Evaluation of the NMP22 test and Comparison with Voided Urine Cytology in the Detection of Bladder Cancer

Kang Hyun Lee

National Cancer Center, Konyang, Korea

The purpose of this study was to assess the clinical performance of the NMP22 test and to compare it with that of voided urine cytology for the detection of bladder cancer. The NMP22 test was evaluated in two groups of patients. The first group was comprised of patients with histologically confirmed active transitional cell carcinoma (TCC) of the bladder, and the second group contained those with a history of bladder TCC but that were considered to have no evidence of disease on the basis of cystoscopic evaluation of bladder and/or biopsy.

Sensitivity was determined in voided urine samples from patients with active TCC of the bladder. Specificity was determined in the urine samples of patients with a history of bladder TCC but no current evidence of disease.

The NMP22 test was positive in 53 of 70 samples from patients with active bladder TCC. The sensitivity of the NMP22 test (75.7%) is significantly better than that of voided urine cytology (55.7%). The specificity of the NMP22 test and of voided urine cytology were 72.2% and 88.9% respectively, in patients with a history of bladder TCC but no current evidence of disease. There was no significant difference between the specificity of NMP22 and that of urine cytology.

The NMP22 test is superior to voided urine cytology in the detection of TCC of the bladder. The results of this study indicate that the NMP22 test is an useful adjunct to cystoscopy in the detection and monitoring of TCC of the bladder.

**Key words:** Bladder cancer, tumor marker, NMP22 test, cytology

**INTRODUCTION**

Bladder cancer is a common genitourinary cancer in Korea. The majority of bladder cancers are superficial or invasive into the lamina propria and amenable to transurethral resection. However, local recurrence rates range from 50% to 80%, and progression rates to muscular invasive bladder cancer range from 10% to 25%\(^2\). For these reasons, intensive surveillance is necessary to detect recurrent bladder cancers early in their natural history. In general patients are routinely monitored by cystoscopy and urine cytology.

Cystoscopy is the gold standard for bladder cancer diagnostics. However, it is an invasive procedure, and limited to tumors that are visible. The sensitivity of urine cytology is on average less than 50% and as low as 30% in low stage, low grade disease\(^3\). In addition, the evaluation of disease status by urine cytology is subjective, and analytical accuracy depends on the skill of the cytopathologist. Furthermore, inconsistencies have been observed among pathologists grading bladder tumors. In addition, intravesical chemotherapy, radiotherapy, repeated catheterization, urinary tract infections and stone disease can cause changes in the exfoliated cells in voided urine, which may interfere with bladder cancer diagnosis\(^5\). Consequently, a noninvasive urine assay that is sensitive, objective and quantitative would be an useful adjunct for an urologist treating patients with transitional cell carcinoma (TCC) of the bladder. The availability of a noninvasive diagnostic test that provides objective, quantitative results, used in conjunction with cystoscopy and urine cytology, would signifi-
cantly improve our ability to take clinical decisions regarding the disease status.

NMP22 is a new noninvasive, quantitative immunocassay that detects nuclear matrix proteins (NMP) in stabilized urine. Nuclear matrix proteins are part of the internal structural framework of the nucleus, and NMP may play important roles in DNA replication, transcription, the processing of RNA and the regulation of gene expression. NMP are present at low levels in the urine of normal individuals and increase in quantity in urothelial neoplasia. The NMP22 test kit is specific for the nuclear matrix protein NuMA (Nuclear Mitotic Apparatus Protein).

This trial was designed to assess the clinical performance of the NMP22 test, in patients with bladder TCC, in comparison with urine cytology.

MATERIALS AND METHODS

NMP22 and urine cytology were evaluated in two groups of patients. The bladder cancer group was comprised of 70 patients with histologically confirmed TCC of the bladder, in the age range 33 to 75 years (mean age 60.2 years).

