



## COMPARISON OF NMP22 URINARY MARKER TEST AND URINE CYTOLOGY IN THE EARLY DETECTION OF RECURRENT TRANSITIONAL CELL CARCINOMA OF URINARY BLADDER

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### ABSTRACT

**Background:** Urinary Bladder Cancer is the second most common cancer treated by urologists. Its incidence is increasing with age and exposure to environmental toxins. Smoking is a known risk factor. The gold standard of diagnosis of bladder cancer non invasively by urine cytology and invasively by cystoscopy with biopsy for histopathological examination. NMP22 is a tumor marker found in urine that had high sensitivity in detecting low grade and early stage urothelial carcinoma. **Aim:** To assess clinical utility of NMP22 Bladder Check Test and to compare it with voided urine cytology in early detection of recurrent Bladder Cancer. **Patients & Methods:** A total of 125 patients of follow up cases of Bladder Cancer were

enrolled in this study. Urine samples were assayed for the presence of NMP22 using NMP22 Bladder Check Test and Cytology was performed by a cytopathologist. The diagnosis, determined from the Cystoscopic findings and biopsy findings of the suspicious lesion was considered as the gold standard. For positive biopsies, the results of the NMP22 Test and cytology were also correlated with tumor grade and Stage. **Results:** From 125 patients, a total number of 59 patients who were positive in cystoscopy with histopathological examination which represent the gold standard in our study. Mean age of the patients was 63 years for males and 67 years for females. The NMP22 test was positive in 45 cases and cytology in 21 cases. The sensitivity of NMP22 Test in recurrent bladder cases was 83.3% which was significantly greater than that of cytology 39.5%. In non-invasive lesions of Bladder Cancer (Ta,T1), NMP22 Test and Cytology was positive in 92.5% and 44% of cases respectively. In muscle invasive lesions, NMP22 Test and cytology were equal(100%). The sensitivity of the

NMP22 test in low grade tumors (GI,GII) was 75%, which was significantly greater than that of cytology 28%, whereas in high grade tumors (GIII) the sensitivity of NMP22 and cytology were nearly equal (90.9% versus 81.8%) respectively. **Conclusion:** The NMP22 Bladder Check is a new point of care diagnostic test for urinary bladder cancer. The results of our study have shown that the NMP22 can be used as a follow up tool with urine cytology as we detected high sensitivity and specificity of NMP22 in recurrent bladder cases especially effective in low grade disease where the cytology sensitivity is low but at this time NMP22 cannot replace cystoscopy surveillance from patients with history of bladder cancer.

**KEYWORD:** NMP22, cell carcinoma of urinary bladder.

## INTRODUCTION

**Background:** Urinary Bladder Cancer is the second most common disease treated by urologists<sup>[1]</sup> urothelial cancer is a cancer of the environment and age, the incidence and prevalence rates increase with age and there is a strong association between environmental toxins and urothelial cancer formation.<sup>[2]</sup> Males are 3 to 4 times more likely to develop bladder cancer than females, presumably because of an increased prevalence of smoking and exposure to environmental toxins. It is generally a disease of the middle aged or elderly person, with a median ages at diagnosis being 69 yr. in Males and 71yr for female.<sup>[2]</sup>

## CAUSES

1-Cigarette smoking: Tobacco is the main known risk factor for urothelial cancer formation, particularly cigarette smoking, accounts for 60% and 30% of all urothelial cancers in males and females, respectively.<sup>[3]</sup> Exposure to arylamines through tobacco smoke has been well recognized as a major risk factor.<sup>[4]</sup>

2-Exposure to chemicals: 2-Naphthylamine, 4-Aminobiphenyl, Benzidine in chemicals, dyes, leather, petroleum factories. Exposure to paint dyes, heavy metals, arsenic in drinking water and polycyclic aromatic hydrocarbons (PAH) have been found to be associated with higher bladder cancer risk.<sup>[5]</sup>

