Comparison of NMP22 BladderChek Test and Urine Cytology for the Detection of Recurrent Bladder Cancer

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Objective: To assess the clinical performance of the NMP22 BladderChek test, which is a qualitative test, and to compare it with voided urine cytology for the detection of recurrent bladder cancer. We also evaluated whether cystoscopy can be omitted from the surveillance protocol by combining the two tests.

Methods: A total of 131 patients with a history of superficial transitional cell carcinoma of the bladder provided urine samples before a cystoscopic examination. Urine samples were assayed for the presence of NMP22 using the NMP22 BladderChek test and cytology was performed by a cytopathologist. Selected patients underwent a biopsy, with appropriate additional therapy. Results of the two tests were compared with that the results of cystoscopy, which was retained as the gold standard. For positive biopsies, the results of the NMP22 test and cytology were also correlated with the tumor stage and grade.

Results: Of the 46 recurrences detected by cystoscopy, the NMP22 test was positive in 39 cases and cytology in 19 cases. The sensitivity of the NMP22 test was 85%, which was significantly greater than that of cytology (41%). In particular, for low-risk tumors it was eight times more sensitive than cytology. The specificities of the NMP22 test and cytology were 77 and 96%, respectively. Combining the two tests increased overall sensitivity to 91%. However, 9% of the tumors were still not detected.

Conclusion: The NMP22 BladderChek test is an in vitro qualitative test that is easily available and cheap; it can be performed by a urologist in the office and results can be interpreted within 30 min. The NMP22 test is superior to cytology for all grades and stages in the detection of recurrence in patients with a history of superficial bladder cancer. Our study indicates that the NMP22 test can be used as a substitute for urine cytology. The NMP22 test cannot replace cystoscopy, but it can be used as an adjunct to cystoscopy in the surveillance protocol for patients with superficial bladder cancer.

Key words: bladder cancer – tumor marker – NMP22 – cytology – surveillance

INTRODUCTION

Cystoscopy and voided urine cytology are effective diagnostic methods for surveillance of superficial bladder cancer. Flexible cystoscopy has made cystoscopy more acceptable to patients, but it remains an invasive procedure. Voided urine cytology also has various drawbacks, such as the requirement for the screener to be trained to make evaluations, the impossibility of quantification and insufficient sensitivity, particularly for low-grade tumors (1).

A wide range of alternative procedures and markers have been proposed and studied for the detection of recurrent bladder tumors. These include flow cytometry, the M 344 antigen (2), nuclear matrix protein 22 (NMP22) (3), the BTA test (4), quantitative fluorescence image analysis (5), the autocrine motility factor (6), activity of ribonuclear protein telomerase (7) and fibrin/fibrin degradation products (8).

The NMP22 BladderChek test uses a specific nuclear matrix protein to detect a bladder tumor via voided urine. We compared the NMP22 BladderChek test with voided urine cytology for the detection of recurrent bladder cancer in patients previously diagnosed with superficial bladder cancer using a qualitative analysis of NMP22 in voided urine, based on a predetermined threshold concentration.

Previous studies have used NMP22 as a quantitative test. Our prospective study is among the first to use NMP22 as a qualitative test for comparison with cytology for the diagnosis of recurrent superficial bladder tumors. We also evaluated whether cystoscopy can be omitted from the surveillance protocol by combining the two tests.

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PATIENTS AND METHODS

A total of 131 patients with previously diagnosed superficial bladder cancer (TNM stages Ta–T1, G1–G3, N0, M0), on follow-up in our institution, were prospectively enrolled in the study. Patients with urinary tract infections, concurrent urolithiasis, a history of bladder interposition and other malignancies were excluded from the study.

A single voided urine specimen was collected just prior to cystoscopy. Two aliquots were separated from this sample, one for the NMP22 test and the other for cytology. The NMP22 assay was performed according to the instructions provided in the NMP22 BladderChek test kit (Matritech, 330 Nevada St, Newton, MA).

The NMP22 BladderChek test uses a lateral flow immunochromatographic strip encased in a plastic cartridge to detect NMP22 in patients’ urine qualitatively. The assay incorporates two different monoclonal antibodies, one capture antibody and one reporter antibody. The test device requires four drops of urine at room temperature and gives the result within 30 min. A colored band in the test position indicates a positive result. All the NMP22 BladderChek test results were interpreted by a single observer (AK).

Voided urine cytology was carried out by a cytopathologist at our institution. The results were classified as malignant, suspicious for malignancy, inconclusive and normal. The last two were classified as negative.