The second group of 36 patients, in the age range 30 to 78 years (mean age 61.7 years), had a history of bladder TCC but were considered free of the disease on the basis of cystoscopic evaluation of the bladder and/or biopsy.

The sensitivities of both tests were determined in the voided urine samples of active bladder TCC patients before resection of the bladder tumor. The specificities of both tests were determined in the voided urine samples of patients with a history of bladder TCC but no current evidence of disease.

The NMP22 test was performed with a nuclear matrix protein testing kit NMP22 (Matritech, Newton, MA). All samples were processed according to the instructions supplied with the kit. Calibrators, controls and stabilized urine samples were added to an antibody coated microplate. The captured nuclear matrix protein antigen was washed and then reacted with a second antibody labeled with digoxigenin. The antigen was washed again and reacted with an antidigoxigenin antibody coupled to horseradish peroxidase and detected using an O-phenylene diamine substrate. The reaction was terminated by the addition of 2M. sulfuric acid. The concentration of antigen in urine was proportional to the intensity of the color developed, and the concentration of digoxigenin labeled NMP22 in the urine was calculated from a standard curve.

According to Kim et al, the cut off level used for the NMP22 test is 7.70 U/mL. Urine cytology was performed by a pathologist blind to the result of NMP22 testing. The diagnostic results of urine cytology testing were categorized as positive when specimens were described as suspicious malignancy and malignancy by pathologist. Bladder TCC was classified according to TNM stage and WHO grade.

The sensitivities of the NMP22 test and urine cytology were calculated according to depth of invasion, T stage (Ta, T1, T2) and the grades and the sensitivities of the two tests were compared. Results were compared using Yates corrected Chi-Squared test. p < 0.05 was accepted as statistically significant.

RESULTS

The NMP22 test was positive in 53 of 70 patients with active bladder TCC, with an overall sensitivity of 75.7%. Urine cytology was positive in 39 of 70 patients with bladder TCC, with an overall sensitivity of 55.7%, which was significantly lower than that of the NMP22 test (Table 1).

Table 1. Comparison of the Overall Sensitivity and Specificity of the NMP 22 Test and Cytology

<table>
<thead>
<tr>
<th></th>
<th>NMP 22 Test</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Sensitivity</td>
<td>75.7% (53/70)*</td>
<td>55.7% (39/70)</td>
</tr>
<tr>
<td>Overall Specificity</td>
<td>72.2% (26/36)</td>
<td>88.9% (32/36)</td>
</tr>
</tbody>
</table>

*significantly higher than cytology (p<0.05)

According to disease stage characteristics of the bladder cancer patients, the sensitivities of NMP22 test and urine cytology were 56.5%, 34.8% in stage Ta, 78.3%, 56.5% in stage T1, and 91.7%, 75.0% in stage ≥ T2, respectively, and the NMP 22 test was more sensitive at all stages, although the differ-
encers were not statistically significant (Table 2).

In patients with superficial bladder TCC (Ta, T1), the NMP22 test was positive in 31 of 46 cases (67.4%) and urine cytology positive in 21 of 46 cases (45.7%). In those with invasive TCC of the bladder (≥T2), the NMP22 test was positive in 22 of 24 cases (91.7%) and urine cytology positive in 18 of 24 cases (75.0%).

According to the disease grade characteristics of bladder cancer patients, the sensitivities of the NMP22 test and urine cytology were 57.9%, and 21.1% in grade 1, 79.4%, and 64.7% in grade 2, and 88.2%, and 76.5% in grade 3, respectively. The NMP22 test was more sensitive than urine cytology in all grades. In particular, the sensitivity of the NMP22 test was significantly higher than that of urine cytology in grade 1 cancers (Table 3).

