UROLOGY – ORIGINAL PAPER

In the cystoscopic follow-up of non-muscle-invasive transitional cell carcinoma, NMP-22 works for high grades, but unreliable in low grades and upper urinary tract tumors

Enis Coskuner · Ibrahim Cevik · Alp Ozkan ·
Ozdal Dillioglugil · Atıf Akdas

Abstract

Introduction and objectives Two percent of the bladder non-muscle-invasive (NMI) transitional cell carcinomas (TCC) are associated with upper urinary tract (UUT) TCC. We evaluated the role of nuclear matrix protein-22 (NMP-22) (BladderChek®) test in the diagnosis of lower urinary tract and UUT-TCC.

Methods From March 2009 to June 2011, 122 patients with bladder NMI-TCC underwent 205 control cystoscopy. A total of 95 (78 men and 17 women, mean age 60.7 years, range, 27–88) patients who were followed regularly with NMP-22 test and with follow-up cystoscopies (145 episodes; min. 1–max. 5) were included in this study. For routine monitoring of the UUT, IVU or CT urography was used once a year for high grades (HG), and once in every other year for low grades (LG). The sensitivity and specificity of NMP-22 were evaluated by ROC curves, and sensitivity, specificity, and positive and negative predictive values were calculated. Chi-square test was used for the differences between the subgroups.

Results Cystoscopy and NMP-22 results of the patients included in the study revealed the sensitivity (44.4%) of the test was very low and the specificity (98.4%) was quite high ($p < 0.001$). Among the 10 cystoscopies where NMP-22 was negative, but cystoscopy was positive for tumor, 8 had LG and 2 had HG TCC. NMP-22 was never positive in low-grade tumors, in other words, all of the NMP-22-positive 8 tumors were high grade. On the other hand, in 20% (2/10) of the cases, NMP-22 can be negative although the tumor was high grade. Two (2.1%) HG UUT-TCC were detected in 95 patients. These 2 patients were within the 125 cystoscopies (75 patients) where both NMP-22 and cystoscopy were negative for tumor.

Conclusions Nuclear matrix protein-22 cannot detect LG TCC. However, it detects overwhelming majority of HG TCC. For this reason, positive NMP-22 test largely indicates HG TCC. NMP-22 is also not reliable in UUT-TCC, even in HG tumors.

E. Coskuner
Department of Urology, School of Medicine,
Acıbadem University, Istanbul, Turkey

I. Cevik (✉)
Department of Urology, School of Medicine,
Yeni Yuzyil University, Istanbul, Turkey
e-mail: cevik@renetworks.net

A. Ozkan · O. Dillioglugil
Department of Urology, School of Medicine,
Kocaeli University, Kocaeli, Turkey

A. Akdas
Department of Urology, School of Medicine,
Marmara University, Istanbul, Turkey

Introduction

More than 75% of patients with bladder cancer are diagnosed as having non-muscle-invasive bladder cancer (NMIBC), including carcinoma in situ (CIS) at presentation [1]. The rate of intravesical recurrence is approximately 75% [2–5]. A significant number of recurrences occur more than 5 years after primary diagnosis [6–8]. In this context, early diagnosis of the disease and prevention of intravesical recurrence and muscle-invasive progression after the initial treatment necessitates lifelong follow-up. The standard follow-up of bladder cancer includes cystoscopy in every 3–4 months during the first 2 years and yearly thereafter, supplemented by cytology [9]. However, up to 10% of all superficial bladder cancers can be missed in cystoscopy, if they are flat lesions or CIS [10–12]. Cystoscopy is also invasive and costly. For overcoming all of these limitations, non-invasive tools for bladder cancer detection and follow-up have been intensively sought.

