KJU
Korean Journal of Urology

www.kjurology.org
http://dx.doi.org/10.4111/kju.2011.52.9.603

Urological Oncology

The Clinical Usefulness of Nuclear Matrix Protein-22 in Patients with Atypical Urine Cytology

Jae Sung Ahn, Hyun-Soo Kim[1], Sung-Goo Chang, Seung Hyun Jeon

Departments of Urology and [1]Pathology, Kyung Hee University, Graduate School of Medicine, Seoul, Korea

Purpose: Difficulty exists in interpreting the significance of atypical urine cytology. This study was performed to assess the diagnostic utility of nuclear matrix protein-22 (NMP-22) testing when atypical cells are detected during urine cytology.

Materials and Methods: Among patients whose urine cytology was reported as atypical between January 2004 and December 2009, a total of 275 who also underwent NMP-22 testing were enrolled in the present study. These patients were further divided into the screening group (143 patients examined as outpatients for hematuria) and the follow-up group (132 patients followed up for previously diagnosed bladder cancer). The sensitivity, specificity, positive and negative predictive values, and accuracy were assessed for atypical cytology alone and in conjunction with NMP-22.

Results: Of the 275 patients exhibiting atypical urine cytology, cancer was confirmed in 85, yielding a positive predictive value of 30.9% (85/275). Of the 96 patients testing positive for NMP-22, 58 were diagnosed with bladder cancer. The positive predictive value in conjunction with NMP-22 was 60.4% (58/96). The sensitivity, specificity, negative predictive value, and accuracy were 68.2% (58/85), 80.0% (152/190), 84.9% (152/179), and 76.2% (210/275), respectively. Testing for NMP-22 in the screening and follow-up groups increased the positive predictive value from 30.0% (43/143) to 64.0% (32/50) and from 31.3% (42/132) to 56.5% (26/46), respectively; there was no significant difference between the screening and follow-up groups (p=0.106).

Conclusions: When only cases with atypical urine cytology were examined, NMP-22 testing increased the detection rate of bladder cancer regardless of whether the test was used in screening hematuria or in following up patients.

Key Words: *Cytology; Nuclear matrix; Urinary bladder neoplasms*

Article History:
received 4 May, 2011
accepted 22 July, 2011

Corresponding Author:
Seung Hyun Jeon
Department of Urology, Kyung Hee University Medical Center, 1, Hoegi-dong, Dongdaemun-gu, Seoul 130-702, Korea
TEL: +82-2-958-8537
FAX: +82-2-959-6048
E-mail: juro@khu.ac.kr

INTRODUCTION

In urine cytology, atypical cells are defined as those that are beyond the limits of description for normal cells but that do not quite fit the description of cancerous cells. Farrow et al reported a positive diagnostic rate of 57% in high-risk patients with atypical urine cytology [1], and Melamed et al reported a positive diagnostic rate of 39% [2]. Thus, the identification of atypical cells on urine cytology does not rule out cancer.

Atypical cells can be found in normal bladder patients diagnosed with cystitis, benign prostatic hyperplasia (BPH), or urinary stones, as well as in those undergoing radiotherapy. Atypical findings in bladder cancer are due to necrotic cancer tissue, high mitotic cancers, and the coexistence of inflammation or hematuria [3].

The presence of atypical cells is often encountered during the assessment of hematuria and in patients with a history of bladder cancer; such a finding usually presents a dilemma to the clinician. In the case of atypical urine cytology, options for clinical evaluation may include repeated cytology, cystoscopy, and surveillance. However, an invasive examination in all patients with hematuria may place a substantial burden on both the patient and the

physician. Therefore, we considered methods to complement urine cytology without the use of more invasive methods.

Noninvasive methods include molecular markers, nuclear matrix protein-22 (NMP-22), bladder tumor antigen, and telomerase, among others [4,5]. Of these, tests for NMP-22 yield a positive predictive value (PPV) of 92% in hematuria patients, are easy to perform, and are inexpensive [6].

Due to these perceived advantages of NMP22, its utility in bladder cancer workup had been investigated in the past. However, few studies have examined its usefulness in the presence of atypical urine cytology. Accordingly, the present study aimed to examine the effect of NMP-22 testing on the detection rate of bladder cancer in patients with atypical urine cytology.

