



Utilization of the NMP22® BladderChek® Urine Test as an Adjunct to Atypical Cytology in the Detection of Bladder Cancer Recurrence

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ABSTRACT

OBJECTIVE: To assess the performance of the NMP22® BladderChek® (NMP22-BC) urine test compared with cytological and histological findings for detecting bladder cancer recurrence in patients with a prior history of bladder cancer.

METHODS: A prospective study was performed, evaluating a total of 112 urine samples from 67 patients. These samples were obtained prior to cystoscopy and were analyzed with the NMP22-BC. The results were compared against those of voided urine cytology (VUC) and cystoscopy.

RESULTS: Of the 112 samples, there was a total of 27 recurrences (24.1%). The overall sensitivity of NMP22-BC alone was 33%, VUC with malignant or suspicious specimens alone was 37%, and the two methods combined was 46%. The specificity of NMP22-BC alone was 92%, VUC alone was 99%, and both methods combined was 92%. The detection rate of bladder cancer recurrence in high-grade tumors increased from 50% for NMP22-BC alone and 43% for VUC alone, to 64% when both tests were used in combination. The detection rate of recurrence for stages Ta, T2, and CIS was also significantly increased when NMP22-BC and VUC were used in combination. The overall positive and negative predictive values were 68% and 84% respectively. Inclusion of atypia as positive for recurrence (VUC+A) increased the overall sensitivity of cytology to 72% but decreased the specificity to 64%. However, in cases where atypical cytology was confirmed with positive NMP22-BC, the sensitivity of the combination was 46% and specificity was 100%.

CONCLUSION: NMP22® BladderChek® is a valuable addition to voided urine cytology for detection of high-risk lesions. Our study shows that once atypical cytology is confirmed by NMP22-BC, the specificity is 100%. Furthermore, the sensitivity of this combination is superior to that of malignant or suspicious cytology alone.

KEYWORDS: Bladder cancer, NMP-22, Cytology, Urinary markers

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INTRODUCTION

Bladder urothelial carcinoma is one of the most common urologic malignancies and the fourth most common malignancy in men. The American Cancer Society estimates 63,210 new cases of bladder cancer were diagnosed in the United States in 2005, with 13,180 resulting in death. The lifetime risk of developing bladder cancer is 1 in 30 for men and 1 in 90 for women [1]. More than 70% of patients with bladder cancer have superficial disease and are managed with transurethral resection with or without intravesical therapy. The probability of recurrence of superficial bladder cancer is 50-70%, most recurring within the first year [2]. The probability of progression from superficial to muscle invasive disease is 10-15%, necessitating aggressive and accurate surveillance [3]. The current surveillance standard is periodic cystoscopy with some doctors advocating voided urine cytology (VUC). Cystoscopy has a reported sensitivity of higher than 90%, but it is less accurate in identifying small, flat, or inaccessible tumors [4]. Furthermore, cystoscopy is invasive, relatively expensive, and increases the risk of urinary tract infection. VUC has a reported specificity of higher than 93% but an inadequate sensitivity of 25-40%, particularly for well or moderately differentiated tumors [5,6]. The results of cytology are also operator dependent, decreasing the cancer detection rate in the hands of inexperienced cytologists. Therefore, there is a need for molecular markers to help monitor patients with a history of superficial bladder cancer.

Nuclear matrix is a non-chromatin structure that supports DNA shape and integrity. It is also involved in DNA replication and the proper distribution of DNA material to daughter cells [7]. Nuclear matrix protein-22 (NMP-22) is released from nuclei of tumor cells during apoptosis. It has been reported that patients with bladder cancer have a 25-fold higher amount of NMP-22 in their urine than control patients, however the clinical significance of this finding remains to be controversial [8,9,10].

Recently the Food and Drug Administration has approved a "point-of-service test", NMP22[®] BladderChek[®] (NMP22-BC) (Matritech, Inc. (Inverness Medical Innovations), Newton, MA), for both the diagnosis of bladder cancer in high-risk or symptomatic patients and as a surveillance tool for bladder cancer recurrence. In this study, we report our results with NMP22-BC in patients with previously diagnosed and treated superficial bladder cancer and compare these results against VUC and cystoscopy.

