Original Article

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Urology Section

Assessment of Diagnostic Accuracy of Nuclear Matrix Protein 22 versus Urine Cytology for Detecting Bladder Cancer keeping Cystoscopy and Biopsy as Gold Standard: A Cross-sectional Study

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ABSTRACT

Introduction: Bladder cancer is a common urological malignancy that requires early and accurate detection for effective treatment and improved patient outcomes. Traditional urine cytology is effective for high-grade tumours but has limited sensitivity for low-grade cancers.

Aim: To compare the sensitivity and specificity of the Nuclear Matrix Protein 22 (NMP22) test and urine cytology for bladder cancer detection, using cystoscopy and biopsy as the gold standard.

Materials and Methods: This cross-sectional validation study was conducted at the Vydehi Institute of Medical Sciences and Research Centre in Bengaluru, Karnataka, India from August 2023 to August 2024, involving 42 patients with Transitional Cell Carcinoma (TCC). Urine samples were analysed using ALERE NMP22® and cytology, with cystoscopy and biopsy serving as the gold standard for diagnosing bladder cancer. Contingency tables were created to calculate sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and diagnostic accuracy of NMP22 and urine cytology, using cystoscopy/biopsy as the gold standard.

Results: The mean age of the study population was 56 years, with the mean age among males and females being 58.5 years and 51.3 years, respectively. NMP22 outperformed urine cytology in diagnosing bladder cancer, with a sensitivity of 76.32% (32/42) versus 31.58% (13/41), and both tests had 100% specificity and PPV. NMP22's NPV was 30.77%, compared to 13.33% for cytology. Overall accuracy for NMP22 was 78.57% (33/42), while the accuracy for cytology was 38.10% (16/42).

Conclusion: The NMP22 is more sensitive than urine cytology for detecting bladder cancer. While cytology remains valuable for its specificity and confirmatory role, NMP22 offers a superior diagnostic option, particularly for the early detection of aggressive tumours. Future research should explore combining NMP22 with other diagnostic methods to further enhance early detection and improve patient outcomes.

Keywords: Biomarkers, Carcinoma, Histopathology, Muscle invasive cancers, Transitional cell carcinoma

INTRODUCTION

Bladder cancer, a malignancy arising from the epithelial lining of the urinary bladder, represents one of the most common urological cancers globally. Early and accurate detection is pivotal for effective treatment and improved patient outcomes; yet, achieving this remains a clinical challenge [1]. The traditional diagnostic method, urine cytology, has been widely used due to its non invasive nature and its ability to detect high-grade tumours. However, its sensitivity for detecting low-grade bladder cancer is limited, leading to potential false-negative results and delayed diagnosis. This limitation underscores the need for more reliable diagnostic methods to enhance the early detection and monitoring of bladder cancer [1,2].

The NMP22 testing has emerged as a promising diagnostic tool in recent years. The NMP22 test measures the levels of NMP22, a protein associated with cell division and structural integrity, which is often elevated in the urine of patients with bladder cancer [3]. Several studies have demonstrated that NMP22 testing offers higher sensitivity and specificity compared to urine cytology, particularly for low-grade tumours [4-6]. NMP22 shows strong potential in early detection and surveillance, offering practical advantages when combined with cystoscopy; although it does not entirely replace cytology or invasive procedures [7,8]. This has spurred interest in its potential to supplement or even surpass urine cytology in the diagnostic pathway for bladder cancer [9].

The gold standard for bladder cancer detection and confirmation remains cystoscopy and biopsy. Cystoscopy allows direct visualisation of the bladder and facilitates the collection of biopsy samples for histopathological examination, providing a definitive diagnosis [10]. However, cystoscopy is invasive, costly, and can be uncomfortable for patients, which limits its feasibility for frequent monitoring. Consequently, there is a critical need to evaluate non invasive tests like NMP22 and urine cytology in comparison to cystoscopy to determine their diagnostic accuracy and potential roles in clinical practice [11].