Nuclear matrix protein-22 (NMP-22) is reportedly an objective, non-invasive, quantitative test with good accuracy in bladder cancer diagnosis [13, 14]. NMP-22 is an urothelial cancer-associated protein, which is released into urine during apoptosis [15]. It has been shown that intracellular nuclear NMP-22 concentration is at least 25-fold greater in bladder cancer cells compared to normal bladder tissue [16]. The U.S. Food and Drug Administration have approved the NMP-22 test for the detection of occult or rapidly recurring disease after transurethral resection of bladder tumor [17]. Commercially, two NMP-22 tests are available; the original quantitative Sandwich-type ELISA (Bladder Cancer Test®) and a qualitative point-of-care test (BladderChek®).

Upper urinary tract TCC is a relatively rare tumor. Approximately 0.7–4% of patients with primary bladder cancer develop upper tract cancers [18]. Most of these tumors are diagnosed between 3 and 6 years after the initial diagnosis of bladder cancer [19]. Although there is no certain monitoring recommendation, positive cytology followed by intravenous urography or abdominal tomography is the only way to detect upper urinary tract tumors [20].

In this prospective study, we evaluated the performance of NMP-22 Bladder Check® test in the cystoscopic follow-up of patients with NMIBC and its value in the diagnosis of upper tract cancers.

Materials and methods

From March 2009 to June 2011, 122 patients with history of non-muscle-invasive transitional cell carcinoma of the bladder (pTa-pT1, low or high grade) underwent 205 control cystoscopy. A total of 95 (78 men and 17 women, mean age 60.7 years, range, 27–88) patients who had regular control cystoscopies and NMP-22 test (BladderChek® Matritech Inc., Germany) were included in the study. We evaluated 145 cystoscopy episodes (min. 1–max. 5 times) in this patient group.

For routine monitoring of the upper urinary tract, intravenous urography or CT urography was used once a year for high grades, and once in every other year for low grades. TNM 2002 system was used for tumor staging [21]. Tumor grading was assigned as low grade and high grade [22].

The BladderChek® test for the nuclear matrix NMP-22 is an immunochromatographic assay utilizing monoclonal antibodies in a lateral flow strip encased in a plastic box. For prevention of false-negative test results, it is suggested to maintain urine in the bladder at least 2 h, before performing the BladderChek test. No intravesical therapy was given within 4 weeks prior to inclusion. Urine samples were also examined for any kind of inflammatory conditions, and the test was not performed in the presence of inflammation. The BladderChek test was performed immediately on fresh voided urine. Four drops were placed in the sample field of the BladderChek test device. After 30–50 min, the result was read in the test field as positive or negative. Data were analyzed using STATA version 11.2 (StataCorp, TX, USA). Sensitivity, specificity, positive, and negative predictive values were calculated. Chi-square test was used for categorical variables. A value of $p < 0.05$ was accepted as statistically significant.

Results

Cystoscopy and NMP-22 results are presented in Table 1. Although the sensitivity (44.4%) of the test

Table 1 Results of NMP-22 and cystoscopy

	Cystoscopy (−)	Cystoscopy (+)	Total
NMP-22 (−)	125	10	135
NMP-22 (+)	2	8	10
Total	127	18	145

was very low, the specificity (98.4%) was quite high ($p < 0.001$). The positive predictive value (PPV) was 80%, and the negative predictive value (NPV) was calculated quite high as 92.6%. Among the 10 cystoscopies where NMP-22 was negative when cystoscopy was positive for tumor, 8 had low-grade and 2 had high-grade TCC. On the other hand, in all of the 8 cystoscopies where both NMP-22 and cystoscopy was both positive, all tumors were high grade. While NMP-22 false positivity occurred only in 1.57% (2/127) of the patients, false negativity was unacceptably high [10/18 (55.6%)] (Table 1). Tumor grades of the 18 recurrent tumors in relation to NMP-22 status are shown in Table 2. Among NMP-22 negative patients, all of the 8 low-grade tumors were Ta stage, while 1 of the high-grade patients was T1 and 1 was Ta. Among the NMP-22-positive patients, 6 of the high-grade ones were T1 and 2 were Ta. NMP-22 was never positive in low-grade tumors (100% false negativity), in other words, all of the NMP-22-positive 8 tumors were high grade. On the other hand, in 20% (2/10) of the cases, NMP-22 can be negative although the tumor was high grade.

