

## RESEARCH COMMUNICATION

# Role of NMP22 Bladder Check Test in Early Detection of Bladder Cancer with Recurrence

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### Abstract

**AIM:** To assess clinical utility of NMP22 Bladder Check Test and to compare it with voided urine cytology and cystoscopy in early detection of Bladder Cancer. **Material & Methods:** A total of 115 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 tumour grade and stage. **Results:** Mean age of the patients was 57.2 years for males and 55.3 years for females. A total of 59 cases of Bladder Cancer (TCC) were diagnosed among which NMP22 test was positive in 48 cases and cytology in 26 cases. The sensitivity and specificities of NMP22 Test in recurrent bladder cases was 81.3% and 92% which was significantly greater than that of cytology 44% and 96.1% respectively. In non-invasive lesions of Bladder Cancer (TCC), NMP22 Test and Cytology was positive in 71.8% and 42.8% of cases respectively. In muscle-invasive lesions, NMP22 Test was positive in 82.2% and 44.4% cases were positive for cytology. The sensitivity of the NMP22 test was 81.3%, which was significantly greater than that of cytology 44%. **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 substitute for urine cytology as we detected high sensitivity and specificity of NMP22 in recurrent bladder cases.

**Keywords:** Bladder cancer - cytology - nuclear matrix protein(NMP) - transitional cell carcinoma

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### Introduction

Urinary Bladder Cancer is the second most common disease treated by urologists. In the United States, it is the fourth most common cancer in men and its current prevalence is approximately 50,000 (Jemal et al., 2005). Advancing age is the major risk factor for the development of Bladder Cancer. It is generally a disease of the middle aged or elderly person, with a median ages at diagnosis being 69 yrs in Males and 71yrs for female (Lynch and Cohen et al., 1995). The majority of Bladder tumours occur in men with a Male/Female ratio of 3:1 (Pashos et al., 2002). The incidence, morbidity and mortality rates vary with country, ethnicity, gender and age (Farley et al., 2000). Transitional Cell Carcinoma (TCC) occurs predominantly in western industrialised countries and Squamous Cell Carcinoma (SCC) in African and Middle East Countries (highest incidence in Egypt). According to U.S. Cancer Institute, white persons have an incidence of 17.7/100000 while black have 9.2/100000 (Ries et al.,

2000). Risk factors include cigarette smoking (Morrison et al., 1984) exposure to chemicals like 2-naphthylamine, 4-aminobiphenyl, benzidine in chemical, dye, leather, petroleum factories (Johanson et al., 1997; Zheng et al., 1992). P53 alterations represent the most commonly identified genetic abnormality (Sai et al., 1990). Hematuria is the most common presentation of patients, either microscopic or macroscopic. In those with macroscopic hematuria, the reported rates of Bladder Cancer range from 13% to 34.5% (Lee et al., 1953; Varkarakis et al., 1974; Sultana et al., 1996; Khadra et al., 2000). Rate of Bladder Cancer in patients with microscopic hematuria has been reported to range from 0.5 to 10.5% in several studies (Golin et al., 1980; Mohr et al., 1986). Irritative symptoms such as frequency, Urgency and Dysuria may be initial presentation of Bladder Cancer particularly in Carcinoma in situ (CIS). Bladder Tumor can present either as superficial disease (75%) or muscle invasive tumor (25%). 98% of Bladder Cancers are Epithelial Malignancies with most being Transitional Cell Carcinoma (TCC)

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(90%). Flexible cystoscopy is the first investigation for the patient suspected of having Bladder Cancer (Young et al., 1998). Flexible Cystoscopy provides equivalent visualization to that provided by rigid cystoscopes. It is helpful to document the exact locations of any abnormality using photography or a Bladder Diagram. Voided Urine cytology is critical adjunct to cystoscopy in the diagnosis of Bladder Cancer. The sensitivity of cytology is known to increase with increasing grade and stage of Bladder Cancer (Planz et al., 2005). Due to invasiveness and expenses of frequent cystoscopies and lack of sensitivity of urine cytology, especially for low grade superficial lesions, Urine based tumor markers are developed. These tests are more accurate in detecting low grade Bladder Cancer. NMP-22 (Nuclear Matrix Protein) Bladder Chek Test is an in vitro immunoassay intended for the qualitative detection of NMP-22 Nuclear Matrix Protein in urine of patients diagnosed with Bladder Cancer. It is the only in-office test approved by FDA for the diagnosis of Bladder Cancer. It is painless and non-invasive assay performed on a single urine sample, that detects elevated levels of NMP22 proteins. Healthy individuals generally have very small amount of NMP-22 protein in the urine. However, the level of NMP-22 protein is often elevated in the urine of patients with Bladder Cancer, even at early stage of disease. It is an in vitro immunoassay intended for the qualitative detection of NMP-22 Nuclear Matrix Protein in urine of patients diagnosed with Bladder Cancer. It is the only in-office test approved by FDA for the diagnosis of Bladder Cancer. It is painless and non-invasive assay performed on a single urine sample that detects elevated levels of NMP22 proteins.

