NMP-22, urinary cytology, and cystoscopy: a 1 year comparison study

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Introduction: *Bladder cancer diagnosis and surveillance is costly and frequent. Urinary cytology is used with cystoscopy in the diagnosis and surveillance of bladder cancer with little evidence to support this practice. Nuclear Matrix Protein-22 (NMP-22) is a marker of urothelial cell death and is elevated in the urine of patients with bladder cancer. Our study compares the performance of NMP-22, urinary cytology and office cystoscopy when utilized in a Veteran Affairs urology practice for 1 year.*
Materials and methods: *A total of 391 consecutive office cystoscopy procedures performed over 1 year were included in the study. NMP-22 and cytology were performed on the urine specimens of patients presenting for cystoscopy. Tumor resection/bladder biopsy was performed when cystoscopy, NMP-22 or urinary cytology were abnormal.*
Results: *Cystoscopy, NMP-22, and urinary cytology data were available in 351 encounters and 69 tumor resections were performed. Urothelial carcinoma bladder (UCB) was identified in 37 bladder specimens. NMP-22, urinary cytology and cystoscopy demonstrated sensitivity and specificity of (51%/96%), (35%/97%), and (92%/88%), respectively. NMP-22 cost $8,750 in the study group and urinary cytology cost $52,500 in the same group.*
Conclusions: *This study demonstrates cystoscopy was the most sensitive test in the diagnosis of UCB. NMP-22 had a higher sensitivity than urinary cytology and similar specificity to cytology. Additional urinary marker testing has a limited role in the management of bladder cancer in the office setting. When adjunct testing is desired in the diagnosis and surveillance of bladder cancer, NMP-22 is a cost effective alternative to urinary cytology.*

Key Words: urinary cytology, cystoscopy, bladder cancer, NMP-22

Introduction

An estimated 69250 adults were diagnosed with bladder cancer in the United States in 2011.[1] Bladder cancer is the fifth most common malignancy in the United States.[1] The diagnosis of urothelial carcinoma bladder (UCB) is based upon cystoscopy and tumor resection. Some practitioners utilize an adjunct test such as urinary cytology. After initial diagnosis, patients with non-invasive bladder cancer require regular follow up for early detection of recurrence and/or progression. Current recommendations in the United States for patients with non-invasive UCB are to undergo surveillance cystoscopy every 3 months for 2 years after initial diagnosis, then cystoscopy every 6 months for the next 2 to 3 years followed by annual cystoscopy.[2,3] European Association of Urology follow up guidelines differ in the frequency of cystoscopy which is based on risk of recurrence.[4] Surveillance for UCB includes urinalysis, cystoscopy, urine cytology, and bladder biopsy/tumor resection when indicated. Due to the burden of frequent surveillance and subsequent treatment, UCB management is costly.[5]

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The high cost of UCB diagnosis and surveillance has generated significant interest in cost saving measures. One method to decrease cost of UCB surveillance and diagnosis would be the substitution of urine cytology with a less expensive urine-based molecular marker, such as Nuclear Matrix Protein-22 (NMP-22). NMP-22 has been shown to be elevated in the urine of patients with UCB.[6] NMP-22 costs $25 per test in our hospital and urinary cytology costs $150 per test. The NMP-22 BladderChek test (Martitech Inc., Newton, MA, USA) is an FDA-approved point-of-care proteomic assay that can be used as an adjunct test in the diagnosis and surveillance of UCB.

The NMP-22 BladderChek test is performed in the office and results are available within 30 minutes. The purpose of our study was to compare the performance and cost of NMP-22 and urinary cytology when both were used over 1 year with flexible cystoscopy in a Veteran Affairs (VA) urology practice.

Materials and methods

The study protocol was approved by the Institutional Review Board, University of Kentucky and the Research and Development Committee, Lexington VA Medical Center. The study was a retrospective review of 1 year of all office cystoscopy procedures in a single VA urology practice after introduction of NMP-22 into the practice. A total of 290 subjects underwent 391 office cystoscopy procedures in our practice from October 2007 to October 2008.

