The Use of NMP22 and Urine Cytology for the Surveilance of Patients with Superficial Bladder Cancer

W Chua, L Tan, R Kamaraj, E Chiong, S Liang, K Esuvaranathan

Citation

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Abstract

Aim To see if NMP22 combined with urine cytology can safely increase the interval between cystoscopies in the surveillance of superficial bladder cancers. Methods Thirty four patients with low grade superficial bladder transitional cell carcinomas were prospectively recruited and followed up with regular flexible cystoscopies over 2 years. Freshly voided urine was collected for urine cytology and NMP22 test prior to cystoscopy. Patients with suspicious cystoscopy findings or suspicious cytology underwent biopsies and/or resection of the suspicious lesions. Results 172 urine samples were collected and analyzed. The NMP 22 results were compared with voided urine cytology, cystoscopy and histological findings. Using the 10U/ml cutoff for quantitative measurement, the sensitivity, specificity, positive and negative predictive values of urinary NMP22 were 50%, 90.7%, 21.0%, and 97.3% respectively. When combined with voided urine cytology, the sensitivity, specificity, positive and negative predictive values were 71.4%, 88.5%, 25.0%, and 98.3% respectively. In comparison, the sensitivity, specificity, positive and negative predictive value of cystoscopy were 85.7%, 97.6%, 66.7%, and 99.2% respectively. Analysis with a receiver operating characteristic(ROC) curve found the ideal threshold of determinance of NMP22 for the detection of recurrences is 8.5U/ml with 100% sensitivity and 73.9% specificity. In our center, replacing alternate cystoscopy examination at 3,9,18 and 30 months with NMP22, results in a cost saving USD$740 or 37% per patient over 5 years. In the event of false positives, the patient will revert to the current surveillance schedule, with no increased costs incurred. Conclusion We propose that the optimal cutoff value for NMP22 is 8.5U/ml. At this level. the combination of the NMP22 BladderChek test and voided urine cytology is a sensitive non-invasive alternative to cystoscopy in the surveillance of patients who have had superficial low grade bladder cancer.

INTRODUCTION

Bladder cancer is the eleventh most common cancer in the world.[1] In Singapore, the age standardized incidence rate is 7.7/100,000 males, and 1.8/100,000 females per year.[2]

Approximately 70% will present with superficial disease, and may be managed with transurethral resection with or without adjuvant intravesical therapy. The recurrence rates for bladder cancer are as high as 50% -70%, with as many as 10-15% progressing to muscle invasive disease.[3] Early diagnosis and treatment of bladder recurrences may permit bladder preservation, prevent progression and may improve survival.

The high incidence and protracted natural history of bladder cancer results in a high prevalence of this disease. The need for frequent cystoscopic surveillance leads to high cost.

In recent years, much research has been directed to finding tumour markers to aid in the screening, diagnosis and surveillance of bladder cancer. Candidate markers have included Nuclear Matrix protein (NMP22), Telomerase, Bladder Tumour Antigen (BTA), BCLA-4, Fibrin Degradation Products (FDP). Konety and Getzenberg [4] have summarised the sensitivities and specificities of these tests in their review of urine based markers of bladder cancer. (Table 1)

Figure 1

Table 1 – Average sensitivities and specificities of bladder tumour markers

Tests	% Sensitivtity	% Specificity
Cytology	49.1	95.7
NMP-22	70.5	75.2
BTA	52.3	84.6
FDP	68.3	77.8
Telomerase	74.3	78.8
BCLA 4	96.4	100

NMP22 is a nuclear mitotic apparatus protein involved in the proper distribution of chromatin to daughter cells that is present in the nuclear matrix of all cell types and located in the mitotic spindle during mitosis. It is thought to be released from nuclei of tumour cells during apoptosis, and its expression is has been shown to be twenty five fold higher in patients with bladder cancer.[5]

As NMP22 is a common protein present in each cell of the body, studies have shown elevated NMP22 in patients with inflammation of the bladder as well as other neoplastic conditions such as renal cell carcinoma. While NMP22 has been shown to be sensitive to the presence of bladder cancer, its utility in the detection of bladder cancer is limited by its lack of specificity. Therefore NMP22 is more accurate in detecting recurrences in patients who already have bladder cancer.[4] It has been approved by the United States Food and Drug Administration (FDA) for the monitoring of patients with non-muscle invasive bladder cancer and for bladder cancer screening in patients with haematuria.

