Detection of Recurrent Bladder Cancer

NMP22 Test or Urine Cytology?

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Purpose: To assess the accuracy of voided urine cytology versus urinary nuclear matrix protein 22 (NMP22) qualitative assay in the diagnosis of various grades and stages of recurrent bladder transitional cell carcinoma (TCC).

Materials and Methods: From July 2007 to February 2009, all patients with history of superficial bladder TCC were included in this multi-center study. Each patient provided three serial voided urine samples for cytologic examination and one sample for the NMP22 qualitative assay prior to urethrocystoscopy. The sensitivity and specificity of urine cytology and the NMP22 test were determined.

Results: The sensitivities of the NMP22 test and cytology for detection of recurrence were 78.8% and 44.2%, respectively ($P = .001$), while the specificities were 69.6% and 83.7%, respectively ($P = .019$). The NMP22 test showed significantly higher sensitivity than cytology in detecting recurrences in low-risk and intermediate-risk groups.

Conclusion: The NMP22 assay could be used for detection of superficial bladder cancer, especially in low- and intermediate-risk groups; however, the value of the test is limited by its low specificity.

Keywords: transitional cell carcinoma, urinary bladder neoplasms, diagnosis, nuclear matrix proteins, tumor markers

INTRODUCTION

The most common genitourinary cancer among our population is the bladder carcinoma with prevalence of 48.3%.[1] Superficial type accounts for 70% of the urothelial cell carcinoma of the bladder that has a high probability of recurrence (60% to 85%).[2,3]

Long-term follow-up is recommended to detect any cancer recurrence or progression. Therefore, cystoscopy, the gold standard test, and urine cytology every 3 to 4 months for the first two years and at a longer interval in subsequent years are recommended as the current standard of care for detection of tumor recurrence.[4,5] Due to invasiveness and high cost of this approach, new techniques and markers, including flow cytometry, quantitative fluorescence image analysis, and nuclear matrix protein 22 (NMP22), etc, have been introduced and studied for their accuracy in detection of recurrent bladder cancers.[6-10]

The NMP22 test detects the nuclear matrix protein qualitatively, which is part of the nuclear mitotic apparatus released from urothelial nuclei upon cellular apoptosis.[11-13] Nuclear matrix protein 22 is shed into the urine and has a 20 to 80-times higher concentration in the urine of the patients with bladder cancer compared to noncancerous controls.[12] This test has been approved by the Food and Drug Administration (FDA) for patient surveillance.[14] This test has sensitivity and specificity as high as urine cytology.

We compared the sensitivity and specificity of voided urine cytology in the diagnosis of various grades and stages of recurrent bladder transitional cell carcinoma (TCC) with the urinary NMP22 qualitative test.

MATERIALS AND METHODS

From July 2007 to February 2009, all patients with history of superficial bladder TCC from seven academic centers were enrolled in this study. Patients with urinary tract infections, concurrent urolithiasis, a history of bladder substitution, and other malignancies were excluded from the study. Written informed consent was obtained from each participant and the study was approved by the Ethics Committee of the Infertility and Reproductive Health Research Center (IRHRC).

Each patient provided three serial voided urine samples within 3 days for cytologic examination and one sample for the NMP22 qualitative assay. In each center, one cytopathologist performed cytologic examination, who was unaware of the cystoscopy and NMP22 results. Malignant and suspicious results for malignancy were classified as positive.

The NMP22 assay was performed according to the instructions provided in the NMP22 point-of-care device (Matritech Inc, 330 Nevada St, Newton, MA). The quality of NMP22 in patients' urine was assessed using a lateral flow immunochromatographic strip. Four drops of urine at room temperature were added to the point-of-care device and results were interpreted within 30 minutes. Positive result yielded a colored band in the test position. All the NMP22 test results were interpreted by a single observer at each center, who was blind to the cystoscopy and cytology results.