Indications for the cystoscopic evaluation were hematuria, surveillance for UCB, and lower urinary tract symptoms. After informed consent for office cystoscopy, urine specimens were collected from all subjects upon presentation to the urology clinic. The urine specimen was divided in to three aliquots. A urine dipstick test along with the NMP-22 BladderChek test was performed on the first two urine aliquots. The NMP-22 BladderChek test was performed by placing four drops of voided urine in the sample well of the device. Results were interpreted by an office urology technician 30 minutes later and compared to a control that is part of the NMP-22 point-of-care test kit. Descriptions, mechanisms of action and details of NMP-22 testing in relation to bladder cancer have been described elsewhere.[6-9] The third urine aliquot was sent to pathology for urinary cytology analysis. One cytotechnologist and two pathologists interpreted the urinary cytology tests. If the urine dipstick test showed evidence of infection (leukocyte esterase or nitrite positive), the office cystoscopy procedure was cancelled and the patient was treated for a urinary tract infection after a confirmatory urine culture was sent. Patients without evidence of urinary tract infection were taken for office cystoscopy which was performed in standard fashion using a sterile, 15-Fr flexible cystoscope and 1% plain lidocaine jelly in the urethra for local anesthesia. Cystoscopy findings, urinary cytology, and NMP-22 results were recorded at each encounter and compared. Patients with negative office cystoscopy, urinary cytology, and NMP-22 were recorded as having no evidence of bladder cancer (negative) in this analysis. Resection of tumor or bladder biopsy were recommended for abnormal cystoscopy (tumor or urothelial abnormality), and/or positive/suspicious urinary cytology, or a positive NMP-22 test. Cystoscopy was recorded as positive (suspicious cystoscopy or obvious tumor was recorded in the analysis as positive cystoscopy) or negative. Cytology was recorded as either positive (positive or suspicious cytology was recorded as positive in this analysis) or negative. NMP-22 was recorded as positive or negative. Patients who went for tumor resection/bladder biopsy had the pathology results recorded as positive (UCB confirmed histologically) or negative (other non-UCB conditions or normal urothelium). Tumors were staged and graded according to the AJCC TNM staging system.[10] Tumor grade was classified as low or high grade. Tumors with stage Ta low grade, and T1 low grade were placed in the low risk category for the analysis. Carcinoma-in-situ (CIS), Ta high grade, T1 high grade, and T2 (any grade) were placed in the high risk category for this analysis.

Sensitivity, specificity, positive and negative predictive values were calculated using standard methods for each office test (NMP-22, cytology, and cystoscopy). All 391 office cystoscopy procedures with markers were initially analyzed as one group. A separate analysis was performed grouping patients without and with history of bladder cancer. Statistical analyses were performed using SPSS statistical software version 18.

Results

There were 290 unique subjects in the study. The age range of the study participants was 31-94 years old. The mean age of study participants was 71.9 years (standard deviation of mean 10.4 years). There were 262 Caucasians, 21 African Americans, 2 Asians, and 5 other/unspecified races in the study group. There were 280 males (96%) and 10 females (4%) in the study group.

A total of 391 office cystoscopies were performed during the study period. Of the 391 cystoscopy encounters, 351 had all three office tests available for analysis. Nine bladder stones and one upper tract

urothelial carcinoma were identified in this group and were excluded from the analysis due to known false positives of NMP-22 with urinary stones.[11-12] One patient with upper tract urothelial cancer was excluded because bladder cancer, not upper tract urothelial cancer, was the endpoint of the analysis. Of the remaining 341 procedures, 143 demonstrated abnormalities in cystoscopy, cytology, or NMP-22 and were recommended for operative tumor resection or biopsy. Sixty-nine bladder biopsies/ tumor resections were performed after informed consent was obtained and medical clearance for surgery was achieved. Seventy-four subjects did not go to bladder biopsy/ tumor resection because they were either medically unfit for surgery or refused surgery. Bladder cancer was identified in 54% (37/69) subjects who underwent bladder biopsy. The study outline is shown in Figure 1.