Patients underwent cystopanendoscopy using a rigid cystoscope and video camera assembly. Any visible tumor or suspicious lesion was biopsied. Biopsies were evaluated using the TNM staging system (9) and WHO grading (10). Findings in cystoscopic biopsies were considered the gold standard and regarded as true positives for comparing the results of the other two tests. Patients with positive isolated NMP22 test or cytology in the absence of a cystoscopic lesion were further evaluated using an intravenous urogram or a contrast-enhanced computed tomogram to rule out an upper-tract lesion or a missed bladder lesion. If none was found, the result was considered a false positive, but the patient remained on follow-up with a higher index of suspicion. These patients were re-evaluated every 3 months for a mean of 8.8 months (range 3–17 months). Any lesion that subsequently developed was recorded. 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, based on the assumption that the lesion was missed on the last cystoscopy.

All observers interpreting the tests were blinded to the findings of other tests.

Statistical analysis was performed using the chi-square test, with Yates’ correction factor, with $P < 0.05$ being considered statistically significant.

RESULTS

Of the 131 subjects, 117 were males and 14 were females. The mean age was 67 years (range 32–91 years). The mean time since diagnosis of transitional cell carcinoma (TCC) of the bladder was 27 months (range 3–112 months). The mean number of previous recurrences was 3.6 (range 1–10).

Of the 46 patients with recurrence on histopathology, 39 were positive for the NMP22 test and 19 were positive for cytology. The sensitivities of the NMP22 test and cytology for the detection of recurrence were 84.8 and 41.3%, respectively; the specificities were 77.6 and 96.4%, respectively (Table 1). For Grade 1 and Grade 2 tumors, the NMP22 test detected more recurrence than cytology (81 versus 23%). This difference was statistically significant ($P = 0.009$). Sensitivity in detecting Grade 3 tumors was almost equivalent for the NMP22 test and cytology (92 versus 84%) (Table 2). When tumors were stratified by T stage, NMP22 was more sensitive than cytology in detecting Ta and T1 tumors (82 versus 30%, $P < 0.05$). Interestingly, all eight T2 stage recurrences were detected by both the NMP22 test and cytology (Table 3).

Nineteen cases were positive for the NMP22 test when no tumor was found on cystoscopy. None of these patients was positive for cytology. However, three were found to have bladder tumors on the first follow-up cystoscopy, suggesting that these may have been missed on the first cystoscopy. Thus, the NMP22 test had an overall false positive rate of 12.2%.

Pathological data were grouped according to risk for recurrence, progression and invasion into a low-risk group (Ta, G1–2), a high-risk group (TaG3, T1) and an invasive group (T2 or higher). The NMP22 test showed significantly higher sensitivity (up to eight times) than cytology in detecting low-risk group recurrences. It detected more recurrences than cytology in the high-risk group, but the difference was not statistically significant ($P = 0.09$) (Table 4).

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

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMP22 test</td>
<td>84.8</td>
<td>77.6</td>
<td>67.2</td>
<td>90.4</td>
<td>80.1</td>
</tr>
<tr>
<td>Cytology</td>
<td>41.3</td>
<td>96.4</td>
<td>86.3</td>
<td>75.2</td>
<td>77.1</td>
</tr>
</tbody>
</table>

Table 2. Sensitivity according to grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>$N = 46$</th>
<th>NMP22 test (%)</th>
<th>Urine cytology (%)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>11</td>
<td>81.8 (9/11)</td>
<td>18.8 (2/11)</td>
<td>0.009</td>
</tr>
<tr>
<td>G2</td>
<td>22</td>
<td>81 (18/22)</td>
<td>27.2 (6/22)</td>
<td>0.0009</td>
</tr>
<tr>
<td>G3</td>
<td>13</td>
<td>92.3 (12/13)</td>
<td>84.6 (11/13)</td>
<td>0.54</td>
</tr>
</tbody>
</table>
On combining the results of the NMP22 test and cytology, 42 of the 46 cystoscopy-positive tumors were detectable, giving an overall sensitivity of 91.3%. However, four patients with recurrence could not be detected using a combination of the tests. Of these four patients, two had TaG1 and the other two had TaG2 recurrences.

**DISCUSSION**

Cystoscopy is the primary diagnostic modality for the diagnosis of bladder carcinoma. Although it is the gold standard for detecting bladder cancer, it is invasive and relatively expensive (11). Voided urine cytology is the standard non-invasive method for diagnosis in the detection of bladder carcinoma (12–14). However, its sensitivity is low: between 11 and 76% in various studies (15). Several factors affect the sensitivity of cytology, including specimen quality, number of exfoliated cells and pathologist expertise. The overall low sensitivity of cytology is due to its low sensitivity in detecting low-grade bladder tumors (16).

Non-invasive urine markers can offer an alternative to the standard mode of detecting bladder cancer or they can be used as an adjunct to cystoscopy (17).