3- family history & Genetic : P53 alterations represent the most commonly identified genetic abnormality in addition to alteration in chromosomes 3,7,17<sup>[6]</sup> in addition to cancer genes and genomic instability, polymorphism of drug-metabolizing enzymes has been reported to influence toxic outcomes of environmental carcinogens, hence, potentially modifying individual cancer risk. Of particular importance are the N-Acetyltransferase (NAT) enzymes,

known to mediate metabolism of bladder carcinogens, mainly aromatic amines and heterocyclic amines.<sup>[7]</sup>

4- chronic infection: shistosomiasis is a known cause of squamous metaplasia with subsequent squamous cell carcinoma of urinary bladder.<sup>[3]</sup>

5-race & ethnicity. Whites get bladder cancer twice as often as African Americans and Hispanics. The lowest rates are among Asians.<sup>[8]</sup>

6-certain drugs: cyclophosphamide, pioglitazone.<sup>[8]</sup>

### **Types of bladder cancer**

1-Transitional cell (urothelial) carcinoma: Urothelial cells also line other parts of the urinary tract, such as the renal pelvis, the ureters and the urethra, so transitional cell cancers can also occur in these places.<sup>[8]</sup> 95% of bladder cancers are epithelial malignancies with most being Transitional Cell Carcinoma (TCC).<sup>[8]</sup>

2-Squamous cell carcinoma: Only about 1% to 2% of bladder cancers are squamous cell carcinomas. Nearly all squamous cell carcinomas are invasive.<sup>[8]</sup>

3-Adenocarcinoma: Only about 1% of bladder cancers are adenocarcinomas. Nearly all adenocarcinomas of the bladder are invasive.<sup>[9]</sup>

4-Small cell carcinoma: Less than 1% of bladder cancers are small-cell carcinomas, which start in nerve-like cells called neuroendocrine cells.<sup>[8]</sup>

5-Sarcoma: Sarcomas start in the muscle cells of the bladder, but they are rare.<sup>[9]</sup>

### **Diagnosis**

#### **Patient history**

Patient history should be taken and recorded for all important information with possible connection to BC, including risk factors and history of suspected symptoms.<sup>[10]</sup>

#### **Sign and symptom of bladder cancer**

Irritative symptoms such as frequency, Urgency and Dysuria may be initial presentation of Bladder Cancer particularly in Carcinoma in situ (CIS).<sup>[10]</sup> Hematuria is the most common presentation of patients, either microscopic or macroscopic.<sup>[11]</sup>

#### **Urinary cytology**

Examination of voided urine or bladder-washing specimens for exfoliated cancer cells has high sensitivity in high-grade tumors but low sensitivity in low-grade tumors, The sensitivity of cytology for CIS detection is 28-100%.<sup>[12]</sup>

### Urinary molecular marker tests

Driven by the low sensitivity of urine cytology, extensive laboratory research has developed numerous urinary tests for BC detection. Considering the frequency of cystoscopy for follow-up, markers for recurrent urothelial cancer would be especially useful and will be discussed later in more details.<sup>[13]</sup>

### Ultrasonography

Ultrasonography (US) is often used as the initial tool to assess the urinary tract. This is not only because it avoids the use of contrast agents, but also because sensitive transducers have improved imaging of the upper urinary tract and bladder. Transabdominal US permits characterization of renal masses, detection of hydronephrosis, and visualization of intraluminal masses in the bladder.<sup>[13]</sup>

### Imaging

-Intravenous pyelography (IVP) is used to detect filling defects in the calyces, renal pelvis and ureters and hydronephrosis, which can indicate the presence of a ureteral tumor. Large exophytic tumors may be seen as filling defects in the bladder.

The necessity to perform routine IVU once a bladder tumor has been detected is questioned because of the low incidence of significant findings obtained with this method. The incidence of upper urinary tract tumors is low (1.8%), but increases to 7.5% in tumours located in the trigone.<sup>[13]</sup>

-CTU: Abdominal and pelvic CT scan with i.v contrast replaced the use of IVP in imaging study of suspected bladder mass. It can detect the location, size, the presence of associated hydroureteronephrosis and the extent of mass to perivesical space.<sup>[13]</sup>

-MRI: Abdominal and pelvic MRI is now increasingly used to detect the site, size, number, state of extravesical extension and the presence of associated lymphadenopathy.<sup>[13]</sup>

### Staging

2009 TNM classification system of bladder tumor.<sup>[15]</sup>

T-primary tumor

Tx primary tumor cannot be assessed.