One hundred ninety eight (198) cystoscopy procedures demonstrated negative findings in all three office studies (cystoscopy, cytology, and NMP-22) and were counted as negative in this analysis. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each office test were analyzed for the entire study group and reported in Table 1. Table 1 also shows the performance of each test in identifying high and low risk tumors. Analysis of different combinations of office testing for UCB was then performed.

NMP-22 combined with cystoscopy yielded a sensitivity and specificity of 97% and 87%, respectively. Urinary cytology combined with cystoscopy revealed sensitivity and specificity of 95% and 87%, respectively. The PPV of the NMP-22/ cystoscopy combination was 55% and NPV was 99%. The cytology and cystoscopy combination revealed a PPV of 54% and a NPV of 99%.

The performance of the office tests was then analyzed according to if patients had any history of bladder cancer at time of office presentation. One hundred twenty eight had no history of bladder cancer on presentation and two hundred thirteen had history of bladder cancer at the time of the planned office cystoscopy. These findings are outlined in Table 2 and Table 3. One hundred twelve subjects in the surveillance group received previous intravesical therapy; the majority (93) had received BCG. Most notable for all three office tests when the groups were separately analyzed in this fashion was the increase in sensitivity, and slight improvement in positive predictive value for the UCB surveillance group and minimal change in negative predictive values.

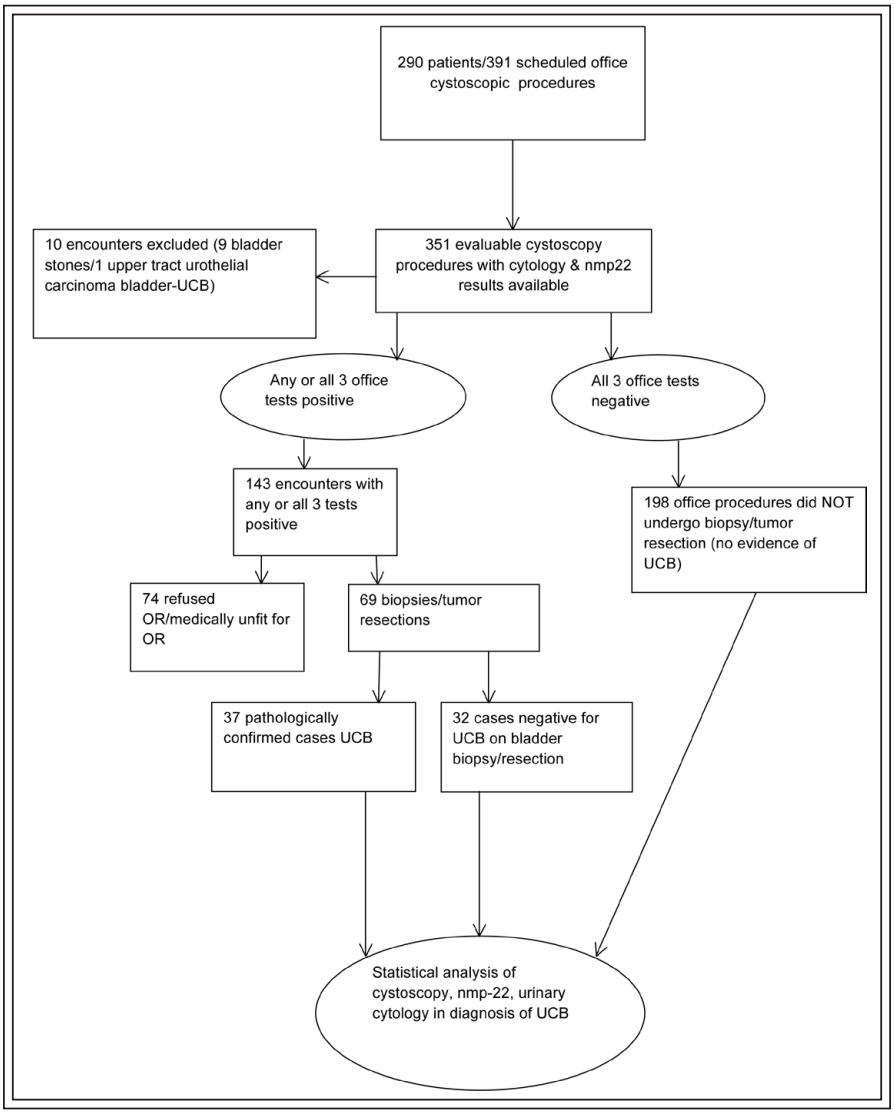

Figure 1. Study outline.

