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Original Article

Performance characteristics of urinary cytology in patients presenting with gross and microscopic hematuria

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Abstract: Purpose: Hematuria investigations presently entail cystoscopy with upper urinary tract imaging albeit without use of urinary biomarkers including cytology. The purpose is to investigate the performance characteristics of urinary cytology in a population of patients presenting with gross (GH) and microscopic (MH) hematuria. Methods: Records for 409 consecutive patients undergoing a complete hematuria evaluation (cystoscopy with upper-tract imaging) who also had urinary cytology were reviewed. Performance characteristics (sensitivity, specificity, PPV, NPV) of cytology for urothelial malignancy were determined. For those with urothelial cancer, the predictive value of a positive cytology for high grade and high stage urothelial cancer was determined. Results: 29 of 409 patients (7.1%) were diagnosed with urothelial carcinoma including 24 (9.2%) and 5 (3.4%) from the GH and MH populations, respectively. Eighteen (62%) of these tumors were high grade of which 5 (28%) were muscle-invasive. The performance characteristics of cytology for urothelial malignancy included a sensitivity of 41%, specificity of 99%, PPV of 75%, NPV of 96%, and diagnostic accuracy of 95%. No observed differences were noted when comparing gender (P=0.55), type of hematuria (P=0.37), or smoking history (P=0.22). For those diagnosed with urothelial malignancy, a positive cytology was not associated with higher grade (P=1.0) or stage tumors (P=0.62). Conclusions: Urine cytology had low sensitivity and PPV for urothelial carcinoma irrespective of smoker status, hematuria type, or gender. These data support the 2020 AUA Microhematuria Guideline emphasizing that urine cytology should not routinely be used in a hematuria screening population.

Keywords: Hematuria, bladder cancer, urothelial cancer, urinary cytology

Introduction

Hematuria, originating from the Greek words *haima* (blood) and *ouron* (urine), is defined by the evidence of blood in the urine and is subdivided into gross, or macroscopic, hematuria (GH) or microscopic hematuria (MH) [1]. Both GH and MH can be caused by an array of urological or nephrological etiologies including (but not limited to): malignancy, infection, urolithiasis, trauma, glomerular causes or benign prostatic hyperplasia [2].

The risk of urinary tract malignancy in a hematuria cohort is approximately 10%, ranging from 3% in the MH population to almost 15% in patients with GH [3]. Factors associated with genitourinary malignancy include advanced age, male sex, smoking, occupational exposure to chemicals, or pelvic irradiation [4]. Owing to the potential risk of cancer, consensus guidelines statements recommend systematic evaluation although variability exists with respect to the type of extent of investigation [5]. In general, however, the core components of hematuria evaluations entail cystourethroscopy with abdominal and pelvic imaging.

The role of urinary cytology in the initial evaluation of hematuria patients has previously been investigated. A prospective study evaluated the role of cytology in a cohort of over 2700 microhematuria patients investigated between 1999 and 2007 [6]. This study noted that cytology had 45.5% sensitivity and 89.5% specificity for urothelial cancer (UC). A more recent study

Table 1. Categories for the Paris criteria for reporting urinary cytology

Category Number	Cytology Description
1	Non-diagnostic or unsatisfactory
2	Negative for high-grade urothelial carcinoma
3	Atypical urothelial cells
4	Suspicious for high-grade urothelial carcinoma
5	High-grade urothelial carcinoma
6	Low-grade urothelial carcinoma
7	Other: primary and secondary malignancies and miscellaneous lesions

Table 2. Clinical and demographic characteristics of 409 patients

Variable	Number of Cases (%)
Median Age (range)	61 (19-98)
Gender	
Male	267 (65)
Female	142 (35)
Smoking history	
Yes	206 (50)
No	203 (50)
Race	
Caucasian	331 (81)
Other	78 (19)
Hematuria	
Gross (macroscopic)	260 (64)
Microscopic	149 (36)
Urothelial Carcinoma	
Bladder	29 (7)
Upper-tract	0 (0)

from the Detect I collaboration reported on a subset of hematuria patients with cytology data (16% of entire cohort) and noted a sensitivity 43.5%, specificity 95.7%, positive predictive value (PPV) 47.6% and negative predictive value (NPV) 94.9% for UC [7].