Accurate and early detection of bladder cancer not only facilitates timely intervention but also improves surveillance strategies, particularly in patients with a history of the disease [10]. The NMP22 test is a urinary biomarker assay that detects elevated NMP22 levels from apoptotic bladder cancer cells. It offers a non invasive, cost-effective alternative to cystoscopy, suitable for routine screening and follow-up. With improved sensitivity for low-grade tumours often missed by cytology or imaging, it enhances early detection. While it is not a standalone solution, its integration into diagnostic workflows enhances early detection, reduces reliance on invasive methods, and improves patient compliance. Research into combining NMP22 with other biomarkers or Artifical Intelligence (AI) driven analyses could further refine its clinical utility.

The present study aims to fill this gap by providing a comprehensive comparison between NMP22 testing and urine cytology, using cystoscopy and biopsy as the gold standard references. By evaluating

the sensitivity and specificity of these non invasive tests, the study seeks to determine their effectiveness and reliability in detecting bladder cancer. The findings could have significant implications for clinical practice, potentially leading to the adoption of more accurate and patient-friendly diagnostic protocols.

Moreover, the study's implications extend beyond diagnostics. Improved early detection methods can lead to better prognosis and survival rates for bladder cancer patients by enabling earlier and more targeted treatments. It can also reduce the need for frequent invasive procedures, thereby improving the quality of life for patients under surveillance for bladder cancer recurrence.

MATERIALS AND METHODS

The present cross-sectional validation study was conducted at the Vydehi Institute of Medical Sciences and Research Centre in Bengaluru, Karnataka, India from August 2023 to August 2024. The study received approval from the hospital's Ethical Review Board (ERB), bearing reference number ECR/747/Inst/KA/2015/RR21.

A non probability consecutive sampling method was employed to enroll 42 participants.

Inclusion criteria:

- Age above 18 years
- Either gender
- Provisionally diagnosed with Transitional Cell Carcinoma (TCC) of the bladder
- Scheduled for cystoscopy

Exclusion criteria:

- Previous diagnosis of TCC of the bladder or upper urinary tract
- Renal malignancy or ongoing dialysis
- Presence of active gross haematuria
- Recent urethral instrumentation or catheterisation within the past two weeks
- Bladder stones
- Active urinary tract infection

Informed written consent was obtained from all participants, and demographic information, including name, age, and gender, was recorded.

Study Procedure

Urine sample collection and analysis: Voided Midstream Urine (MSU) samples were collected during outpatient visits prior to any treatment, following the standard protocol set by the Human Kidney and Urine Proteome Project (HKUPP) network [11]. The urine samples were divided into two aliquots: one for NMP22 analysis using the ALERE™ NMP22® BladderChek® test [11], conducted in the urology laboratory at VIMS & RC, and the other for cytopathology analysis at the CDL at VIMS & RC.

For urine cytology, both air-dried and wet-fixed slides (ThinPrep slides, Cytyc Corporation, Marlborough, MA) were prepared using direct smearing and cytocentrifugation. After centrifugation at 2000 rpm for five minutes, the supernatant was removed, and the cell pellets were washed with Cytolyt® Solution. Drops of each patient sample were transferred into PreservCyt® Solution and fixed for 15 minutes. Air-dried slides were stained with Diff-Quik® stain (MICROPTIC SL, Barcelona, Spain), while Papanicolaou stain was used for wet-fixed slides. A consultant cytopathologist evaluated all specimens using the Paris classification system for reporting urine cytology [12]. Classes 1 and 2 were considered negative, while classes 4 and 5 were considered positive for bladder cancer. Patients with class 3 (atypical urothelial cells) cytological findings but negative cystoscopy results were excluded.

Confirmation of bladder cancer: The presence or absence of bladder cancer was confirmed through rigid cystoscopy and biopsy performed under spinal or general anaesthesia by a consultant urologist, assisted by a researcher. All relevant data were recorded on a specially designed pro forma by the researcher.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM Statistiical Package of Social Sciences (SPSS) Statistics for Windows, Version 24.0 (Armonk, NY: IBM Corp). Descriptive statistics were used to calculate means and Standard Deviations (SD) for continuous variables such as age, and an Independent sample t-test was utilised for these continuous variables. Categorical variables, including gender and TCC detection by NMP22, urine cytology, and cystoscopy, were presented as frequencies and percentages. The chi-square test was applied to determine significance. Contingency tables were created to calculate sensitivity, specificity, PPV, NPV, and diagnostic accuracy of NMP22 and urine cytology, using cystoscopy as the gold standard. Receiver Operating Characteristic (ROC) curve analysis was performed to compare the Area Under the Curve (AUC) among these tests. McNemar's test was used to compare sensitivity and specificity, with a p-value of ≤0.05 considered significant.