TABLE 1. **Markers and cystoscopy statistical performance**

	Sensitivity	Specificity	PPV	NPV	High risk tumors	Low risk tumors
NMP-22	51%	97%	68%	93%	13/18 (72%)	6/19 (32%)
Cytology	35%	97%	65%	90%	12/18(67%)	1/19 (5%)
Cystoscopy	92%	90%	55%	99%	15/18 (83%)	19/19(100%)

PPV = positive predictive value; NPV = negative predictive value.

The cost savings of the NMP-22 test over urinary cytology was a significant finding in this study. Considering 351 patient encounters had cystoscopy, urinary cytology and NMP-22 testing completed, we calculated the cost of urinary cytology ($150/test) and NMP-22 ($25/test) for the study group. A total cost of $52,500 was calculated for urinary cytology performed during 351 office cystoscopy procedures and $8,750 for NMP-22 testing performed in the same 351 office cystoscopy procedures.

Discussion

NMP-22 has been evaluated as both a screening and surveillance tool in the detection of UCB.[7-9,11,13] Advantages of NMP-22 include low cost, reproducibility, rapid results and elimination of pathologist/cytotechnologist interpretation.[11] Disadvantages of NMP-22 include false positives associated with benign urologic pathology such as benign prostatic hyperplasia, urolithiasis, urinary tract infection, hematuria, and pyuria.[12,13] The objective of our study was to compare cost and performance of NMP-22 and urinary cytology when both were used for 1 year as adjuncts to flexible cystoscopy in a VA urology practice. Our analysis demonstrated similar findings to other investigators who have compared NMP-22, cytology, and cystoscopy in the detection of UCB as outlined in Table 4.[7,14,15]

Urinary cytology, though specific, suffered from low sensitivity (35%) as a stand-alone test in the detection of all stages and grades of UCB in this study. NMP-22 performed better as a stand-alone test with higher sensitivity than urinary cytology (51%).

The specificity of NMP-22 (96%) was the nearly the same as urinary cytology (97%). Cystoscopy was the best as a stand-alone overall test to detect UCB in this analysis with a sensitivity of 92% and specificity of 88%. The addition of NMP-22 or urinary cytology to cystoscopy provided slight improvement in sensitivity when compared to cystoscopy alone and virtually no change in specificity when compared to cystoscopy. Lotan et al demonstrated an increase in PPV of NMP-22 with increasing patient age, presence of gross hematuria, male sex and smoking history.[16] NMP-22 in our study group showed a higher overall PPV than the Lotan study and a similar NPV of NMP-22 when compared to the same study. The Lotan study group also differed significantly from our population in size (1328 versus 290), presentation (initial evaluation versus initial evaluation and bladder cancer surveillance patients) and performance of the NMP-22 test based on population demographics and symptoms (age, smoking history, symptoms).

With high risk tumors, NMP-22 was positive in 73% of the cases in our study group. By comparison, urinary cytology was positive in only 60% of patients in high risk tumors. Our data is consistent with

TABLE 2. **No history of urothelial carcinoma bladder – 128 encounters**

	Sensitivity	Specificity	PPV	NPV
NMP-22	64%	96%	69%	95%
Cytology	43%	95%	55%	92%
Cystoscopy	100%	89%	58%	100%

PPV = positive predictive value;
NPV = negative predictive value.