**Table 2. Comparison of the Sensitivity of the NMP 22 Test and Cytology, According to the Disease Stage Characteristics of Bladder Cancer Patients**

<table>
<thead>
<tr>
<th>Stage</th>
<th>NMP 22 test</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta</td>
<td>56.5% (13/23)</td>
<td>34.8% (8/23)</td>
</tr>
<tr>
<td>T1</td>
<td>78.3% (18/23)</td>
<td>56.5% (13/23)</td>
</tr>
<tr>
<td>≥T2</td>
<td>91.7% (22/24)</td>
<td>75.0% (18/24)</td>
</tr>
<tr>
<td>Total (n=70)</td>
<td>75.7% (53/70)*</td>
<td>55.7% (39/70)</td>
</tr>
</tbody>
</table>

*significantly higher than cytology (p<0.05)*

**DISCUSSION**

The greatest challenge in the management of superficial TCC of the bladder is to prevent its progression to invasive disease. Although 70 to 80% of newly diagnosed tumors have superficial disease, stage and/or grade progression occurs in a significant number of patients.

Because progression and recurrence rates are high, extreme vigilance is necessary.

The standard of methodologies for the early detection of recurrent TCC of the bladder are cystoscopy and cytology, but cystoscopy is an invasive procedure and urine cytology is subject to variation among cytopathologists and is relatively insensitive to low grade disease.

The development of tumor markers that could reliably predict the presence of bladder TCC would considerably aid bladder cancer detection and management. The BTA stat test, BTA test, BTA TRAK assay, telomerase test, and immunostaining of Lewis X antigen have been introduced and studied.

Recently the NMP22 test, an enzyme immunoassay for nuclear mitotic apparatus protein in voided urine, was introduced for the detection of occult or rapidly recurring disease following transurethral resection of bladder tumor. Nuclear matrix proteins are part of the internal structural framework of the nucleus and have a role in DNA replication, transcription and processing of RNA, and possible the regulation of gene expression. The nuclear matrix protein testing kit, NMP22, measures a nuclear mitotic apparatus protein, a specific class of nuclear matrix proteins. This protein associates with the mitotic spindle apparatus during mitosis and may be involved in the proper distribution of chromatids to daughter cells. If improper distribution of chromatids takes place during mitosis, such as bladder cancer, there is 25-fold increase in nuclear mitotic apparatus protein when compared with the concentration in the urothelial cells of the normal bladder. At least a tenfold increase in nuclear mitotic apparatus protein has been shown to occur in cancer tissue and in transformed cell lines.

Carpinito and co-workers evaluated the utility of urinary NMP22 immunoassay as an indicator for the presence of urinary tract TCC. Among the
TCC patients examined, those with active disease showed significantly higher urinary NMP22 levels (median 6.04 U/mL) than those with no evidence of disease (median 4.11 U/mL). In addition, there was a statistically significant difference between the NMP22 levels of healthy volunteers (median 2.86 U/mL) and subjects with active TCC (median 6.04 U/mL). Studies upon the relationship between TCC and urinary NMP22 values demonstrated that the effect of disease far outweigh the possible effects of sex on the NMP22 assay results, and no difference in urinary NMP22 values was found between male and female TCC patients. They concluded that the NMP22 assay showed considerable promise as a tumor marker.

In a previous pilot study, colleagues and I studied the significance of urinary NMP as a tumor marker for TCC of the bladder. The exercise showed that the mean NMP22s of healthy volunteers and bladder TCC patients were 4.04 and 186.9 U/mL, respectively. The difference was statistically significant (p=0.028). In terms of detecting bladder TCC, the sensitivity of urine cytology was 68% and that of NMP22 test 80%, whereas a combination of the two gave a sensitivity of 88%, when a value of urinary NMP22 exceeding 7.70 U/mL was considered as positive. Specificity was not studied at that time. The finding suggested that NMP22 could increase the diagnosis and detection of the recurrence of the bladder TCC if the technique was combined with urine cytology as the urinary tumor marker of bladder TCC.