We aim to see if NMP22 combined with urine cytology can be used to safely increase the interval between cystoscopy in the surveillance of superficial bladder cancers, in order to reduce morbidity and cost.

METHODS
NMP22 ELISA TEST

This a quantitative enzyme immunoassay that detects activity and is reported as a numeric value of activity per mililitre(units/mL). The immunoassay detects complexed nuclear matrix proteins over 1 million d (base sequences) and also in their fragmented form, in the range of 30kd. The soluble portions of these complexed and fragmented forms are excreted in the urine, allowing the assay to be performed. The test is performed by trained personnel at a reference laboratory.

The immunoassay values (units/mL) have been studied in different demographic populations. Normal women have higher NMP22 levels than normal age-matched men. The median value for a normal woman aged 50-70 years is 3.90 versus 2.38 U/mL for men. This difference is statistically significant. [6]

BLADDERCHEK TEST

The NMP22 BladderChek Test is a qualitative point of care device, utilizing lateral flow immunochromatography. The 10-U/ml threshold of determination for the qualitative point-f-care test for NMP22 protein corresponds to the cutoff previously approved by the FDA for quantitative measurement of the marker.[7] The NMP22 BladderChek Test has the advantages of being rapid, office based, non-invasive, and relatively inexpensive. It is simple to administer, with test results available during the patient visit.

Consecutive patients with histology-proven low grade superficial bladder transitional cell carcinomas (grade G1 or G2 and stage Ta or T1) were prospectively recruited and followed up with regular flexible cystoscopies according to our surveillance schedule. We reviewed the patients at three-monthly intervals for the first two years, then at six-monthly intervals from the third to fifth years, and at yearly intervals from the sixth year thereafter. At each visit, freshly voided urine was collected for urine cytology and NMP22 test prior to cystoscopy. Patients with muscle invasive disease, carcinoma-in-situ, G3 tumours, previous radiotherapy, and clinically or microbiologically diagnosed urinary tract infection were excluded from this study. We included patients who received BCG immunotherapy.

This study was approved by our institution's Ethics Review Board, and all patients gave informed consent.

The NMP22 tests were performed according to the manufacturer's instructions. The test specimens were identified by study identification numbers in this single blind study so that the clinicians involved were blinded to the results. The NMP22 ELISA test was used initially. Subsequently, when the NMP22 BladderChek test was made available, the urine samples were tested with both tests to ensure equivalency. The latter urine samples were tested solely using the NMP22 BladderChek test.

Patients with suspicious cystoscopy findings or suspicious cytology were evaluated further with transurethral biopsies and/or resection of the suspicious lesions. Those with biopsies/ resection specimens positive for malignancy on histology, or cytology with malignant cells were considered to have a bladder cancer recurrence. Conversely, patients were considered negative for cancer if no tumours were found on cystoscopic surveillance, or if tissue or cytology removed did not reveal any malignancy. These patients were followed up according to our surveillance schedule.

STATISTICAL ANALYSIS

Sensitivity of the NMP22 test to detect recurrent bladder tumours was calculated as the number of patients with true positives divided by the total number of patients with malignancy (as determined by the presence of histologically proven tumour detected by cystoscopy or cytology). Specificity was the percentage of true negatives defined as the percentage of patients with a negative NMP22 test result who were not diagnosed with a malignancy.

The Statistical Package for Social Sciences (SPSS), release 12.0 was used for analysis.