RESULTS

The study included 42 bladder cancer patients, with a mean age of 56.47 years (range 26-82). Most patients (47.61%) were aged 41-60, followed by 38.09% over 60 years, and only 14.29% aged 40 or younger [Table/Fig-1]. The average Body Mass Index (BMI) was 21.25, which falls within the normal weight range (18.9-24.4). Despite obesity being a risk factor for bladder cancer, this cohort had a lower average BMI.

Parameters	Number of cases (n)	Percentage (%)
Age group (years)		
≤40	6	14.29
41-60	20	47.61
>60	16	38.09
Gender		
Male	32	76
Female	10	24

[Table/Fig-1]: Age and gender distribution of the patients.

The comparison of preoperative and postoperative haematological parameters, including haemoglobin, white blood cell count, and platelet count, showed no statistically significant differences, indicating stability in these parameters postsurgery [Table/Fig-2].

Parameters	Preoperative Mean±SD	Postoperative Mean±SD	p-value
Haemoglobin n (gm%) (mean±SD)	10.29±1.40	10.43±1.07	0.184
White Blood Cells (WBC) (per cubic mm)	8.40±1.03	8.98±1.55	0.045
Platelet (lac/cumm)	208.81±52.61	207.40±47.06	0.752

[Table/Fig-2]: Patient baseline characteristics.
Student independent sample t-test

Diagnostic accuracy of NMP22 versus cytology: NMP22 demonstrated a higher sensitivity (76%) compared to cytology (32%), indicating that NMP22 is more effective in detecting true positives in bladder cancer cases. The specificity of both NMP22 and cytology was 100% [Table/Fig-3].

Parameters	Sensitivity	Specificity	Positive Predicted Value (PPV)	Negative Predicted Value (NPV)	Accuracy
NMP22	76%	100%	100%	31%	79%
Cytology	32%	100%	100%	13%	38%

[Table/Fig-3]: Diagnostic accuracy between NMP22 and urine cytology with histopathology as gold standard.

Association of NMP22 and cytology with tumour stage and grade: The NMP22 demonstrated strong performance in detecting advanced tumour stages. It was positive in 100% of lamina propria tumours (12/12) and 92% of muscle-invasive tumours (11/12), with only one false negative in the muscle-invasive category. The p-value for the difference in detection across various stages was <0.001, indicating a significant association between NMP22 positivity and tumour stage, particularly in more advanced stages.

Urine cytology, however, showed lower effectiveness. It was positive in only 17% of lamina propria tumours (2/12) and 83% of muscle-invasive tumours (10/12). Notably, cytology failed to detect any subepithelial tissue tumours (0/14), highlighting its limitations in identifying early-stage bladder cancer [Table/Fig-4].

	NMP22 positive	Cytology	p-value
Sub-epithelial tissue	43% (6/14)	0	--
Lamina propria	100% (12/12)	17% (2/12)	<0.001
Muscle invasive	92% (11/12)	83% (10/12)	0.054

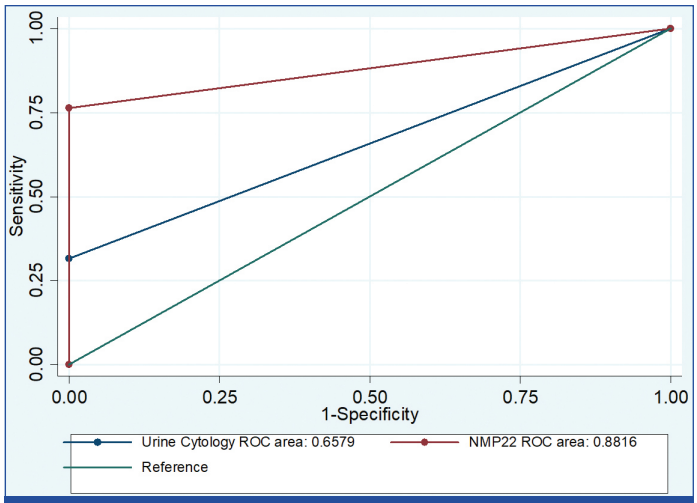
[Table/Fig-4]: Sensitivity according to t-stage.
Chi-square test