METHODS

This study was approved and overseen by the Institutional Review Board of Hackensack University Medical Center. We prospectively monitored 67 consecutive patients with previously treated urothelial cell carcinoma of the bladder for recurrence with 112 NMP22-BC, VUC, and cystoscopy events. The study group included 45 men and 22 women with a mean age of 70 years (range 47 to 91 years). Of the patients, 34 (51%) were active smokers or had a history of smoking. All patients provided a voided urine specimen prior to undergoing cystoscopy. Four drops of the voided urine specimen were applied to the NMP22-BC test according to the instructions in the package insert. The result was recorded after 30 minutes. The remainder of the urine specimen was sent for cytological analysis. Cytology was defined as positive if the specimen contained malignant or suspicious cells in one analysis (VUC) or if the specimen contained atypical cells in addition to malignant or suspicious cells in the second analysis (VUC+A). Results of both NMP22-BC and VUC and VUC+A alone and in combination were compared with cystoscopy and the final histological diagnosis. All patients were monitored for upper tract recurrence with either computed tomography urogram or intravenous pyelogram. In order to minimize reporting bias, the physician performing cystoscopy was blinded to the results of the NMP22-BC test result at the time of the examination. In addition, the pathologist interpreting the NMP22-BC test was blinded to the results of the cystoscopic examination. The first and second voided cytologies were reviewed independently and were blinded.

RESULTS

Of 112 monitored events, there was a total of 27 recurrences (24.1%) confirmed with histology. Thirteen recurrences were low-grade (Grade 1-2) tumors and 14 were high-grade (Grade 3-4). Fifteen tumors were stage Ta, 5 were stage T2, 1 was stage T3, 1 was stage T4, and 5 were carcinoma in situ (CIS). There were no stage T1 recurrences in the series. Event monitoring was distributed evenly amongst the patients and was not adjusted based on symptoms, pathology, or recurrence rate.

The overall sensitivity of NMP22-BC was 33%, specificity was 92%, positive predictive value was 60%, and negative predictive value was 81%. The sensitivity of VUC alone was 37%, specificity was 99%, positive predictive value was 91%, and negative predictive value was 83%. The sensitivity of the combination NMP22-BC/VUC was 46%, specificity was 92%, positive predictive value was 68%, and negative predictive

value was 84%. As expected, inclusion of atypia as a positive result for recurrence increased the sensitivity of VUC+A to 72% and decreased the specificity to 64%. However, in cases where atypical cytology was confirmed by positive NMP22-BC, the sensitivity of the combination was 46% and the specificity was 100% (Table 1).

When detection results were stratified by grade, NMP22-BC alone detected 3/13 (23%) low-grade lesions and 7/14 (50%) high-grade lesions. VUC detected 4/13 (31%) low-grade and 6/14 (43%) high-grade tumors. In combination, 4/13 (31%) low-grade and 9/14 (64%) high-grade tumors were detected (Table 2).

The results of detection rate when stratified by stage of the recurrent tumors are summarized in Table 3.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
NMP22-BC	33	92	60	81
VUC	37	99	91	83
NMP22-BC/VUC	46	92	68	84
VUC+A	72	64	34	90
VUC+A confirmed by NMP22-BC	46	100	12	86

Table 1. Overall results for NMP22[®] BladderChek[®], voided urine cytology, and NMP22-BC/VUC combination

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DISCUSSION

Bladder cancer is associated with a high rate of recurrence as well as a potential for disease progression. There is a well established need for the development of a reliable urine test that would be able to supplement cystoscopy and bladder biopsies for monitoring bladder cancer recurrence. An ideal test for surveillance of patients with bladder cancer needs to be sensitive and have a high negative predictive value that would allow for detection of recurrence during screening. VUC carries a low sensitivity in low-grade and low-stage tumors. It is also established that cytology suffers from operator dependent

	Low-grade	High-Grade
NMP22-BC	23	50
VUC	31	43
NMP22-BC/VUC	31	64

Table 2. Cancer detection rate (%) by grade

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interpretation of the results. Furthermore, the ambiguity of "atypical cytology" poses a management dilemma for practitioners as well as psychological unease for patients. The current study evaluates the NMP22[®] BladderChek[®] test for the detection of bladder cancer recurrence in patients with a history of prior bladder cancer and compares the results against those of VUC and cystoscopy.

Cystoscopy remains the gold standard for bladder cancer detection. In our study cystoscopy detected 24 of 27 recurrences and resulted in the highest sensitivity and specificity.

Urine cytology evaluates the appearance of the nuclei in exfoliated malignant cells seen under light microscopy. Historically, this evaluation has a reported sensitivity of 30-40% and a specificity higher than 90%. The results of the present study are consistent with these reports, as VUC correctly identified 10 out of 27 recurrences. There were 2 false positive results identified by VUC in this study. When the inclusion criteria for positive cytology were extended to atypical specimens in VUC+A group, cytology correctly identified 18 of 27 recurrences, however the false positive rate increased to 35 of 112 specimens.