In this study, the NMP22 test was positive in 53 of 70 (75.7%) cases of active bladder cancer, and is significantly better than the sensitivity of voided urine cytology (55.7%). Stampfer et al also reported that the sensitivity of NMP22 for the detection of bladder TCC was as much as twice that of cytology when a reference value of 6.4U/mL was used.

However, NMP22 is not a perfect bladder tumor marker. As is true for cytology, the NMP22 test has a lack of specificity for certain urologic conditions and a lack of sensitivity, especially for the lower grades and stages of TCC.

When analyzing the sensitivity of NMP22 in this study, in terms of disease stage and grade, its sensitivity to invasive cancers (≥ T2) proved significantly higher than that of stage Ta cancers. According to Soloway et al, a comparison between NMP22 and cytology in recurrent bladder cancers showed similar sensitivity to high grade or muscle invasive cancers but that NMP22 was 2 to 3 times more sensitive in detecting recurrent low stage/low grade cancers.

In this study, the sensitivities of NMP22 test were higher than those of urine cytology in all stages, although the differences were not statistically significant.

The sensitivity of NMP22 test was higher than that of urine cytology in all stages, and this difference was statistically significant in grade 1 cancers. These findings suggest that the urinary NMP22 test is better than urine cytology at detecting bladder TCC, especially in low grade cancers.

Reports to date suggest that novel diagnostic tumor markers show a lower specificity and lower positive predictive value than is desirable, and have high false positive rates. The effectiveness of the NMP22 test can be significantly enhanced by adopting exclusion criteria, which include, urolithiasis, urinary tract infection, non TCC genitourinary malignancy, genitourinary tract instrumentation, internal urinary stent, and bowel interposition. Our present study was undertaken using these exclusion criteria to reduce the false positive rate. In our study, the specificity of the NMP22 test and urine cytology were 72.2% and 88.9%, respectively, in cases with a history of bladder cancer but no current evidence of disease. The specificity of urine cytology was higher than that of the NMP22 test, although the difference was not statistically significant.

Though NMP22 offers advantages over urine cytology in terms of its increased detection rate, it may be argued that its decreased specificity is a disadvantage. However, one is generally more concerned about missing a clinically significant tumor than in subjecting a patient with a false positive result to additional cystoscopy. Consequently, in terms of a screening test, the maximization of sensitivity, rather than specificity, is paramount.

NMP22 has a role in tumor monitoring as it is a noninvasive, quantitative test with good accuracy in predicting disease status following cystos...
copy. Soloway and co-workers examined the recurrence of bladder cancer when a primary tumor was resected. Urinary NMP22 levels were measured more than 5 days after tumor resection or during follow up surveillance cystoscopy. When recurrence rates were compared, patients with NMP22 values of less than 10 U/mL were found to have a lower recurrence rate than those with values greater than 10 U/mL. Patients with post-resection NMP22 values greater than 20 U/mL had very high recurrence rates. These findings provide the urologist with an additional quantitative, noninvasive tool for the evaluation of a patient with TCC shortly after surgery, and might allow the urologist to afford the patient better treatment. Moreover, the use of NMP22 may reduce the frequency of diagnostic cystoscopy in future.

In conclusion, NMP22 is a new noninvasive, quantitative test performed on voided urine and is superior to voided urine cytology in the detection of bladder TCC. The sensitivity of the NMP22 test was 75.7% in patients with active bladder cancers, and is significantly higher than that of voided urine cytology (55.7%). The urinary NMP22 test shows considerable promise as a tumor marker.

The results of this study indicate that the NMP22 test is superior to voided urine cytology in the detection of bladder TCC, an useful adjunct to cystoscopy and an useful monitoring tool for bladder TCC.

Kang Hyun Lee, M.D.
National Cancer Center, 809 Madu 1-Dong, Ilsan-Gu, Koyang, Kyonggi 411-351, Korea.
Tel: 82-31-920-1676, Fax: 82-31-920-1759,
E-mail: kanghlee@kcchsun.kcch.re.kr

REFERENCES