Two (2.1%) upper urinary system tumors were detected in 95 patients. Both of these ureteral tumors were high grade. They were within the 125 cystoscopies (75 patients) where both NMP-22 and cystoscopy were negative for tumor.

Discussion

Patients with NMIBC are initially treated by transurethral resection. Because of its high recurrence rate

Table 2 Tumor grades and NMP-22 status of recurrent tumors

	NMP-22 (−)	NMP-22 (+)	Total
Low grade	8	0	8
High grade	2	8	10
Total	10	8	18

and possibility of progression to a muscle-invasive stage or metastasis, close surveillance of patients is mandatory. A lifelong monitoring after initial treatment of NMIBC should be done. It has been estimated that one in 1,450 people in the Western world is under surveillance for bladder cancer [23]. Routine continuous follow-up for bladder cancer is a standard care of NMIBC treatment. It is also important to define risk of recurrence and progression of bladder cancer in a surveillance population [24]. Adherence to surveillance has been evaluated among a population-based sample of 6,716 Medicare enrollees diagnosed with NMIBC. According to this study, only 39.7% of patients had all of the 5 follow-up visits [25]. About 60% of patients with NMIBC did not regularly adhere to the follow-up protocol.

It is easy and relatively comfortable to detect or monitor bladder cancer in an office setup with a flexible cystoscopy for an urologist. However, on the patients' side, it is not. The lack of compliance to surveillance necessitates the utilization of more comfortable methods rather than cystoscopy. Although urine biomarkers have higher sensitivity than cytology, their sensitivity is lower than cystoscopy [26]. Therefore, cystoscopy cannot be replaced by more comfortable urine biomarkers.

Cystoscopy is the gold standard for monitoring of patients with bladder cancer. However, it has some drawbacks like difficulties to identify flat lesions, no diagnostic value for upper urinary tract [11]. Several biomarkers have been developed to detect recurrent bladder cancer in voided urine samples as an alternative for invasive and expensive cystoscopy procedure. Sensitivities of these biomarkers are low, especially in patients under surveillance [27]. Particularly for NMP-22, range of sensitivity is from 47 to 100% and its specificity from 60 to 90% [28]. Shariak T. et al. [29] performed NMP-22 test on 302 patients at risk for recurrence, and the sensitivity and specificity of the test were reported as 66 and 73%, respectively. The sensitivity was low for Ta and G1 tumors 46%. Another large study was performed by Grossman et al. [11] in monitoring of 668 patients with a bladder cancer history and found steadily increasing sensitivity of NMP-22 for identification of recurrences with increasing stage from pTa and pT1 to pT2 as 36, 65, and 88%, respectively. Cytology and cystoscopy is standard in the surveillance of bladder cancer. In a meta-analysis, sensitivity and specificity of cytology

were 34 and 99%, respectively. In the same meta-analysis, sensitivity and specificity of NMP-22 were 73 and 80%, respectively. In this meta-analysis, all biomarkers for bladder cancer (NMP-22, BTA-TRAK and Stat, FDP, CYFRA, BCLA-4, etc.) were individually more sensitive and less specific than cytology [26]. In a study where NMP-22 test was evaluated as a tool for surveillance of recurrent bladder cancer, sensitivity of cystoscopy and NMP-22 were reported as 91.3% (94/103) and 45.7% (43/94), respectively. Eight of 9 tumors that were overlooked by cystoscopy were high grade, but NMP-22 was positive in 7 of the 8 of these high-grade tumors. Thus, combination of cystoscopy and NMP-22 test resulted in a sensitivity of 99% (102/103) [30]. In the present study, the overall sensitivity of NMP-22 test was 44.4% for recurrent tumors. Our specificity was 98.4% and quite higher than the older studies and also almost equal to cytology. Because all of the recurrent tumors were detected by cystoscopy, statistical analysis for combination of cystoscopy with NMP-22 test is not possible in the present study.