Various reports have shown the NMP22 quantitative enzyme immunoassay test to have a sensitivity of 48-80% in detection

Stage	Total number of recurrences	NMP22-BC	VUC	Combination NMP22-BC & VUC
CIS	5	2/5 (40%)	2/5 (40%)	3/5 (60%)
Ta	15	3/15 (20%)	4/15 (27%)	5/15 (33%)
T2	5	3/5 (60%)	3/5 (60%)	4/5 (80%)
T3	1	0/1 (0%)	0/1 (0%)	0/1 (0%)
T4	1	1/1 (100%)	1/1 (100%)	1/1 (100%)

Table 3. Cancer detection rate (%) by stage

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of bladder cancer recurrence. NMP22-BC is a novel “point of service”, qualitative assay. It has been reported that NMP22-BC carries a superior sensitivity to voided urine cytology in screening populations with risk factors or symptoms of bladder cancer [11]. This test detected 9 out of 27 recurrences with 6 false positive results. One of these 6 was also misdiagnosed with cystoscopy, and pathology revealed a benign urothelial papilloma. Two more false positive results revealed multiple bladder calculi in one case and chronic cystitis in the other. It could be postulated that a high turnover of inflammatory cells can mimic malignancy with a high release of nuclear matrix protein into the urine, thus causing false positive results with the NMP22-BC assay. When used as an adjunct to atypical cytology (VUC+A group), the combination correctly identified all 85 events without a recurrence, a specificity of 100%.

The overall sensitivity and specificity of NMP22-BC is similar to that of cytology. Parallel use of multiple screening tests can

potentially increase the detection rate of the disease. Combining NMP22-BC with cytology did provide additional adjunctive diagnostic information in detecting high-grade tumors as well as stage pT2 and CIS lesions. In addition, the NMP22-BC test drastically improved the specificity of VUC+A. Based on the findings of this study, NMP22-BC is a useful diagnostic tool that may be a powerful adjunct when combined with VUC.

CONCLUSION

NMP22-BC is a valuable addition to VUC for detection of high-risk lesions. Our study shows that once atypical cytology is confirmed by NMP22-BC, the specificity is 100%. Furthermore, the sensitivity of this combination is superior to that of malignant or suspicious cytology alone.

REFERENCES

- [1] American Cancer Society. (2005) www.cancer.org
- [2] Abel PD. Prognostic indices in transitional cell carcinoma of the bladder. *Br J Urol.* 1988 Aug;62(2):103-9.
- [3] Lutzeyer W, Rübber H, Dahm H. Prognostic parameters in superficial bladder cancer: an analysis of 315 cases. *J Urol.* 1982 Feb;127(2):250-2.
- [4] Walker L, Liston TG, Lloyd-Davies RW. Does flexible cystoscopy miss more tumors than rod-lens examination? *Br J Urol.* 1993 Oct;72(4):449-50.
- [5] Landman J, Chang Y, Kavalier E, Droller MJ, Liu BC. Sensitivity and specificity of NMP-22, telomerase and BTA in the detection of human bladder cancer. *Urology.* 1998 Sep;52(3):398-402.
- [6] Zippe C, Pandrangi L, Agarwal A. NMP22 is a sensitive and cost effective test in patients at risk for bladder cancer. *J Urol.* 1999 Jan;161(1):62-5.
- [7] Gordon JN, Shu W-P, Schluskel RN, Droller MJ, Liu BC-S. Altered extracellular matrices influence cellular process and nuclear matrix organization of overlying human bladder urothelial cells. *Cancer Res.* 1993 Oct 15;53(20):4971-7.
- [8] Keesee SK, Briggman JV, Thill G, Wu YJ. Utilization of nuclear matrix protein for cancer diagnosis. *Crit Rev Eukaryot Gene Expr.* 1996;6(2-3):189-214.
- [9] Chang YH, Wu CH, Lee YL, Huang PH, Kao YL, Shiau MY. Evaluation of nuclear matrix protein-22 as a clinical diagnostic marker for bladder cancer. *Urology.* 2004 Oct;64(4):687-92.
- [10] Lekili M, Sener E, Demir MA, Temeltas G, Muezzinoglu T, Buyuksu C. Comparison of the nuclear matrix protein 22 with voided urine cytology in the diagnosis of transitional cell carcinoma of the bladder. *Urol Res.* 2004 May;32(2):124-8. Epub 2003 Dec 19.
- [11] Grossman HB, Messing E, Soloway M, Tomera K, Katz G, Berger Y. Detection of bladder cancer using a point-of-care proteomic assay. *JAMA.* 2005 Feb 16;293(7):810-6.

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