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Patients with a negative cystoscopy and negative Nmp22® Bladderchek® test are at low risk of missed transitional cell carcinoma of the bladder: a prospective evaluation

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ABSTRACT

Objectives: Urine based tumor markers have uncertain utility in diagnosis or surveillance of patients with bladder cancer while cytology is commonly used. We evaluated whether cytology provides additional diagnostic information in patients with a negative NMP22® BladderChek® test (BladderChek) and negative cystoscopy.
Materials and Methods: We performed subset analyses of 2 large prospective multi-center databases evaluating BladderChek for UCB detection and surveillance. These cohorts were analyzed for presence of cancer and result of urine cytology in setting of a negative cystoscopy and negative BladderChek. Subsequently, we prospectively performed cystoscopy, cytology and BladderChek on 434 patients at our institution being evaluated for UCB.
Results: In the detection database (n = 1331), 1065 patients had a negative cystoscopy and BladderChek. There were 3 cancers (stages Ta, Tis and T1) and cytology was atypical in one and reactive in two. In the surveillance cohort (n = 668) patients, 437 patients had negative cystoscopy and BladderChek. Cancer was found in 2 patients (stages Tis and Ta). The patient with Tis has dysplastic cytology and Ta tumor had reactive cytology. In our cohort of 434 patients, 288 pts had negative cystoscopy and BladderChek. One cancer was missed, a Ta ureteral urothelial carcinoma with a reactive cytology.
Conclusions: In patients with negative cystoscopy and BladderChek, very few cancers are missed and cytology was not effective in detection. Use of a point-of-care test in conjunction with cystoscopy in lieu of cytology could decrease cost, provide immediate results, improve negative predictive value and reduce the uncertainty that results from inconclusive cytologic results.

Key words: *Kidney Neoplasms; Population Surveillance; Diagnosis; Nuclear Matrix Protein 22; Cytology*
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INTRODUCTION

Urothelial carcinoma of the bladder (UCB) is the 4th most common cancer in men and the 5th most common cancer overall (1). Up to 70% to 80% of patients diagnosed with UCB present with either gross or microscopic hematuria, and muscle invasion is present in 25% of patients at diagnosis (2). Overall UCB survival rates are stage dependent and 5-year survival for tumors confined to the mucosa are significantly higher than for cancers that are muscle invasive or metastatic (3).

Unfortunately, despite successful treatment, 50%-70% of non-muscle invasive tumors will recur within 5 years and 10%-30% will progress to invasive cancer (4). Due to the aggressive and recurrent nature of UCB, diligent hematuria evaluation and surveillance is required. Currently, cystoscopy along with urine cytology (UC), are the most commonly utilized tests for both UCB detection and surveillance. However, these tests are not perfect. Cystoscopy has been reported to have up to a 30% false negative rate (5,6). Likewise, cytology suffers frequently from inconclusive findings, a low sensitivity

(especially for low grade tumors), an increased expense and a delay in result availability (7,8). These shortfalls can create a clinical conundrum for the physician and increased anxiety for the patient especially when the cytology is inconclusive or atypical. An increased understanding of the biology of urothelial carcinoma has lead to the development and evaluation of multiple urinary markers to help aid in both cancer detection and surveillance (9). One such marker is NMP22 (Nuclear Matrix Protein 22). The NMP22 BladderChek Test (Matritech Inc, Newton, Mass) is Food and Drug Administration approved for the diagnosis of UCB in high risk patients (include patients with hematuria and patients undergoing UCB surveillance). The BladderChek Test is a point-of-care test that provides immediate results without the need for a clinical laboratory for evaluation (9). It detects elevated amounts of nuclear mitotic apparatus protein, a component of the nuclear matrix essential for cell division that is released into the urine during cell death. Previous reports have noted a very high negative predictive value for the BladderChek test and we hypothesized that patients with a negative BladderChek test and negative cystoscopy would have a very low risk of missed malignancy (10,11). In this prospective analysis we examined the databases of 2 published multi-institutional studies with a cohort of over 2000 patients who had cystoscopy, cytology and BladderChek either in the setting of detection or surveillance of bladder cancer. The goal was to evaluate how often patients with both a negative NMP22 BladderChek test and a negative cystoscopy have missed cancer and whether urine cytology provides any additional diagnostic role in these patients. We then prospectively evaluated 434 consecutive patients who presented to our clinic for evaluation for hematuria or surveillance for bladder cancer to determine the value of cytology in the setting of a negative cystoscopy and negative BladderChek test.