Tumour grade comparison {World Health Organisation (WHO) classification}: NMP22 also outperformed urine cytology in detecting tumour grades. It was positive in 100% (16/16) of Grade III tumours. Conversely, urine cytology showed reduced sensitivity, detecting only 31% (11/16) of Grade III tumours [Table/Fig-5].

Parameters	Positive	Negative	Total	p-value
NMP22				
Tumour cannot be assessed	0	4 (100%)	4	<0.001
Sub-epithelial tissue	6 (43%)	8 (57%)	14	
Lamina propria	12 (100%)	0	12	
Muscle invasive	11 (92%)	1 (8%)	12	
Cytology				
Tumour cannot be assessed	0	4 (100%)	4	<0.001
Sub-epithelial tissue	0	14 (100%)	14	
Lamina propria	2 (17%)	10 (83%)	12	
Muscle invasive	10 (83%)	2 (17%)	12	
Histopathology				
Tumour cannot be assessed	1 (25%)	3 (75%)	4	<0.001
Sub-epithelial tissue	13 (93%)	1 (7%)	14	
Lamina propria	12 (100%)	0	12	
Muscle invasive	12 (100%)	0	12	
NMP-22				
Grade-I	9 (53%)	8 (47%)	17	0.006
Grade-II	4 (75%)	1 (25%)	5	
Grade-III	16 (100%)	0	16	
Cytology				
Grade-I	1 (6%)	16 (94%)	17	<0.001
Grade-II	0	5 (100%)	5	
Grade-III	11 (31%)	5 (69%)	16	
Histopathology				
Grade-I	16 (94%)	1 (6%)	17	0.530
Grade-II	5 (100%)	0	5	
Grade-III	16 (100%)	0	16	

[Table/Fig-5]: Comparison of t-stage and Grade with NMP-22, urine cytology and histopathology.
Chi-square test was used

This ROC curve compares the diagnostic performance of urine cytology and NMP22 in detecting a condition. The x-axis represents 1-Specificity (False Positive Rate), while the y-axis represents Sensitivity (True Positive Rate). The AUC for urine cytology was 0.6579, indicating moderate diagnostic accuracy, whereas NMP22 achieved a higher AUC of 0.8816, reflecting strong diagnostic performance. The red curve (NMP22) was consistently closer to the top-left corner of the plot, outperforming the blue curve (urine cytology) in sensitivity and specificity. In conclusion, NMP22 demonstrates superior overall accuracy compared to urine cytology for identifying the condition [Table/Fig-6].



[Table/Fig-6]: ROC curve showing the AUC for diagnostic performance of urine cytology and NMP22.

DISCUSSION

The current study underscores the diagnostic value of NMP22 compared to traditional cytology in detecting bladder cancer, particularly in higher stages and grades of the disease. NMP22 exhibited a sensitivity of 76%, which is significantly higher than the 31.58% sensitivity observed with cytology. The specificity of both tests was 100%, confirming that both are highly reliable in ruling out non cancer cases when a negative result is obtained.

For instance, a study by Grossman HB et al., reported that the NMP22 assay was positive in 44 of 79 patients with cancer (sensitivity, 55.7%; 95% Confidence Interval [CI], 44.1%-66.7%), whereas cytology test results were positive in 12 of 76 patients (sensitivity, 15.8%; 95% CI, 7.6%-24.0%). The specificity of the NMP22 assay was 85.7% (95% CI, 83.8%-87.6%) compared with 99.2% (95% CI, 98.7%-99.7%) for cytology [4]. The higher sensitivity observed in present study (76.32%) could be attributed to the population's characteristics or differences in sample handling and processing methods. Nonetheless, the trend remains consistent across studies, confirming NMP22's reliability in detecting bladder cancer.