T0 No evidence of primary tumor.

Ta Non invasive papillary carcinoma.

Tis Carcinoma in situ, flat tumor.

T1 Tumor invade sub epithelial connective tissue.

T2 Tumor invade muscle

T2a Tumor invade superficial muscle(inner half)

T2b Tumor invade deep muscle(outer half)

T3 Tumor invadeperivesical tissue:

Microscopically

Macroscopically(Extravesical)

T4 Tumor invade any of the following: prostate, uterus, vagina. pelvic wall, abdominal wall.

T4a prostate, uterus, vagina

T4b pelvic wall, abdominal wall.

N-Lymph node

Nx Lymph node cannot be assessed

No no lymph node metastasis

N1 Metastasis in asinle lymph node in atrue pelvis (hypogastric, obturator, externaliliac, presacral)

N2 Metastasis in multiple lymph node in true pelvis.

N3 Metastasis in common iliac lymph node.

M-Metastasis

Mx Metastasis cannot be assessed

M0 No evidence of distant metastasis

M1 Distant metastasis

### **Histological grading of non-muscle-invasive bladderurothelial carcinomas**

1973 WHO grading Urothelial papilloma

Grade I: Well differentiated.

Grade II: Moderately differentiated.

Grade III: Poorly differentiated.

In 1998, a new classification of non-invasive urothelial tumors was proposed by the World Health Organization (WHO) and the International Society of Urological Pathology (ISUP) (1998 WHO/ISUP classification) and published by the WHO in 2004. Its major contribution

is a detailed histological description of the various grades, which uses specific cytological and architectural criteria.<sup>[16,17]</sup>

\*1973 WHO Grade 1 carcinomas have been reassigned to PUNLMP and Low-grade carcinomas in 2004 WHO classification and Grade 2 carcinomas to Low-grade and High-grade carcinomas. All 1973 WHO Grade 3 carcinomas have been reassigned to High-grade carcinomas in 2004 WHO classification.<sup>[18]</sup>

### **Urinary cytology**

Examination of voided urine or bladder-washing specimens for exfoliated cancer cells has high sensitivity in high-grade tumors but low sensitivity in low-grade tumors. As a result of loss of cell cohesion in the epithelial lining of the bladder in CIS, there is a larger number of floating cells in the urine, as well as a high degree of anaplasia. The sensitivity of cytology for CIS detection is 28-100%.

Cytology is thus useful when a high-grade malignancy or CIS is present.<sup>[19]</sup> However, urinary cytology often is negative in the presence of low grade cancer. Positive voided urinary cytology can indicate a urothelial tumor anywhere in the urinary tract, from the calyx to the ureters, bladder and proximal urethra. Negative cytology, however, does not exclude the presence of a tumor in the urinary tract.

Cytological interpretation is user-dependent. Evaluation can be hampered by low cellular yield, urinary tract infections, stones, or intravesical instillations.<sup>[20]</sup>

Cytology should be performed on fresh urine with adequate fixation. Morning urine is not suitable because of the frequent presence of cytolysis. Atypical cells are defined as those that are beyond the limits of description for normal cells but that do not quite fit the description of cancerous cells.<sup>[21]</sup>

Atypical cells can be found in normal bladder patients diagnosed with cystitis, benign prostatic hyperplasia (BPH), or urinary stones, as well as in those undergoing radiotherapy. Atypical findings in bladder cancer are due to necrotic cancer tissue, high mitotic cancers, and the coexistence of inflammation or hematuria.<sup>[22]</sup>

As indicated before, however, the main shortcoming of voided cytology is the low sensitivity (approximately 20-40%) for detecting low grade neoplasms including benign papilloma,

urothelial carcinoma with low malignant potential (borderline) and low grade papillary urothelial carcinoma. There are two main reasons for such low sensitivity. First, tumor cells of the low grade tumors are not routinely shed into the urine because of their cohesive nature. Second and probably more important, is the fact that low grade tumor cells by definition have similar cytomorphology to normal urothelial cells microscopically.<sup>[23]</sup>