In another study, the sensitivity of NMP-22 increased from 36 to 100% from pTa stage to pT4 and increased from 31.6 to 75.0% from well-differentiated tumors to poor differentiated ones [30]. In the study performed by Hwang et al. [31], the sensitivity of NMP-22 was reported as 26.5% for Ta and T1 tumors and 61.5% for T2 and higher stages. In both of these studies, sensitivity increased steadily with increasing T stage and tumor grade. In the present study, all NMP-22-positive patients had high-grade tumors, and all low-grade tumors were NMP-22 negative (Table 2).

History of TCC of bladder is a known risk factor for development of TCC in the upper urinary tract. Most guidelines do not recommend routine monitoring of the upper urinary tract for all patients with a history of bladder cancer but favor imaging strategies based on risk stratification of primary bladder tumor. For example, in patients who received intravesical BCG for NMIBC, the incidence of upper urinary tract cancer increases to 20–25%. High-risk patients (T1, high grade, multifocal, and CIS) were suggested to undergo upper tract imaging annually for the first 5 years and then every 2 years, and low risk (Ta, low grade) patients may be offered UUT imaging upon symptoms or stage or grade progression [32]. Intravenous contrast morbidity and radiation exposure are the

disadvantages of these imaging technics. Urinary cytology and other urinary biomarkers in the early diagnosis of the upper tract cancers are optional. Also postradical cystectomy, urine cytology, and imaging are used for primary monitoring of UUT tumors. Urine-based biomarkers are increasingly used, but the efficacy and accuracy in these patients remain unclear [33]. In a study by Jovanovic et al., diagnostic value of cytology and NMP-22 was evaluated in 34 patients with UUT tumors. Two samples were collected from each patient, one from urine obtained by ureteral catheterization from affected ureter and one from voided urine. According to the results, sensitivity and specificity of NMP-22 test from catheterized urine were 73.5 and 88%, and from voided urine were 70.5 and 92%, respectively. For cytology, although the sensitivity of catheterized urine (64.7%) and voided urine (58.8%) was lower than NMP-22, its specificity was higher both in catheterized (96%) and voided (96%) urine samples. These results are promising for detecting UUT tumors with a urine biomarker. However, combining NMP-22 test with UUT imaging may help monitoring UUT for patients with NMIBC. In the same study, high-grade (Grade 3) UUT tumors were detected more (91.7%) than low grades (Grade 1 28.6%, Grade 2 80%) by NMP-22 test from catheterized urine samples [34]. In our patient group, upper urinary tract cancer ratio is 2.1% consistent with the literature. Both of these two patients had high-grade multicentric bladder tumors and had intravesical BCG treatment following their initial tumor resection. Upper urinary tract cancer was diagnosed by imaging in the third year after the initial diagnosis of bladder cancer; NMP-22 and cystoscopy were negative in both of these two patients, although they had high-grade ureteral tumors. Pathology of the nephroureterectomy specimen confirmed the diagnosis in both of them.

It is obvious from the present study that NMP-22 test has unacceptably high false negativity rate for the whole patients with positive cystoscopy (55.6%), and for low (100%) and high-grade (20%) tumors (Table 2). Similar to our study, false negativity has been reported to be quite high (43%) in another study examining the role of NMP-22 in the follow-up of bladder cancer [35]. Because of this high false negativity, NMP-22 should be accompanied by cystoscopy in the monitoring of bladder cancer.

In conclusion, although NMP-22 BladderChek® test is easy to perform, user independent, and used in

office, it is not reliable in the detection of low-grade TCC. On the other hand, it detects overwhelming majority of high-grade TCC. For this reason, positive NMP-22 test largely indicates high-grade TCC. NMP-22 is not reliable in UUT-TCC, even in high-grade tumors. Because of high false-negative rates, NMP-22 should be accompanied by cystoscopy for the monitoring of bladder cancer.

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