The NMP22 test demonstrates a higher sensitivity for detecting bladder cancer, particularly in identifying low-grade and high-grade non muscle invasive tumours. A study by Kumar A et al., showed that out of the 46 recurrences detected by cystoscopy, the NMP22 test was positive in 39 cases, while cytology was positive in 19 cases. The sensitivity of the NMP22 test was 85%, which was significantly greater than that of cytology (41%) [5].

The UBC rapid test, another urinary biomarker, also has moderate sensitivity but is less specific than NMP22. In contrast, NMP22 achieves a balanced profile, offering a good trade-off between sensitivity and specificity, especially for high-grade and muscle-invasive cancers [7].

The correlation of NMP22 with tumour stage is particularly noteworthy. In present study, NMP22 was positive in 92% of muscle-invasive tumours, consistent with the findings of Shariat SF et al., who reported a strong association between NMP22 positivity and advanced tumour stages [12].

A negative NMP22 result might also lead to false reassurance, reducing patient compliance with follow-up protocols. To address this, combining NMP22 with other diagnostic methods, such as

cytology or molecular markers, improves overall accuracy and mitigates the risk of false negatives. Clinicians must use NMP22 as part of a broader diagnostic strategy, particularly for high-risk patients, to enhance detection rates while minimising delays in treatment [6,8].

When considering tumour grade, NMP22 again demonstrated superior diagnostic capability, with 100% positivity in Grade III tumours. This finding is supported by results from Tetu B and Tiguert R, who also reported high NMP22 sensitivity in detecting high-grade tumours [13]. NMP22's lower sensitivity for detecting subepithelial tumours, especially in the early stages of bladder cancer, presents a significant limitation. These early tumours, such as Ta-stage non muscle invasive bladder cancer, shed fewer cancer cells, leading to lower levels of NMP22 proteins in the urine. This results in a higher risk of false-negative results, potentially delaying diagnosis and treatment when early detection is crucial. Additionally, benign conditions like haematuria or urinary tract infections may interfere with the test, complicating its diagnostic reliability.

To overcome these challenges, future directions could include combining NMP22 with other biomarkers, such as UroVysion or cytokeratin assays, which may enhance sensitivity. Moreover, refining assay techniques, such as quantitative NMP22 tests, and adjusting cutoff values based on patient-specific factors could improve detection, especially for early-stage tumours. Incorporating NMP22 into a multimodal diagnostic approach, particularly in high-risk groups, and using it in longitudinal surveillance settings may also help capture early signs of disease progression, thereby improving overall diagnostic performance [6,8].

The present study has several notable strengths. It provides a comparative analysis of NMP22, cytology, and histopathology, focusing on their performance in detecting different t-stages and grades of bladder cancer. By highlighting the non invasive nature of NMP22 testing, the study emphasises its potential as a cost-effective diagnostic tool, particularly for advanced-stage and high-grade tumours. Furthermore, the statistically significant results reinforce the reliability of the findings and their relevance in clinical practice.

Limitation(s)

A small sample size restricts the generalisability of the conclusions to broader populations. Additionally, potential selection bias and the single-center nature of the study may reduce its applicability across diverse clinical settings. The study did not focus on BMI's impact on prognosis or treatment outcomes, which may require further research. Future research should focus on larger, multicentre studies to confirm these findings and ensure their applicability across different populations. Exploring the combination of NMP-22 with other diagnostic modalities, such as imaging or molecular biomarkers, could improve the detection of early-stage and low-grade tumours. Long-term, longitudinal studies are also recommended to evaluate the role of NMP-22 in monitoring disease recurrence. Furthermore, a cost-benefit analysis could assess the feasibility of incorporating NMP-22 into routine diagnostics.

CONCLUSION(S)

In conclusion, the data from this study reaffirm the diagnostic advantages of NMP22 over cytology, particularly in detecting high-grade and advanced-stage bladder cancers. While cytology remains useful due to its high specificity and role in confirming positive cases, NMP22 offers a more sensitive alternative, especially for identifying more aggressive forms of the disease. These findings suggest that NMP22 could play a pivotal role in the diagnostic pathway for bladder cancer.

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