MATERIALS AND METHODS

All studies were performed with the approval and oversight of the Institutional Review Board for the Protection of Human Subjects. Initially we performed subset analyses of 2 large prospective multi-center databases evaluating the BladderChek test. Subsequently, the results were validated in a prospective cohort of subjects at our institution who had a BladderChek test prior to evaluation for bladder cancer with cystoscopy and barbotage cytology.

Database analyses and the prospective evaluation at our institution were to establish if urine cytology would have provided any addition diagnostic role in the setting of both a negative BladderChek test and cystoscopy.

The first prospective multi-center database evaluated the role of the BladderChek test for bladder cancer detection (10). This study involved 23 geographically dispersed clinical sites, including academic, Veteran's Affairs Hospitals, and private practice facilities that prospectively recruited 1331 consecutive patients with bladder cancer risk factors or symptoms, such as hematuria, dysuria, or smoking from September 2001 to May 2002. None of these subjects had a prior history of bladder malignancy. This cohort of subjects provided a voided urine sample for BladderChek test prior to cystoscopy as well as cytology specimens.

The second prospective multi-center database evaluated BladderChek test utility in bladder cancer surveillance (11). This study also involved 23 clinical facilities in 9 US, states, including academic, Veteran's Affairs Hospitals, and private practice facilities. From September 2001 to February 2002, 668 consecutive subjects with a history of bladder cancer were enrolled. In this cross-sectional study, each participant provided a voided urine sample for cytology and BladderChek prior to cystoscopy.

Lastly, a total of 434 consecutive subjects were solicited from urology clinics from the University of Texas Southwestern Medical Center at Dallas and Parkland Health and Hospital Systems (Dallas, TX) from January of 2008 to September of 2009. Subjects recruited were presenting for initial hematuria workup (gross and microscopic), dysuria, other suspicious symptoms, and for routine BC surveillance. Only participants with known, active bladder cancer at the time of evaluation were excluded. Each subject provided a voided urine sample in order to perform the BladderChek test. The test was performed by adding 4 drops of voided urine to the sample well of the point-of-care device. Posi-

tive or negative results were read 30 to 50 minutes later in the test window. A built in control indicated that the assay was complete. Four patients were omitted from the study because of 2 consecutive inconclusive findings on BladderChek test. Subjects undergoing the BladderChek test subsequently had a cystoscopy and a barbotage urine cytology. The cytology sample was sent and examined according to institutional protocols by staff pathologists with expertise in genitourinary cytopathology.

RESULTS

In the first multi-center database of 1331 subjects examining NMP22 BladderChek test's ability to detect bladder cancer in high risk individuals, 1065 subjects had both a negative cystoscopy and BladderChek test. There were only 3 cancers (stages Ta grade 2, Tis and T1 grade 1) diagnosed in this group. In these patients, cytology was atypical in one and reactive in 2 of the subjects.

With respect to the second multi-center database that examined the BladderChek test's ability to detect bladder cancer during surveillance for recurrent bladder cancer, 437 of 668 subjects met inclusion criteria with a negative cystoscopy and BladderChek test. Cancer was diagnosed in 2 patients (stages Tis and Ta grade 2). The patient with Tis had a history of BCG and cytology showed dysplasia. The patient with the Ta tumor had a reactive cytology.

Validation was then performed in our institution's cohort of 434 subjects. Of the original cohort, 288 patients met the study inclusion criteria of having both a negative cystoscopy and BladderChek test. There were 83 patients undergoing evaluation for hematuria (gross (n = 43) and microscopic (n = 40)). There were 203 patients undergoing cystoscopy for bladder cancer surveillance. One patient had evaluation for suspicious imaging and one for unclear reasons. There were 176 and 112 men and women, respectively. The mean age was 64 (range 20-92). Further evaluation showed the presence of only one diagnosed cancer. The tumor was a Ta ureteral urothelial carcinoma with a reactive cytology.

Cumulative analysis of the data for these 3 prospective studies revealed only 6 subjects with cancer from a cohort of 1790 subjects having both a negative cystoscopy and a negative BladderChek test (Table-1). Of these 6 subjects diagnosed with cancer, only 1 patient with a history of BCG and Tis had dysplasia on cytology. In the remainder of these patients, cytology was either atypical or reactive.

DISCUSSION

This study evaluated a large cohort of patients with a negative cystoscopy and negative BladderChek test and found that these patients are at an exceedingly low risk for missed malignancy (n = 6, 0.3%). The addition of cytology in these patients did not detect any of these missed malignancies except for finding dysplasia in one patient with Tis. The strength of this study is the fact that all the patients were prospectively evaluated both in the setting of detection and surveillance using cystoscopy, cytology and the BladderChek test. This type of design reduces the risk of selection bias in the cohort. The data from the 2 large multicenter studies was performed in 23 centers and their results were confirmed at our institution in a prospective fashion.