### **Common indications for urinary cytology**

- 1- It has been used as a screening tool to detect urothelial cancers in high risk populations, especially in populations exposed to chemical carcinogens through occupational means.<sup>[23]</sup>
- 2-It has been used as an initial test for patients presenting with hematuria to rule out (or rule in) the possibility of urothelial malignancy.<sup>[23]</sup>
- 3-It has been used as a monitoring and follow-up tool for patients with a previous diagnosis of urothelial cancer to rule out tumor recurrence.<sup>[23]</sup>
- 4-It has been used after transurethral resection for assessment of the completeness of tumor removal.<sup>[24]</sup>
- 5- Recently it has been applied as a test for detecting inflammation or infection, especially in kidney transplant patients where the presence or absence of polyoma virus infection may have significant clinical implications for rejection.<sup>[25]</sup>

### **Urine sample processing**

Sample fixation: The urine specimen should be processed immediately or refrigerated at 4 degrees Celcius for no longer than 24 hours. If a delay of greater than 24 hours is anticipated, the specimen should be fixed with an equal volume of 50% ethanol, or the specimen should be centrifuged and the sediment mixed with an ethanol-based fixative for liquid-based cytology or with 50% isopropyl alcohol or denatured alcohol. Low pH appears to favor preservation of urothelial cells.<sup>[26]</sup>

### **Specimen processing**

Specimens should be processed by cytocentrifugation or by a liquidbased preparation. Fifty milliliters of specimen are transferred to individual centrifuge tubes and spun down at 10 min / 1500 rpm. The supernatant is aspirated off and the sediment is resuspended in a balanced salt solution.<sup>[27]</sup>

### **Specimen adequacy**

Exact adequacy guidelines for urine specimens have not been established. In general, slides should contain at least fifteen well-visualized basal and intermediate cells to be classified as adequate.<sup>[27]</sup>

### **Tumour markers**

Diagnosis of bladder cancer has long relied on cystoscopy. This has been aided with the use of urine cytology; however, low sensitivity, particularly for low-grade disease, is associated with this test and has resulted in significant limitations. The advent of noninvasive urine-based markers, including fluorescence in situ hybridization (FISH), nuclear matrix protein 22 (NMP22), bladder tumor antigen (BTA), Immunocyt, as well as other novel modalities, has yielded improved diagnostic accuracy. These tests are more accurate in detecting low grade Bladder Cancer and in earlier stage of disease.<sup>[29]</sup>

### **NMP-22 (Nuclear Matrix Protein)**

NMP-22 (Nuclear Matrix Protein) BladderChek Test is an in vitro immunoassay intended for the qualitative detection of Nuclear Matrix Protein in urine of patients diagnosed with Nuclear matrix proteins (NMP) consist of a three -dimensional web of RNA and proteins that supports the nuclear shape, organizes DNA and coordinates DNA replication, transcription, and gene expression. NMP-22 is released from the nuclei of tumor cells during apoptosis.<sup>[32]</sup> NMP-22 is a 240-kDa protein that is transitional cells specific that shed into the urine and has a 25-times higher concentration in the urine of bladder cancer patients than in non-cancer controls.

The Alere NMP22®BladderChek® Test is an in vitro immunoassay intended for the qualitative detection of the nuclear mitotic apparatus protein (NUMA), which is an abundant component of the nuclear matrix proteins, in urine of persons with risk factors or symptoms of bladder cancer or with a history of bladder cancer. There are a variety of NMP-22 cutoff levels for bladder cancer detection, but typically 10 units/mL is used to identify patients with or without cancer.<sup>[33]</sup>

The Alere NMP22®BladderChek®Test technology uses a lateral flow immunochromatographic strip encased in a plastic cartridge to detect nuclear matrix protein qualitatively in the patient's urine sample. The antibodies in the lateral flow immunochromatographic strip are monoclonal antibodies (MAbs) raised against NUMA



extracted from cancer cell line by the method of Fey and Penman. Two different MABs are used, one as a capture antibody and one as a reporter antibody.<sup>[34]</sup>

## PATIENTS AND METHODS

A total of 125 patients with previously diagnosed superficial bladder cancer (TNM stages Ta–T1, G1–G3, N0, M0), on follow-up in urology consultation and department in Ghazi AL Hariri surgical specialty hospital of Baghdad medical city, were prospectively enrolled in the study. The study started from 1<sup>st</sup> of October 2012 to 4<sup>th</sup> of October 2014 with total patients number of 125 patients being divided to 113 male with and 12 female.