Urethrocystoscopy, using a rigid cystoscope and video camera, was performed for all the patients. Any visible tumor or suspicious lesion was biopsied for histopathologic examination, using the TNM staging system[15] and World Health Organization grading.[16] Findings from histopathologic evaluation of biopsies were considered as a gold base for comparing the results of other two tests. Either normal appearance in endoscopy or histopathologically nonmalignant tissue in biopsy was defined as negative for TCC. If no tumor was observed endoscopically, patients with positive isolated cytology or NMP22 test were further evaluated by random biopsies from the bladder and urethra and by imaging, like intravenous urography or contrast-enhanced computed tomography,

Table 1. Sensitivity, specificity, positive predictive value, and negative predictive value of the NMP22 test and voided urine cytology

	Urine Cytology	NMP22 Test	*P*
Sensitivity (%)	44.2 (23/52)	78.8 (41/52)	.001
Specificity (%)	83.7 (77/92)	69.6 (64/92)	.019
Negative Predictive Value (%)	72.6 (77/106)	85.3 (64/75)	NS*
Positive Predictive Value (%)	60.5 (23/38)	59.4 (41/69)	NS

*NS indicates non-significant

in order to rule out any missed lesion in the genitourinary system. Patients with a higher index of suspicion were re-evaluated every 4 weeks until 6 months. Patients who were found to have an upper tract lesion or a bladder lesion in the next cystoscopy were considered true positives for the test.

Data analysis was performed by using SPSS software (the Statistical Package for the Social Sciences, Version 15.0, SPSS Inc, Chicago, Illinois, USA) using Chi-Square, Fisher's Exact, and Mann-Whitney *U* tests and by calculating the 95% confidence interval (CI) to determine the sensitivity and specificity of urine cytology and the NMP22 assay.

RESULTS

Of 320 recruited participants, 144 patients, 125 men and 19 women, met the inclusion criteria, diagnostic tests, and follow-up period. The mean age of the entire group was 61.8 years (range, 26 to 86 years).

Of 144 patients, 52 (36.11%) were diagnosed with recurrent bladder TCC; 48 patients were detected with cystoscopy and 4 after the first follow-up cystoscopy. One of these 4 patients had carcinoma in situ (CIS) in his pathology and was positive for cytology; but other 3 subjects were NMP22-positive with small tumor size, 2 of them had T1G2 and the other one had T1G3.

Of 52 patients with tumor recurrence, 16 had grade I, 20 had grade II, and 16 had grade III disease, while 18 had stage Ta, 22 had stage T1, 3 had CIS, and 9 had stage T2 or more. On histopathology, 41 were positive for the NMP22 test and 23 were positive for cytology.

The sensitivity, specificity, positive predictive value, and negative predictive value of the NMP22 test and voided urine cytology for detection of recurrence are presented in Table 1. The sensitivities of urine cytology and urinary NMP22 regarding the stage, grade, and risk stratification are shown in Table 2.

Pathological data were grouped according to risk for recurrence, progression, and invasion into a low-risk group (Single Ta, G1 < 3 cm), a high-risk group (Multiple T1G2, Tis, T1G3, and TaG3), and an intermediate-risk group (rather than two other groups). The NMP22 test showed significantly higher sensitivity than cytology in detecting low-risk and intermediate-risk groups recurrences. However, this test detects recurrence as same as cytology in the high-risk group (Table 2).

After combining the results of the NMP22 test and cytology, NMP22 test and cystoscopy, and cytology and cystoscopy, the overall sensitivity increased to 88.5% (46/52), 98.1% (51/52), and 94.2% (49/52), respectively, rather than cytology or NMP22 test alone.