Nuclear Matrix Protein makes up the structural framework of nucleus (Berezney et al., 1974; Fey et al., 1986). and are associated with such functions as DNA replication and RNA synthesis (Pardoll et al., 1980; Kumara-Siri et al., 1986) as well as regulation and co-ordination of gene expression (Berrios et al., 1985; Zeitlin et al., 1987; Nakayasu et al., 1989). NMP-22 is specific for transitional cells in the urinary tract. Malignant transitional cells contain up to 80 times higher concentration of NMP-22 than normal transitional cells and release it into urine upon cell death. Typically, patients with transitional cell carcinoma of Bladder have higher concentration of NMP-22 in their urine than patients without TCC. Based on previous studies, NMP-22 levels above 10 U/ml in the urine is associated with a high probability of transitional cell Carcinoma of Bladder NMP-22 is specific for transitional cells in the urinary tract.

We investigated a non-invasive urine based test for the nuclear matrix protein NMP22 proteomic marker for detection of recurrent bladder cancer in patients on follow up which has been reported to have greater clinical efficiency as an aid in diagnosis of bladder cancer and compared its utility to detect cancer with that of voided urine cytology.

## Materials and Methods

This randomized, double blind, prospective study was conducted in the Department of Urology and Pathology,

Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar. Institutional ethical committee approval and written informed consent was taken. A total of 115 patients were enrolled in this study from July 2006 to Nov 2008. All 115 patients were following up cases of Bladder Cancer. Urine samples were assayed for the presence of NMP22 using NMP22 Bladder Chek 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.

A single voided urine sample was collected just prior to cystoscopy. Two aliquots were separated from the sample, one for NMP22 test and other for cytology. Urine sample was tested within two hours of collection. Test device and dropper from foil package were opened just prior to use. Dropper provided with the kit was used to add patient urine to well of test device. Four drops of patient's urine were added to well of test device. Results of test were read with in 30 minutes. Patient with urinary tract infections, indwelling urinary catheters, foreign bodies in urinary bladder were excluded from the study. Patient having undergone bowel interposition surgery and Prostate and renal malignancy were also excluded from the study. Materials required for the procedure were NMP22 Bladder Chek test kit (Matritech, Inc.USA), Plastic urine collecting container, watch or timer.

## Results

The total number of studied patients was 115 follow up cases of Bladder cancer (Table 1). The number of males were 89 (77.4%) and females were 26 (22%). 72 patients (62.6%) were in age group of 40-60yrs and 7(6.1%) patients were below 40yrs of age and 36 patients (31.3%) were more than 60yrs of age. 56 patients (48.7%) in our study were asymptomatic while as hematuria was seen in 53 patients (46.1%) followed by dysuria in 25 patients (21.7%) and urgency in 9 patients (7.8%). Table 2 depicts results as per the grade of tumor by NMP22 Bladder Chek Test was positive in 19 out of 24 patients (79%) with grade I TCC, 17 out of 20 patients (85%) with grade II TCC and 12 out 15 patients (80%) with grade III lesion. Voided urine cytology was positive for M cells in 7 of 24 patients (29.16%) in grade I lesions, 8 of 20 patients (40.0%) in grade II and 11 of 15 patients (73.%) in grade III lesions. The difference in G-I and G-II between NMP22 and urine cytology was statistically significant (P=0.001 and 0.008 respectively) while as statistical association observed in

**Table 1. Showing General Characteristics of the Studied Subjects**

General characteristics		N	%
	Total	115	100.0
Gender	Male	89	77.4
	Female	26	22.6
Age	< 40	7	6.1
	40 to 60	72	62.6
	≥ 60	36	31.3

**Table 2. Sensitivity of NMP22 and Urine Cytology in Various Grades of Bladder Cancer**

Grade	NMP22	Sensitivity %	Urine Cytology	Sensitivity %	p-value
G-1	19/24	79.16	7/24	29.16	0.001
G-2	17/20	85	8/20	40	0.008
G-3	12/15	80	11/15	73.3	0.62
Overall	48/59	81.35	26/59	44.06	0.04

**Table 3. Sensitivity of NMP22 and Urine Cytology with Reference to Tumor Stage of Bladder Cancers**

Tumor stage	NMP22	Sensitivity %	Urine Cytology	Sensitivity %	P value (Fisher exact test)
Non- invasive	11/14	78.57	6/14	42.85	0.060
Muscle-invasive	37/45	82.22	20/45	44.44	<0.001
Overall	48/59	81.35	26/59	44.06	<0.001

G-III between the two was insignificant ( $p=0.62$ ). Overall positive picked up by both NMP22 and M cells were 48 of 59 (81.45%) and 26 of 59 (44.06%) patients respectively.