Limitations of these prior studies, however, include (1) older data sets using different conventions of cytology reporting schemes; (2) multi-hospital collaborations lending variability in pathologic interpretation; and (3) subset analysis of larger hematuria cohorts with potential selection bias of those patients having cytology performed. Therefore, the objective of our study was to investigate the performance characteristics of urinary cytology for UC in a cohort of patients undergoing complete hematuria evaluation for GH or MH at a single institution using a standardized contemporary reporting system [8].

Materials and methods

The charts of consecutive patients undergoing a complete hematuria evaluation (cystoscopy with upper-tract imaging) by a single surgeon who also had a urinary cytology performed were reviewed. Upper-tract imaging in all patients entailed either a computed tomography (CT) or magnetic resonance (MR) urogram. All patients undergoing a cystoscopic evaluation had a urinary cytology collected. Collections occurred on the day of the cystoscopic procedure prior to instrumentation via a voided specimen. Urinary cytology was collected in all patients irrespective of symptoms, degree of hematuria as part of two prospectively accruing urinary biomarker trials (IRB # 9914 and 15942). Cytologic interpretation was performed by dedicated genitourinary cytopathologists. The Paris System for reporting urinary cytology was used (**Table 1**) [8-10]. The inclusion criteria were patients with a presenting symptom of gross or microscopic hematuria on a quantitative urinalysis who received both upper urinary tract imaging as well as cystoscopic evaluation. The exclusion criteria were: patients with non-diagnostic or unsatisfactory samples (group 1) and those with non-urothelial lesions (group 7). This yielded a final cohort of 409 patients. Additionally, patients with cytology negative for high-grade urothelial carcinoma (group 2) and atypical urothelial cells (group 3) were classified as negative in our analysis. Conversely, cytologies reporting suspicious (group 4), low-grade (group 5), or high-grade (group 6) carcinoma were classified as positive.

All variables were summarized prior to analysis to determine their distributions. A Chi-square test was used to look for associations between patient characteristics and bladder cancer diagnosis (**Table 2**). Test performance measures including sensitivity, specificity, positive predic-

Table 3. Cohort characteristics stratified by bladder cancer diagnosis

Variable	Bladder Cancer	No Bladder Cancer	*P* value
Median Age (range)	76 (49-88)	60 (19-98)	<0.001
Gender			0.004
Male	26 (10)	241 (90)	
Female	3 (2)	139 (98)	
Smoking History			0.02
Yes	21 (10)	185 (90)	
No	8 (4)	195 (96)	
Race			1.00
Caucasian	24 (7)	307 (93)	
Other	5 (6)	73 (94)	
Hematuria			0.03
Gross	24 (9)	236 (91)	
Microscopic	5 (3)	144 (97)	
Overall	29	380	

tive value, negative predictive value, and accuracy were calculated overall and by hematuria type, gender, and smoking history for urinary cytology for urothelial malignancy. A Chi-square test was used to compare the sensitivity between the hematuria types, genders, and smoking history groups. A significance level of 0.05 was used for all statistical analyses which were performed using SAS software 9.4 (SAS Institute, Cary, NC).

Results

Table 2 highlights the clinical characteristics of our cohort. 267 men and 142 women with a median age of 61 (range, 19 to 98 years) were included in this study. Approximately 80% of the cohort was Caucasian and 50% noted a smoking history with a median pack year of 25. 260 patients (64%) underwent evaluation for GH, while 149 for MH. Overall, 29 patients (7.1%) were diagnosed with urothelial carcinoma all of which were bladder cancer. Eighteen (62%) of these tumors were high grade of which 5 (28%) were muscle-invasive. Specific distribution of tumors included 11 low grade (LG) Ta, 5 high grade (HG) Ta, 5 HG T1, 5 HG T2, and 3 carcinoma in situ (CIS).