TABLE 3. **With history of urothelial carcinoma bladder – 213 encounters**

	Sensitivity	Specificity	PPV	NPV
NMP-22	57%	96%	72%	93%
Cytology	30%	96%	78%	90%
Cystoscopy	87%	87%	53%	98%

PPV = positive predictive value;
NPV = negative predictive value

previous studies that demonstrate the sensitivity of NMP-22 for aggressive tumors to be in the range of 68%-90%.[7,17,18]

The characteristics unique to our study include the patient population and practice setting. Our study population was largely male and Caucasian, reflecting the Veteran population in the Central/Eastern Kentucky region. Selection bias was negligible since all patients presenting to our clinic for cystoscopy were based on bladder cancer surveillance and/or lower urinary tract symptom/signs at initial presentation (i.e. hematuria). The UCB cystoscopy surveillance protocol in our practice (cystoscopy plus urinary cytology) had been in place for over 15 years before the NMP-22 test was introduced at the Lexington VA Medical Center. In 2005, Grossman et al[7] performed a similar study involving multiple centers including VA, private and university practice settings. Our study differed from the Grossman study in size, practice setting, and demographics.

Our study could have been strengthened by performing bladder biopsies in all subjects (whether positive or negative on office testing), however this attempt to achieve a gold standard was not safe or practical. Performing bladder biopsies in all patients, regardless of office test findings, is a significant deviation from the standard of care in bladder cancer diagnosis and surveillance. This practice would place many subjects at risk of complications from the bladder biopsy procedure, especially when office testing was negative. Additional limitations include our small study population size, the mixed population of new patients and bladder cancer surveillance patients, and the large number patients with positive office testing who did not undergo bladder biopsy. On the other hand, we believe this study provides a realistic "snap-shot" of a typical urology practice as we changed our office cystoscopy protocol and introduced a newer, less expensive test into the practice. Though our analysis showed NMP-22 to be as accurate as urinary cytology in the diagnosis and follow up of bladder cancer, urinary cytology still maintains a useful role among some urologists when following patients with CIS or high grade tumors involving the prostatic urethra. In addition, cytology can be helpful in the decision to perform biopsy of erythematous lesions in the bladder.

A financial analysis of our data revealed savings of $43,750 when NMP-22 was compared to urinary cytology in this study {calculated as follows: $52,500 (cytology total cost in study) -$8,750 (NMP-22 total cost in study) = $43,750}. Statistical analysis demonstrated elimination of urinary cytology would have been a safe practice without loss of bladder cancer diagnostic accuracy. Zippe et al showed a similar range of potential cost savings of $26,400 when NMP22 was used in place of urinary cytology in a study of 330 patients examining cytology and NMP-22 in the diagnosis of UCB.[19]

TABLE 4. **Study demographics and sensitivity/specificity comparison**

Lexington VAMC		
Number subjects (n)	290	
Mean age years	71.9	
Sex		
Male	280 (96%)	
Female	10 (4%)	
Race		
White	262 (90.3%)	
Black	21 (7.3%)	
Hispanic	0 (0%)	
Asian	2 (0.7%)	
Other/unknown	5 (1.7%)	

		Sensitivity	**Specificity**
Lexington VAMC	NMP-22	51%	96%
	Cytology	35%	97%
Grossman et al[7]	NMP-22	55.7%	85.7%
	Cytology	15.8%	99.2%
Eissa et al[14]	NMP-22	85%	91%
	Cytology	44%	100%
Lekeli et al[15]	NMP-22	52.6%	82.5%
	Cytology	31.6%	100%

Conclusions

Our findings demonstrate cystoscopy is the best single test in the diagnosis and follow up of patients with bladder cancer. Some urologists choose urine based testing in addition to cystoscopy in these patients. When an adjunct test to cystoscopy is desired, NMP-22 is an acceptable, cost-effective alternative to urinary cytology. When cystoscopy is abnormal, additional office testing such as cytology and NMP-22 may be useful in deciding if a bladder biopsy is indicated. In the present medical economic climate where cost control is paramount in urology practice, these findings support revision of surveillance and diagnostic regimens for bladder cancer. □

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