### Inclusion criteria

Any patient previously diagnosed with superficial bladder TCC.(Ta,T1,CIS).

### Exclusion criteria

- 1- Patients with invasive bladder TCC (T2 and above)
- 2- Patients with upper tract TCC (urothelial tumors of renal pelvis and ureter).
- 3- Bladder tumors other than TCC (squamous, adenocarcinoma).
- 4- Patients with urinary tract infections, or catheterized patients.
- 5- Patients with urinary stone disease.
- 6- Patients with other organs malignancy.

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 (Matriech, 330 Nevada St, Newton, MA). The NMP22 BladderChek test is a point of care immunoassay 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 Bladder Check test results were interpreted by a single observer. Voided urine cytology was carried out by a cytopathologist in Ghazi AL Hariri surgical specialty hospital. The results were classified as

malignant, suspicious for malignancy, inconclusive and normal. The last two were classified as negative. Patients underwent cystoscopy using a rigid cystoscope and video camera assembly. Any visible tumor or suspicious lesion was biopsied. Biopsies were evaluated using the TNM staging system and WHO grading.

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 abdominal and pelvic US 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 6.7 months (range 3–13 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 value < 0.05 being considered statistically significant.

## RESULTS

Of the 125 subjects, 113 were males and 12 were females. The mean age was 63 years for males (range 27–89 years) and 67.5 for females (range 34–71 year). The mean time since diagnosis of transitional cell carcinoma (TCC) of the bladder was 12 months (range 3–36 months). The mean number of previous recurrences was 1.6 (range 0–3). Of the 59 patients with recurrence on histopathology, 45 were positive for the NMP22 test and 21 were positive for cytology. The sensitivity of the NMP22 test and cytology for the detection of recurrence was 83.3 and 39.5%, respectively; the specificity was 78.9% and 95.1% respectively. (Table1) Sensitivity, specificity, positive predictive value, negative predictive value of the NMP22 test and voided urine cytology.

	Sensitivity %	Specificity %	Ppv%	Npv%
NMP22 test	83.3	78.9	55.5	93.7
CYTOLOGY	39.5	95.1	80.9	75

For Grade 1 and Grade 2 tumors, the NMP22 test detected more recurrence than cytology (75 versus 28%). This difference was statistically significant (P = 0.0004). Sensitivity in

detecting Grade 3 tumors was almost equivalent for the NMP22 test and cytology (90.9 versus 81.8%) and the p value was statistically not significant ( $p=0.53$ ).

**(Table 2.) Sensitivity according to grade.**

N=59	NMP22 %	Urine cytology %	P value
G1(16)	75 (12/16)	18 (3/16)	0.001
G2(32)	71 (23/32)	28.1 (9/32)	0.0004
G3(11)	90.9 (10/11)	81.8 (9/11)	0.53

When tumors were stratified by T stage, NMP22 was more sensitive than cytology in detecting Ta and T1 tumors (83% versus 46%,  $P < 0.05$ ). Interestingly, all three T2 stage recurrences were detected by both the NMP22 test and cytology (Table3).

**(Table 3). Sensitivity according to T stage.**

N 59	NMP22% 45	NMP22% 45	P value
Ta(n=26)	65.3 (17/26)	65.3 (17/26)	0.0002
T1(n=30)	83.3 (25/30)	83.3 (25/30)	0.002
T2 or higher	100 (3/3)	100 (3/3)	

17 cases were positive for the NMP22 test when no tumor was found on cystoscopy. None of these patients was positive for cytology. However, nine 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%. Pathological data were grouped according to risk for recurrence, progression and invasion, also taking in consideration the size ( $>3\text{cm}$ ), multiplicity and previous recurrence, into a low-risk group (Ta, G1–2), a high-risk group (TaG3,Tis, T1) and an invasive group (T2 or higher not listed in table). The NMP22 test showed significantly higher sensitivity (up to five 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 ( $P = 0.06$ ) (Table 4).