DISCUSSION

Cystoscopy is the gold standard modality for the diagnosis of bladder carcinoma; however, it is invasive and relatively expensive.[17] Voided urine cytology is based on morphologic assessment in intact cells shed in urine; hence, small tumors

Table 2. Diagnostic value of urine cytology and NMP22 test in each tumor grade and stage, and risk of recurrence and progression

Tumor Class (n = 52)	Sensitivity of Urine Cytology (%)	Sensitivity of NMP22 Test (%)	*P*
Stage			
CIS (n = 3)	100 (3/3)	66.7 (2/3)	.00
Ta (n = 18)	11.1 (2/18)	61.1 (11/18)	.002
T1 (n = 22)	45.5 (10/22)	95.5 (21/22)	.021
≥ T2 (n = 9)	88.9 (8/9)	77.8 (7/9)	NS
Grade			
G I (n = 16)	0.0 (0/16)	68.8 (11/16)	.00
G II (n = 20)	40.0 (8/20)	85.0 (17/20)	.022
G III (n = 16)	93.8 (15/16)	81.3 (13/16)	NS
Risk stratification			
Low (n = 15)	0.0 (0/15)	66.7 (10/15)	.00
Intermediate (n = 20)	40.0 (8/20)	85.0 (17/20)	.022
High (n = 8)	87.5 (7/8)	87.5 (7/8)	NS

* CIS indicates carcinoma in situ; and NS, non-significant.

or well-differentiated ones are difficult to recognize because cells less likely exfoliate spontaneously. This fact explains its low sensitivity (15% to 30%) in low-stage cancers.[13] Therefore, many new urine-based tests for substitution of urine cytology have been developed. Of which, BTA stat, BTA trak, NMP22, FDP, ImmunoCyt, and FISH (UroVysion) have been approved by the FDA.[18]

Nuclear matrix, first described in 1974, is a non-chromatin structure that supports nuclear shape, organizes DNA, and takes part in DNA replication and transcription, and in RNA processing. Nuclear matrix protein 22 is a nuclear protein which plays a role in control of the chromatid regulation and cell separation during replication. The NMP22 test is an office-based procedure that can be interpreted by a urologist within 30 minutes; therefore, it can be used as an alternative to urine cytology.[19,20]

Various published studies have reported NMP22 (sensitivity: 70% to 80%) to be at least twice more sensitive than urine cytology (sensitivity: 10% to 40%) in detecting bladder cancer.[8,21-23] In our study, the sensitivity of the NMP22 test was significantly higher, but the specificity was lower than that for cytology (77.8% versus 44.2% and 69.6% versus 83.7%, respectively). These findings are compatible with the quantitative analyses of NMP22 performed by other investigators.[8,22,23] It was demonstrated that the quantitative NMP22 test had an overall sensitivity of 70% to 80% for the detection of recurrent superficial bladder TCC in approximately 400 patients. In comparison, cytology showed sensitivity of 10% to 40%.[20,24,25] A higher sensitivity for urine cytology rather than other published articles was detected in this study, may be due to examining of three urine samples instead of one.

Few drawbacks have been mentioned for NMP22, including lack of exact cutoff point for quantitative assessment.[26] Jamshidian and colleagues suggested a cutoff point of 10.1 U/mL for Iranian patients for detection of the bladder cancer.[19] Using NMP22 point-of-care device (Matritech BladderChek Test), bladder cancer can be evaluated qualitatively by cutoff point of 10 U/mL, as a positive result for test.

Our study showed that the NMP22 test had consistently higher sensitivity than cytology in detecting

different stages and grades of recurrence in patients with a history of superficial bladder cancer. But in detection of high-grade or advanced tumor, there was no significant difference between two tests. It was already demonstrated that there is no significant difference in risk of progression in the low- and intermediate-risk groups. Kumar and associates showed that the NMP22 test was eight times more sensitive than cytology to detect the low-risk group, but not intermediate- and high-risk groups. [20] Tendency of the NMP22 assay to detect recurrent bladder tumor in patients with intermediate-risk in our study is in contrast to Kumar's study. Traditionally, diagnosis of these groups has been the greatest challenge for non-invasive assays. By use of NMP22 test as a surveillance marker, repetition of cystoscopy or transurethral resection of the bladder tumors may be avoided or can be delayed in such patients.

CONCLUSION

The NMP22 test is a non-invasive and rapid test for the diagnosis of low-stage bladder cancer.

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CONFLICT OF INTEREST

None declared.

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