Nuclear matrix, first described in 1974, is the non-chromatin structure that supports nuclear shape and organizes DNA, and it takes part in DNA replication and transcription and in RNA processing (18–20). NMP22 is involved in the proper distribution of chromatin to daughter cells during cell division and is found in the nuclear matrix of all cell types. NMP22 is thought to be released from the nuclei of tumor cells after they die, and it can be detected in the urine. Research has found that patients with bladder cancer may have urinary levels of NMP22 that are 25-fold greater than levels in healthy subjects (21).

The NMP22 BladderChek test is an *in vitro* immunoassay intended for the qualitative detection of NMP22 in urine. It determines whether NMP22 is present in urine and provides an absolute positive or negative test result, in the same manner as a pregnancy test. Stamfer et al. (22) evaluated 231 patients with a history of superficial TCC of the bladder and found that NMP22 was two times more sensitive than cytology for the detection of TCC when using a reference value of 6.4 U/ml. In three separate studies, involving ~400 patients, Soloway et al. (23), Miyanaga et al. (24) and Landman et al. (25) demonstrated that the quantitative NMP22 test had an overall sensitivity of 70–80% for the detection of recurrent TCC. In comparison, cytology showed sensitivity of ~10–40%. The three sets of investigators used an NMP22 cut-off reference value of 6–20 U/ml.

Our study, using the NMP BladderChek test for qualitative analysis, with a predetermined cut-off value of 10 U/ml, showed that the NMP22 test had significantly higher sensitivity than cytology for detecting all 46 patients with recurrence. NMP22 showed an overall sensitivity of 85% compared with 42% for cytology. These findings correlate with the quantitative analyses of NMP22 performed by the authors mentioned above.

Superficial bladder tumors frequently recur after resection (50% or more), and the disease may progress in 30% of patients. Takashi et al. (26) performed a multivariate analysis of survival in 264 cases of primary superficial bladder cancer and noted certain prognostic factors and relative risks. By combining these, they identified six risk groups according to the number of any of the factors, including T2 or T3 stage, tumor size of >3 cm, irritative symptoms, age >70 years and Grade 3 disease. They concluded that the presence of a greater number of these factors correlated with worse evolution. Rodriguez et al. (27) performed a multivariate analysis on 1529 patients with superficial bladder cancer and identified risk groups that could determine recurrence, progression and mortality. They found that low-risk groups had a 37% rate of recurrence but no progression or mortality, whereas high-risk groups carried a 15% risk of progression to muscle-invasive disease and 9.5% mortality.

Traditionally, detection of these low-risk groups has been the greatest challenge for non-invasive assays. This is important particularly because repeat cystoscopy or transurethral resection of bladder tumors may be avoided or can be delayed in such patients (28).

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. In particular, in the low-risk group, the NMP22 test was eight times more sensitive than cytology. This finding can be utilized in low-risk group patients with a

<table>
<thead>
<tr>
<th>Pathological result</th>
<th>Number of patients</th>
<th>Sensitivity of NMP22 test (%)</th>
<th>Sensitivity of cytology (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk TaG1–2</td>
<td>18</td>
<td>83.3 (15/18)</td>
<td>11.1 (2/18)</td>
<td>0.001</td>
</tr>
<tr>
<td>High-risk TaG3,T1</td>
<td>20</td>
<td>80 (16/20)</td>
<td>45 (9/20)</td>
<td>0.09</td>
</tr>
<tr>
<td>Invasive T2 or higher</td>
<td>8</td>
<td>100 (8/8)</td>
<td>100 (8/8)</td>
<td>–</td>
</tr>
</tbody>
</table>
negative NMP22 test to modify the currently used rigorous surveillance protocol of cystoscopy.

Of our patients, 12.2% showed a false positive NMP22 result. Of these, 11 patients had received prior adjuvant intravesical therapy in the form of either intravesical chemotherapy or immunotherapy. Prior intravesical therapy is a known confounding factor that can lead to a false positive NMP22 test (29,30). Moreover, it is also possible that these patients harbor non-visible disease which will become clinically evident later. This can be clarified only with a longer follow-up.

Using a combination of NMP22 and cytology, the overall sensitivity increased to 91%. However, 9% of the recurrences were still not detected. Thus, even the combination of the NMP22 test and cytology cannot replace cystoscopy in the surveillance protocol.

CONCLUSION

The NMP22 BladderChek test is an in vitro immunoassay used for the qualitative detection of NMP22 in urine. It is quick, easily available and economical, and it causes no patient discomfort. Compared with cytology, it has higher sensitivity for all stages and grades of superficial bladder cancer. It is eight times more sensitive than cytology in detecting recurrence of low-risk superficial bladder tumors. However, even the combination of NMP22 and cytology may not detect 9% of the tumors. Thus, these tests cannot be used to omit cystoscopy from the surveillance protocol.

References