Histologically two groups of cases were assessed while observing the staging as non-invasive and invasive bladder cancer Table 3. Out of 14 non-invasive cases 11 were positive by NMP22 (78.57%) while as with same number of samples, 6 were positive by urine cytology (42.85%) and this showed statistically a mild significant association ( $p=0.06$ ) as compared to the invasive cases where 34 of 45 cases were positive (82.22%) by NMP22 and 20 of 45 cases were positive by urine cytology (44.4%) which showed a strong statistical association ( $p<0.001$ ). The overall performance of NMP22 and urine cytology was observed to be 81.35% (48 of 59) and 44.06% (26 of 59) respectively which showed a strong statistical association with  $p<0.001$ .

The overall sensitivity and specificity of NMP22 in our study was 81.3% and 100% respectively. The urine cytology shows sensitivity and specificity to be 35.5% and 96.1% with an accuracy of 68.7%. There were a 100% positive predictive values by NMP22 as compared to urine cytology with PPV as only 66.7%. On the other hand Negative predictive values were slightly higher by NMP22 (87.4%) and lower NPV was observed by urine cytology (68.7%).

## Discussion

Cystoscopy is the primary diagnostic tool for diagnosis of Bladder Carcinoma. Although, it is gold standard for detection of Bladder Cancer, it is invasive and relatively expensive.

Voided Urine Cytology is the standard non-invasive method for diagnosis of Bladder Carcinoma. However, it has limitation of low sensitivity. Significant Institutional variability exists in the accuracy of urinary cytology in predicting recurrence of TCC of the Bladder. Its accuracy has been reported as 38% to 65% in a large multi-institutional cohort from 4 continents (Karakiewicz et al., 2006). Also, voided urine cytology has some major drawback, including the requirement of a trained cytopathologist, low sensitivity and greater cost. The sensitivity of urinary cytology is even lower for low grade bladder TCC. These shortcomings of urinary cytology have resulted in a search for alternate markers for screening and follow-up of TCC of the Bladder.

Newer diagnostic techniques involving urine bound

markers can offer an alternative to the standard modes of detecting Bladder Cancer or they can be used as an adjunct to Cystoscopy. The NMP22 Bladder Check is a new point of care diagnostic test for urinary bladder cancer. It is a dipstick technology that can detect  $6 \times 10^{-17}$  moles of nuclear matrix protein within urine. It is normally present in low concentrations, however it is elevated upto 80-fold in tumor cells; tumor cells within the urinary tract shed relatively high concentration of NMP22 into the urine.

It is an in vitro immunoassay intended for the qualitative detection of NMP-22 Nuclear Matrix Protein in urine of patients diagnosed with Bladder Cancer. It is the only in-office test approved by FDA for the diagnosis of Bladder Cancer. It is painless and non-invasive assay performed on a single urine sample that detects elevated levels of NMP22 proteins. 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 (Berezney et al., 1974, Pardoll et al., 1980; Gordan et al., 1993). 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 (Keese et al., 1996).

A study evaluating the detection of Bladder Cancer with this test and cytology found the NMP-22 Test to have a specificity of 85.7% overall. The sensitivity was significantly better than that of cytology (55.7% vs 15.8%) (Landman et al., 1998).

Stamfer et al., (1998) 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., (1996), Miyanaga et al., (1997) and Landman et al., (1998), 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 showed more or less consistent results as the above studies with a sensitivity of 71.8% for NMP22 and 35.5% for cytological examination on 59 recurrent bladder cancer cases. The interval between

surgery and follow up was 2 months to 28 months. Our study observed the detection of high and almost equal percentage of both non-muscle invasive and invasive bladder cancers by NMP22 (78% and 82% respectively) as compared to low percentage of both stages by urine cytology. This predicts NMP22 as an efficient diagnostic marker to diagnose recurrent bladder tumours. A notable finding in our study was that NMP22 had almost equal sensitivity for all grades (79% < 85 > 81%; G-I < G-II > G-III). This was in contrast when compared to urine cytology where sensitivity was low in low grade tumours (29% < 40%; G-I < G-II) and high in higher grade (73% for G-III). This shows that NMP22 can detect bladder cancer of all grades while as cytology lacks the efficiency in low grades.

The results in our study demonstrate that the NMP22 detected a higher percentage of bladder cancer than cytology. Overall NMP22 identified 22 recurrent cases of bladder cancer that cytology missed while cytology was positive in only one patient for whom NMP22 was negative.

In conclusion, both high sensitivity and specificity of NMP22 test depicts it superior to cytology for all grades and stages in the diagnosis recurrent bladder cancers. The results of our study have shown that the NMP22 can be used as a substitute for urine cytology as it can be conveniently performed at point of care and results can be obtained within 30 minutes.

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