Even though cystoscopy has a very high sensitivity and specificity, most urologists use adjunct testing such as cytology to assure that no tumors were missed. NMP22 BladderChek test has a very high negative predictive value (96.8% in setting of detection and 90.5% in setting surveillance) (10,11). Our hypothesis was that combining the 2 tests would miss very few cancers and in fact this was the case. While cytology is usually used as an adjunct to cystoscopy, it provided little benefit in patients with negative cystoscopy and negative BladderChek with the exception of the patient with history of CIS who had dysplastic cells in his urine. The combination of cystoscopy and BladderChek had a higher sensitivity and negative predictive value than cystoscopy and cytology in both prospective multicenter studies (10,11). There is also evidence that the sensitivity of cytology has decreased over time (12).

Several other studies have evaluated the utility of cytology. Falebita et al. retrospectively evaluated urine cytology over a 6 year period at their institution (13). They found that out of 2,568 urine cytological examinations, only 25 were positive for

Table 1 *- Study Cohorts.*

Study	Total Number of Patients	Number of patients with Negative Cytology and Negative BladderChek	Missed Cancers (n)	Missing Tumor Pathology	Cytology
Detection (16)	1331	1065	3	Ta, Tis, and T1	1 atypical 2 reactive
Surveillance (17)	668	437	2	Ta, Tis	1 reactive 1 dysplastic
UTSW Validation Cohort	434	288	1	Ta Ureteral	1 Reactive

malignant cells with a cost of 210,000 euros. They concluded that routine urine cytology was not cost effective and did not add to the diagnostic yield beyond cystoscopy and diagnostic imaging. Feifer et al. evaluated 200 consecutive low-risk patients with microscopic hematuria and found that none had a positive cytology, 23 (11.5%) had atypical cytology, and 177 (88.5%) had negative urinary cytology (14). Cytology missed 8 bladder cancers and the cost of performing urinary cytology was estimated at $262 per patient.

There are additional disadvantages to cytology including atypical findings which raise concern for malignancy especially in patients with a history of bladder cancer (15). Furthermore, cytology is not a point of care test such that urologists must contact patients with results and possibly schedule additional visits to discuss outcomes. Patients also have the added anxiety of waiting for the result. In addition, cytology is more costly than a BladderChek test. Cytology can cost from $50-100 while the BladderChek test costs $20-$24 (16). While these differences in cost are not very large, they can add up considering the frequency of cystoscopy and the high prevalence of patients who are evaluated for bladder cancer based on hematuria or undergoing routine surveillance. Adjunct tests contribute to bladder cancer being the most expensive cancer from diagnosis to death (17).

One potential unanswered question in our study is what to do with a positive BladderChek test and a negative cystoscopy. The specificity of urine-based tumor markers is inferior to cytology and false positive results can raise a concern of missed malignancy. In the absence of imaging to evaluate the upper tract, one should consider whether the abnormal marker is detecting a malignancy in the kidney or ureter. Imaging of the upper tracts may be helpful in this setting. The use of a secondary marker such as the Urovysion FISH assay or cytology may be of value and is something that we are currently evaluating.

Close surveillance of these patients is probably advisable as some studies suggest the possibility of a higher risk of cancer recurrence in patients with positive urine markers even if a tumor is not identifiable (18,19).

In patients with low grade disease, we are planning to initiate a study comparing use of cystoscopy with BladderChek compared to cystoscopy and cytology. At this time, it is unclear whether cytology should not remain standard in patients with high grade disease or CIS. There was one patient with a history of CIS whose recurrence was identified based on a dysplastic urine cytology despite a negative cystoscopy and negative BladderChek. At the same time, a meta-analysis of the literature found a similar sensitivity for detecting high grade bladder cancer for cytology and most urine-markers (including NMP22) (9).

CONCLUSIONS

In patients with a negative cystoscopy and BladderChek, there are very few missed cancers. Cytology also missed of these tumors except one

patient with a history of CIS. Use of a point of care test in conjunction with cystoscopy in lieu of cytology could decrease cost, provide immediate results, improve negative predictive value and reduce the uncertainty that results from inconclusive cytologic results. This may be particularly valuable in patients with history of low grade cancers.

CONFLICT OF INTEREST

None declared.

ABBREVIATIONS

BladderChek: NMP22® BladderChek®
UCB: Urothelial carcinoma of the bladder
CIS: Carcinoma in situ

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