MATERIALS AND METHODS

The study group consisted of 275 consecutive patients with urine samples collected between January 2004 and December 2009 that had been categorized as atypical because of the presence of either cell clusters or cytologic atypia. These patients had all undergone NMP-22 testing after the observation of atypia in urine cytology. Patients who did not undergo follow-up after their first diagnosis of cytologic atypia were excluded. Of the patients, all of those being followed up for bladder cancer had undergone cystoscopy, whereas not all of the patients who were being evaluated for hematuria had undergone cystoscopy. Patients in whom bladder cancer was diagnosed within 1 year after the observation of atypical cytology were considered cancer-positive.

Of the experimental groups in the present study, the first group, the screening group, consisted of subjects with an initial presentation of hematuria. This group included patients with gross or microscopic hematuria and excluded all patients undergoing follow-up for bladder cancer. The second group, the follow-up group, consisted of patients previously pathologically diagnosed with bladder cancer confirmed by transurethral resection of bladder tumor (TUR-BT).

Cytologic samples were obtained from bladder washings in patients followed up for bladder cancer. In the case of hematuria patients, bladder washings were used when cystoscopy was carried out, and voided urine was used otherwise; all tests were interpreted by an experienced pathologist. Qualitative assessment of NMP-22 was carried out by using the NMP-22® BladderChek® test kit (Matritech, Newton, MA, USA).

NMP-22 testing was performed according to the recommendations of the manufacturer. Drops of fresh urine were applied to the NMP-22 kit within 2 hours of collection, and results were read after 30 minutes. Tests showing a vertical line in both the control window and the test window were considered to be positive. A vertical line appearing in the control window indicated that the test was performed correctly.

Urine cytology and NMP-22 testing were carried out concurrently in both groups. TUR was carried out when mass lesions were identified on cystoscopy, and cytology was repeated when no such lesion was observed.

The sensitivity, specificity, PPV, negative predictive value, and accuracy were calculated overall and for the screening and follow-up groups. The sensitivity of NMP-22 in assessing specific stages and grades was compared with that of urine cytology. For statistical analysis, the authors used the chi-square test, Fisher's exact test, and McNemar test run on SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA), and p-values less than 0.05 were considered to be indicative of statistical significance.

RESULTS

A total of 275 patients presented with atypical cells in urine cytology. Of these, 85 (30%) were diagnosed with bladder cancer according to pathology. Of the 275 patients, 96 tested positive for NMP-22. Of these, 58 (60%) were diagnosed with bladder cancer, indicating a two-fold increase in PPV ($p < 0.001$) (Table 1).

The 275 patients were divided into the hematuria screening group (143 patients) and the bladder cancer follow-up group (132 patients) for analysis. Of the 143 patients in the screening group who also exhibited atypical urine cytology, 50 were diagnosed with bladder cancer. The PPV of atypical cells alone was 34.9%. Of the 132 patients in the follow-up group, 46 were diagnosed with bladder cancer. The PPV for atypical cytology alone in this group was 34.8%. No significant difference was observed in the cancer detection rates of the screening and follow-up groups (PPV) by use of atypical cytology (p=0.106).

A total of 50 patients in the screening group tested positive for NMP-22; of these, 32 were diagnosed with bladder cancer, yielding a PPV of 64% (32/50). On the other hand, 46 patients in the follow-up group tested positive for NMP-22; of these, 26 were diagnosed with bladder cancer, yielding a PPV of 56.5% (26/46) following stratification of subjects on the basis of NMP-22 test results. Comparison of the screening and follow-up groups showed no significant difference in the cancer detection rate when

TABLE 1. Overall results in patients with the NMP22 BladderCheck test and atypical cytology and in patients with atypical urine cytology only

	NMP22BC with atypical cytology		Atypical cytology only		p-value
	Positive	Negative	Positive	Negative	
Bladder cancer (+)	58	27	85	450	
Bladder cancer (−)	38	152	190	1,423	
PPV	60.4% (58/96)		30.9% (85/275)		<0.001

NMP-22: unclear matrix protein-22, PPV: positive predictive value

NMP-22 testing was used (p=0.294) (Table 2); the cancer detection rates (PPV) of NMP-22 were 60.4% and 56.5%, respectively.

As also shown in Table 2, NMP-22 testing in subjects with atypical cytology had an overall sensitivity of 68.2% (58/85), specificity of 80% (152/190), PPV of 60.4% (58/96), negative predictive value of 84.9% (152/179), and accuracy of 76.2% (210/275).