Table 3 summarizes the cohort characteristics stratified by bladder cancer diagnosis. Consistent with prior publications, a bladder cancer diagnosis was associated with older patient age (P<0.001), male gender (P=0.004), smoking history (P=0.02), and gross (vs. microscopic) hematuria (P=0.03).

Sixteen of 409 (3.9%) patients had a positive cytology at evaluation. The performance characteristics of cytology for urothelial malignancy in the entire cohort included a sensitivity of 41%, specificity of 99%, positive predictor value (PPV) of 75%, negative predictor value (NPV) of 96%, and diagnostic accuracy of 95% (**Table 4**). No statistical differences were noted when comparing gender (P=0.55), type of hematuria (P=0.37), or smoking history (P=0.22).

For patients diagnosed with urothelial malignancy, positive cytology was observed in 9/24 (38%) patients with non-muscle invasive cancer and 3/5 (60%) patients with muscle invasive bladder cancer (P=0.62). With respect to tumor grade, positive cytology was noted in 5/11 (45%) with low grade bladder cancer and 7/18 (39%) with high grade bladder tumors (P=1.0). Finally, 2 of 3 patients (67%) with CIS had a positive cytology.

Discussion

We investigated the performance characteristics of urinary cytology for UC in hematuria patients undergoing complete evaluation. Our study is unique in that it is comprised of a contemporary cohort of GH and MH patients whose cytology was interpreted by dedicated genitourinary cytopathologists using the standardized Paris grading system. We observed that urine cytology had low sensitivity (45.5%) and PPV (75.0%) for urothelial carcinoma irrespective of smoker status, hematuria type, or gender. These results are concordant with some prior studies and further support the 2020 American Urological Association and the Society of Urodynamics (AUA/SUFU) Microhematuria Guideline indicating that urine cytology should not be used routinely in the initial diagnostic algorithm [2].

Prior studies have also attempted to evaluate the clinical utility of urinary cytology in patients presenting with hematuria. Compared to our study, these studies are older and, in general, comprised of smaller patient cohorts than our analysis. Urine cytology that was positive or highly suspicious for malignancy was reported in 76 patients from 1987 to 1995 [11]. All

Table 4. Predictive characteristics of urinary cytology for urothelial malignancy in the entire cohort and subgroups

	Sensitivity (%)	Specificity (%)	Positive Predictor Value (%)	Negative Predictor Value (%)	Accuracy (%)
Hematuria					
Gross	46	98	73	95	97
Microscopic	20	100	100	95	99
Gender					
Male	39	100	91	94	94
Female	67	98	40	99	97
Smoking History					
Yes	33	100	88	93	93
No	63	99	63	99	97
Overall	41	99	75	96	95

patients subsequently underwent cystoscopy, bladder biopsies and radiographic studies of the upper tracts. Of the 76 patients with positive urine cytology, only 9 had UC at initial work-up, while 5 were diagnosed with UC at a median follow-up of 97 months. They reported a sensitivity (77%), specificity (31%), PPV (13%) and NPV (91%) of cytology. Although the performance characteristics varied respectively in comparison to our findings, the authors also concluded that in patients without a history of UC, the diagnostic value of cytology is insignificant and not cost effective to be included as part of the routine work-up of UC.

1000 consecutive patients who were examined with cytology, upper tract imaging and flexible cystoscopy, were prospectively studied from 2003 to 2004 presenting with hematuria [12]. Of the 986 samples sent for cytology, 126 came back abnormal, 71 of which were found to have UC after flexible cystoscopy. Three had upper tract UC diagnosed radiologically. No cases of UC were diagnosed on the basis of urine cytology alone and the cost for cytology and additional investigations totaled $68,495.90. They concluded that urine cytology does need not be used routinely in the initial diagnostic workup for hematuria. Thus, the financial burden of urinary cytology on the healthcare system in addition to its unreliable performance characteristics demonstrates that it does not need to be routinely used in a hematuria screening.

