagnosis of high-grade urothelial carcinoma at 80% and 90%.

For 16 cases of low-grade urothelial lesions diagnosed by followup biopsies, only 2 of 16 were correctly diagnosed as LGUC by urine cytology, while 12 were diagnosed as AUC-US, one as AUC-H, and one as NUAM . The cytologic features of LGUC include papillary-like clusters, irregular nuclear membrane, increased nucleus-to-cytoplasm ratio, and homogenous cytoplasm; however, these features are overlapping with reactive changes secondary to calculi,

inflammation, and instrumentation that contribute to the difficulty in diagnosing LGUC by urine cytology.[9] The two cases of LGUC diagnosed in our study relied on the cell block preparation in which well-defined papillary clusters with fibrovascular core were easily identified.

In our study, none of the nonurothelial cancers including 2 adenocarcinomas of the urinary bladder, 3 small cell carcinoma, 2 metastatic colonic adenocarcinomas, 1 squamous carcinoma of cervical origin, 1 prostatic adenocarcinoma, and 1 leiomyosarcoma were diagnosed by urine cytology. The corresponding urinary cytologic diagnoses of these 10 cases were NUAM in 4 cases and AUC-US in 6 cases. All of the urine samples of these cases were voided urines. For cases with the presence of atypical cells in the urine cytology, the number of atypical cells was scant and they were not diagnostic of malignancy.

The AUC-US diagnostic category encompasses a heterogenous group of lesions. In our study, 40% were HGUC, 39% were benign nonneoplastic and 21% showed LGUC or other nonurothelial cancers including adenocarcinoma of the urinary bladder, small cell carcinoma, and metastatic colonic adenocarcinoma. In contrast, the cytologic diagnoses of AUC-H and HGUC are highly associated with high-grade urothelial lesions in the urinary tract. None of the nonurothelial cancers and only one LGUC were diagnosed in the followup cases of AUC-H or HGUC. If we considered AUC-US as negative, the sensitivity, specificity, positive predictive rate, and negative predictive rate for detecting cancer would be 49%, 91%, 85%, and 93% versus 86%, 67%, 73%, and 82%, respectively, if we included AUC-US as an abnormal result. The study by Brimo et al also showed a similar pattern of sensitivity and specificity of 46.3% and 85%, respectively, if AUC-US was considered as negative and 82% and 45.4% if AUC-US was considered as an abnormal result.[10]

The cytologic features of HGUC were well preserved with the liquid-based SurePath preparation.[11] In our laboratory, the most important cytologic feature of high-grade urothelial lesions, hyperchromasia, was well visualized on the SurePath slides that also contributed to the high correlation of AUC-H and HGUC with high-grade urothelial lesions in the followup biopsies.

The Johns Hopkins Template for Reporting Urinary Cytology was created for targeting patients with high-grade urothelial lesions who need to undergo cystoscopy. We have confirmed the effectiveness of using this template for the interpretation of urinary cytology samples that were prepared by the liquid-based SurePath method. In this study, the urine cytology diagnosis of AUC-H or HGUC is highly associated with a histologic diagnosis of high-grade urothelial lesions and shows statistical significance ($P<0.05$) when compared with the diagnosis of AUC-US. We agree that the diagnosis of AUC-H and HGUC warrants a cystoscopic examination, but the diagnosis of AUC-US was also noted to have cancers in 61% of the followup biopsies that also requires close clinical correlation and followup.

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Table 1 Diagnostic categories of the Johns Hopkins Template for urinary tract cytologic samples

NUAM	No urothelial atypia or malignancy identified
AUC-US	Atypical urothelial cells of uncertain significance
AUC-H	Atypical urothelial cells, cannot exclude HGUC
HGUC	High-grade urothelial carcinoma
LGUC	Low-grade urothelial carcinoma
Other	Squamous carcinoma, adenocarcinoma, etc.
Inadequate	Absence of urothelial cells or washing with low cellularity, etc

Table 2 Followup histologic correlation of urinary cytology cases using the Hopkins Template

Hopkins Template Followup Histology	NUAM (*n*=110)	AUC-US (*n*=83)	AUC-H (*n*=49)	HGUC (*n*=29)	LGUC (*n*=2)	Total (*n*= 273)
BNN	90	32	7	3		132
HGUC	15	33	39	26		113
LGUC	1	12	1		2	16
Cancer – Others	4	6				10
Nephrogenic adenoma			2			2
Malignancy Rate	18%	61%	82%	90%	100%	51%
HGUC Rate	14%	40%	80%	90%	0%	41%

BNN, Benign nonneoplastic; AUC-H, atypical urothelial cells, cannot exclude high-grade urothelial carcinoma; AUC-US, atypical urothelial cells of undetermined significance; HGUC, high-grade urothelial carcinoma; LGUC, low-grade urothelial carcinoma; NUAM, negative for urothelial atypia or malignancy

Table 3 Urine cytology includes AUC-US as abnormal.

Urine Cytology Followup Histology	Negative (NUAM) (*n*=110)	Positive (AUC-US + AUC-H + HGUC + LGUC) (*n*=163)
Malignant	20	119
Benign	90	44
Sensitivity	86%	
Specificity	67%	
Positive predictive value	73%	
Negative predictive value	82%	

AUC-H, atypical urothelial cells, cannot exclude high-grade urothelial carcinoma; AUC-US, atypical urothelial cells of undetermined significance; HGUC, high-grade urothelial carcinoma; LGUC, low-grade urothelial carcinoma; NUAM, negative for urothelial atypia or malignancy

Table 4 Urine cytology includes AUC-US as negative.

Urine Cytology Followup Histology	Negative (NUAM + AUC-US) (*n*=193)	Positive (AUC-H + HGUC +LGUC) (*n*=80)
Malignant	71	68
Benign	122	12
Sensitivity	49%	
Specificity	91%	
Positive predictive value	85%	
Negative predictive value	63%	

AUC-H, atypical urothelial cells, cannot exclude high-grade urothelial carcinoma; AUC-US, atypical urothelial cells of undetermined significance; HGUC, high-grade urothelial carcinoma; LGUC, low-grade urothelial carcinoma; NUAM, negative for urothelial atypia or malignancy