The sensitivity of NMP-22 with atypical cells was also analyzed after stratification on the basis of tumor stage and grade. For stratification on the basis of T stage, each subject was categorized as Ta, T1, or T2 or higher. For stratification on the basis of tumor grade, each case was categorized as low (grades 1 and 2) or high (grade 3). Stratification on the basis of T stage showed that sensitivity increased with T stage, yielding a sensitivity of 66.7% for Ta, 81.8% for T1, and 90% for T2 or higher stages; however, this increase was not statistically significant (p=0.165). On the other hand, the increase in sensitivity with increasing grade following grade-wise stratification was shown to be statistically significant, with a sensitivity of 58.1% for low-grade tumors and of 87.0% for high-grade tumors (p=0.002) (Table 3).

DISCUSSION

The standard practice when encountering atypical urine cytology is to repeat the urine cytology or perform cystoscopy (in patients undergoing bladder cancer follow-up). Normal findings in these tests will most often result in overlooking the finding of atypical urine cytology [4-6].

The sensitivity of urinary cytology in screening a symptomatic population is too low (30% to 50%) [7-9]. Even among patients undergoing treatment for bladder cancer and follow-up, satisfactory results have not been obtained in regular cystoscopic examination or urine cytology. Hence, additional tests are needed.

NMP is present in low concentrations in the urine and in normal cells. However, in tumor cells, NMP levels are increased 80-fold. Thus, in cases of bladder cancer, NMP is excreted in the urine in high concentrations. The target antigen NMP-22 or the nuclear mitotic apparatus protein of the NMP-22 test is present within epithelial cells and during cell division [10-12]. The NMP-22 test used in our study is advantageous because the test can be performed immediately after the collection of urine from a patient in an outpatient clinic, allowing for rapid results to be obtained and therefore helping to immediately determine the need for additional testing.

In the present study, of the 275 patients with atypical urine cytology, 85 were subsequently diagnosed with bladder cancer, yielding a relatively high cancer detection rate of 30.9% (85/275) for atypical cytology alone. Therefore, atypical findings in urine cytology should not be dismissed as normal. Atypical findings in bladder cancer can be due to necrotic cancer tissue, highly mitotic cancer, and the coexistence of inflammation or hematuria. Findings of atypical urine cytology should thus be backed up with additional tests.

The NMP-22 test was carried out in the subjects of this study to augment the cancer detection rate in patients presenting with atypical urine cytology. As shown in the results, NMP-22 testing used in combination with urine cytology yielded a two-fold increase in the cancer detection rate (30.9% [85/275] to 60.4% [58/96]). These results were the same for both the screening and the follow-up groups.

However, testing for NMP-22 in the presence of atypical urine cytology yielded a cancer detection rate of 60.4% (58/96), and 37 of the patients who tested negative for NMP-22 were subsequently diagnosed with bladder cancer. These points suggest that NMP-22 testing alone cannot fully complement urine cytology in the presence of atypical cytologic findings [13,14].

The findings of the present study show that the NMP-22 test is noninvasive and easy to carry out, demonstrating its use as a tool for clinical examination in the presence of atypical urine cytology results [15-18].

Additionally, although statistical significance was not observed, the sensitivity of the NMP-22 assay increased with increasing T stage, and the sensitivity of the NMP-22 assay was significantly higher for high-grade cancer than it was for low-grade cancer. Previous studies on NMP22 have mostly examined the utility of NMP22 in diagnosis in hematuria patients and as follow-up tests in cases of

TABLE 2. Sensitivity, specificity, PPV, NPV, and accuracy in patients with atypical cytology when indexed to NMP22 in the incident and prevalent group

	Overall	Screening	Follow-up	p-value
Sensitivity (%)	68.2 (58/85)	74.4 (32/43)	61.9 (26/42)	0.142
Specificity (%)	80.0 (152/190)	82.0 (82/100)	77.7 (70/90)	0.291
PPV (%)	60.4 (58/96)	64.0 (32/50)	56.5 (26/46)	0.294
NPV (%)	84.9 (152/179)	88.1 (82/93)	81.3 (70/86)	0.146
Accuracy (%)	76.2 (210/275)	79.7 (114/143)	72.7 (96/132)	0.111

PPV: positive predictive value, NPV: negative predictive value, NMP-22: unclear matrix protein-22

TABLE 3. Sensitivity of atypical cytology indexed with NMP22 to detect specific stages

Bladder cancer stage	Overall	p-value
		Ta-T2
Ta (%)	66.7 (28/42)	0.165
T1 (%)	81.8 (27/33)	
T2 or higher (%)	90 (9/10)	
Low grade (%)	58.1 (18/31)	0.002
High grade (%)	87.0 (47/54)	

bladder cancer. However, the present study differs from the other investigations in that it focused on the utility of the NMP22 assay in patients with atypical urine cytology.