152 patients greater than 50 years of age from 2010 to 2012 who presented with hematuria and a suspected lesion of UC on ultrasound were studied [13]. Urinary cytology, bladder biopsy and cystoscopy, reported as positive, negative or suspicious, were obtained. 133 (87.5%) patients had UC in histopathological examination. The sensitivity of cytology was 53.4% and only 5 patients had suspicious cystoscopy findings. The percentage of positive cytology was highest among patients with gross hematuria (51.3%), posterior wall lesions (75%), papillonodular configuration (81.8%) and invasive cancer (59.1%). They also concluded that cytology did not add any more significant information in this group of patients, which we also agree with. In our study, for those diagnosed with urothelial malignancy, a positive cytology was not associated with higher grade (P=1.0) or stage tumors (P=0.62). Instead, we recommend the use of urinary cystoscopy with more severe urothelial carcinomas due to its higher sensitivity and specificity in patients.

Recently, newer bladder tumor markers such as bladder tumor antigen (BTA), nuclear matrix proteins (NMP) and fibrinogen degradation products (FDP) have been investigated as potential screening tools. However, they share lower specificities, higher false positive rates and variable improvements in sensitivities compared to traditional urine cytology [14]. In one study investigating NMP in 79 patients, the sensitivity and specificity of NMP were 55.7% and 85.7%, respectively compared to 15.8% and 99.2%, respectively of cytology [15, 16]. In 2 studies investigating BTA, only the specificities of 69% and 73% could be recorded because no malignancies were detected in urine specimens [17]. In 2001, the FDA approved a fluorescence in situ hybridization (FISH) assay called UroVysion that detects chromosomal 3, 7, and 17 aneuploidies and the loss of the 9p21 locus of the P16 tumor suppressor gene consistent with bladder cancer in urine specimens of patients with MH [18]. Three studies reported the average UroVysion sensitivity ranging from 61% to 100% and specificity ranging from 71% to 93% for the detection of all grades and stages of UC

[19]. Compared to our sensitivity and specificity of 41% and 99%, respectively, in urinary cytology, these new urinary biomarkers potentially demonstrate more utility as a screening tool in the clinical evaluation of patients presenting with hematuria. However, more research is necessary in evaluating these biomarkers as they currently lack sufficient reliability to be used routinely. Future prospective studies may better define the role of cytology or newer urinary biomarkers in the hematuria screening population.

Recently, prospective trials present some novel areas of investigation to integrate biomarkers into the hematuria algorithm. For example, a randomized, two-arm clinical trial that uses a multiplexed molecular biomarker test called Cxbladder Triage currently is accruing to evaluate UC in 600 adult participants presenting with hematuria in the United States and Canada [2]. The primary outcome measure includes the increased utility, defined by the reduction in cystoscopy procedure count 6-months post-trial, between active and control arms when Cxbladder is used. Secondary outcome measure includes the performance characteristics of the Cxbladder test compared with that of cytology and the total anxiety and pain score of Cxbladder compared with cystoscopy using a patient-reported outcome questionnaire. Such a trial will present data regarding the merits of biomarkers to better screen patients presenting with hematuria.

Limitations of our study include: (1) the study is retrospective and biases associated with this design are inherent; (2) although cytology was interpreted by dedicated cytopathologists, there is potential variability in application of the Paris criterion between individual clinicians; (3) our cohort originates from a single medical center and observations may not be generalizable across a larger cohort. Nonetheless, our data is contemporary compared to prior UC cytology analyses with more robust application of a standardized system than previously utilized. Further studies comparing the performance characteristics of novel biomarkers with urinary cytology may help shed more light on the optimal diagnostic tool in patients presenting with hematuria.

Conclusion

Urine cytology had low sensitivity and PPV for urothelial carcinoma. These data support the 2020 AUA Microhematuria Guideline emphasizing that urine cytology should not routinely be used in a hematuria screening population. Future prospective studies may better define the role of cytology or newer urinary biomarkers in the hematuria screening population.

Disclosure of conflict of interest

None.

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