**(Table 4). Sensitivity according to stage and grade, grouped by risk and degree of recurrence, progression and invasion.**

Pathological result	Number of patients	Sensitivity of NMP22 test (%)	Sensitivity of cytology (%)	P-value
Low-risk TaG1–2	25	73 (19/25)	15 (2/25)	0.0002
High-risk TaG3,T1	31	76.6 (23/31)	53 (16/31)	0.065
T2 or higher	3	100 (3/3)	100 (3/3)	

On combining the results of the NMP22 test and cytology, 51 of the 59 cystoscopy- positive tumors were detectable, giving an overall sensitivity of 89.8%. However, eight patients with recurrence could not be detected using a combination of the tests. Of these eight patients, five had TaG1 and the other three had TaG2 recurrences.

## DISCUSSION AND CONCLUSIONS

### Discussion

Cystoscopy is the main diagnostic modality for the diagnosis of bladder carcinoma. Although it is the gold standard for detecting bladder cancer, it is invasive and relatively expensive.<sup>[39]</sup> Voided urine cytology is the standard non-invasive method for diagnosis in the detection of bladder carcinoma. However, its sensitivity is low: between 11 and 76% in various studies.<sup>[40]</sup> 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.<sup>[41]</sup>

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. NMP22 is thought to be released from the nuclei of tumor cells after they die and it can be detected in the urine.<sup>[42]</sup> Research has found that patients with bladder cancer may have urinary levels of NMP22 that are 25-fold greater than levels in healthy subjects.<sup>[43]</sup>

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<sup>[44]</sup>, 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%.<sup>[45]</sup> The investigator used an NMP22 cut-off reference value of 6–20 U/ml. Our study, using the NMP22 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 59 patients with recurrence.<sup>[46]</sup> NMP22 showed an overall sensitivity of 83.3% compared with 39.5% 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.<sup>[47]</sup> 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<sup>[48]</sup> and his colleagues carried a study on 131 patients who are known cases of TCC of urinary bladder and compare the result of recurrence which are based on cystoscopy and histopathology with the result of NMP22 and urine cytology and found that sensitivity of NMP22 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<sup>[48]</sup> and his colleagues found on 1,070 patients who had hematuria or who were being followed up for bladder cancer, who compared the sensitivity and specificity of the NMP22BC test with those of urine cytology and the sensitivity of the NMP22BC test (77.5%) was significantly higher than that of urine cytology (46.3%). The specificity of the NMP22BC test was 88.8%, compared with 97.9% for urine cytology.<sup>[49]</sup>

The sensitivity of the NMP22BC test (81.8%) in non-muscle-invasive bladder cancer was higher than that of cytology (36.4%). However, the sensitivity of the NMP22BC test and of urine cytology in invasive bladder cancer were 57.1% and 92.9%, respectively. The sensitivity of the NMP22BC test was higher for low-grade bladder cancer (83.9%) than for high-grade (62.5%) and the sensitivity of cytology was higher for high-grade bladder cancer (66.7%) than for low-grade (37.5%). This is important particularly because repeat cystoscopy or transurethral resection of bladder tumors may be avoided or can be delayed in such patients.<sup>[50]</sup>

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 five times more sensitive than cytology. This finding can be utilized in low-risk group patients with a negative NMP22 test to modify the currently used rigorous surveillance protocol of cystoscopy.<sup>[51]</sup> Of our patients, 12% showed a false positive NMP22 result. Of these, 16 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 especially when it is accompanied by severe inflammatory response of bladder like in intravesical BCG.<sup>[52]</sup>

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 90%(53/59). However, 10% (6/59) of the recurrences were still not detected. Thus, even the combination of the NMP22 test and cytology cannot replace cystoscopy in the surveillance protocol.<sup>[52]</sup>

## 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 five 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 10% of the tumors. Thus, these tests cannot be used to omit cystoscopy from the surveillance protocol.

## Recommendation

We recommend to more extensive study of NMP22 in superficial tumors of urinary bladder and to use it as an adjunctive method beside urinary cytology and cystoscopy for surveillance of patients with superficial bladder cancer.

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