CONCLUSIONS

The present study examined the utility of the NMP22 assay in evaluating patients with atypical urine cytology. Although numerous studies have previously expounded on the utility of NMP22 assays in diagnosing bladder cancer, no study so far has assessed the utility of such assays in the evaluation of patients with atypical urine cytology. This study demonstrated that NMP22 assays help in diagnosing bladder cancer in patients whose urine cytology was atypical.

On the other hand, this study could not enroll a sufficient number of subjects, which remains a crucial shortcoming. Furthermore, because only patients with atypical urine cytology were examined, the proportion of patients with atypical cytology among those with hematuria or those being followed up for bladder cancer in whom urine cytology was carried out could not be assessed. Further in-depth studies thus appear necessary to address these limitations.

Conflicts of Interest

The authors have nothing to disclose.

REFERENCES

1. Farrow GM. Pathologist's role in bladder cancer. Semin Oncol 1979;6:198-206.
2. Melamed MR, Koss LG. Developments in cytological diagnosis of cancer. Med Clin North Am 1966;50;651-66.
3. Jo MK, Chung JS, Lee SE. The significance of atypical cell in urinary cytology. Korean J Urol 1998;39:9-14.
4. Farrow GM. Pathologist's role in bladder cancer. Semin Oncol 1979;6:198-206.
5. Melamed MR, Koss LG. Developments in cytological diagnosis of cancer. Med Clin North Am 1966;50;651-66.
6. Raina R, Pahlajani G, Ponsky LE, Agarwal A, Zippe CD. The clinical utility of atypical cytology is significantly increased in both screening and monitoring for bladder cancer when indexed with nuclear matrix protein-22. BJU Int 2008;102:297-300.
7. Ponsky LE, Sharma S, Pandrangi L, Kedia S, Nelson D, Agarwal A, et al. Screening and monitoring for bladder cancer: refining the use of NMP22. J Urol 2001;166:75-8.
8. Sharma S, Zippe CD, Pandrangi L, Nelson D, Agarwal A. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. J Urol 1999;162:53-7.
9. Gupta NP, Sharma N, Kumar R. Nuclear matrix protein 22 as adjunct to urine cytology and cystoscopy in follow-up of superficial TCC of urinary bladder. Urology 2009;73:592-6.
10. Khadra MH, Pickard RS, Charlton M, Powell PH, Neal DE. A prospective analysis of 1,930 patients with hematuria to evaluate current diagnostic practice. J Urol 2000;163:524-7.
11. Nabi G, Greene DR, O'Donnell M. How important is urinary cytology in the diagnosis of urological malignancies? Eur Urol 2003;43:632-6.
12. Miyanaga N, Akaza H, Ishikawa S, Ohtani M, Noguchi R, Kawai K, et al. Clinical evaluation of nuclear matrix protein 22 (NMP22) in urine as a novel marker for urothelial cancer. Eur Urol 1997;31:163-8.
13. Deshpande V, McKee GT. Analysis of atypical urine cytology in a tertiary care center. Cancer 2005;105:468-75.
14. Brown FM. Urine cytology. It is still the gold standard for screening? Urol Clin North Am 2000;27:25-37.
15. Feifer AH, Steinberg J, Tanguay S, Aprikian AG, Brimo F, Kassouf W. Utility of urine cytology in the workup of asymptomatic microscopic hematuria in low-risk patients. Urology 2010;75:1278-82.
16. Kapila K, Kehinde EO, Anim JT, Mojiminiyi OA, Vinsu A, George SS, et al. Could nuclear matrix 22(NMP22) play a role with urine cytology in screening for bladder cancer? – experience at Kuwait University. Cytopathology 2008;19:369-74.
17. Choi HS, Lee SI, Kim DJ, Jeong TY. Usefulness of the NMP22BladderCheck test for screening and follow-up of bladder cancer. Korean J Urol 2010;51:88-93.
18. Karakiewicz PI, Benayoun S, Zippe C, Lüdecke G, Boman H, Sanchez-Carbayo M, et al. Institutional variability in the accuracy of urinary cytology for predicting recurrence of transitional cell carcinoma of the bladder. BJU Int 2006;97:997-1001.