RESULTS

Thirty four consecutive patients (27 men, 7 women), median age of 62 years (41 to 77 years) with histology-proven low grade superficial bladder transitional cell carcinomas were recruited and followed up for 2 years.

A total of 172 urine samples were collected and analyzed. NMP22 ELISA test was used for the first 61 voided urine samples. When NMP22 BladderChek test was made available, the subsequent 35 voided urine samples were tested with both tests. There was a 100% concordance using 10U/ml as cutoff. The last 41 urine samples in our series were tested with the NMP22 BladderChek test. The NMP22 results were compared with voided urine cytology, cystoscopy and histological findings.

Using the 10U/ml cutoff for quantitative measurement of the marker as previously approved by the FDA, the sensitivity, specificity, positive and negative predictive values of urinary NMP22 were 50%, 90.7%, 21.0%, and 97.3% respectively. When combined with voided urine cytology, the sensitivity, specificity, positive and negative predictive values were 71.4%, 88.5%, 25.0%, and 98.3% respectively (Table 2). In comparison, the sensitivity, specificity, positive and negative predictive value of cystoscopy were 85.7%, 97.6%, 66.7%, and 99.2% respectively.

Figure 2

Table 2 – Overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for cancer recurrence (NMP22 cutoff ⏃ 10U/ml)

	NMP22 10U/ml	Cytology	Cystoscopy	Combination of NMP22 and Cytology
Sensitivity	50.0	40	85.7	71.4
Specificity	90.7	100	97.6	88.5
PPV	21.0	100	66.7	25
NPV	97.3	97.5	99.2	98.3

Seven biopsy proven recurrences were detected. None of the recurrences had progressed to muscle invasion. One of these recurrences was extravesical - from a ureteric TCC.

Of the 172 urine specimens, there were 15 false positives (Table 3) and 3 false negatives. The range of NMP22 values for the 3 false negatives were from 8.5 – 9.6U/ml.

Figure 3

Table 3 – False positives

	No. of patients	Ranges (U/ml)
BCG cystitis	2	17.6 to 111.1
Non specific cystitis	5	12.3 to 43.5
Unknown	8	10.3 to 53.3

DISCUSSION

Superficial transitional cell carcinoma require early detection and close surveillance. Low-grade Ta lesions recur at a rate of 50% to 70% and have approximately a 5% chance of progression, whereas high-grade T1 lesions recur in more than 80% of cases and progress in 50% of patients within 3 years.[8] The inability to differentiate between aggressive and indolent Ta, T1 bladder cancer results in excessively frequent cystoscopic evaluation of patients who may have indolent disease.

Cystoscopy is our current gold standard for detection of recurrences. It is invasive, uncomfortable and may cause complications such as infections and urethral strictures. Urine cytology on the other hand is good for detecting high

grade tumours, but it is lacking in the diagnosis of low grade malignancy. Wiener showed sensitivity rates of 17%, 61% and 90% in grade 1, 2 and 3 tumours respectively.[9] In addition, it is highly dependent on the expertise of the cytopathologist.[10]

Our results with the NMP22 correlate well with others who have used it in the detection of recurrent disease.[9,11-18] (Table 4)

Figure 4

Table 4 – Comparison of reported sensitivity and specificity of NMP22 (at various cutoff values) and urine cytology in monitoring of recurrent bladder cancer

	Test (cut off value)	Sensitivity	Specificity
Soloway(1996)[12]	NMP22 10U/ml	69.7	78.5
Stampfer (1998)[13]	NMP22 6.4U/ml	68.2	79.8
Wiener (1998)[9]	NMP22 10U/ml	48	70
Giannopoulos A (2000)[14]	NMP22 8.0U/ml	62.6	73.9
Poulakis V(2001)[15]	NMP22 10U/ml	79	70
	NMP22 8.25U/ml	85	68
	NMP22 6.4U/ml	91	56
Miyanaga (2003)[16]	NMP22 12.0U/ml	18.6	85.1
	NMP22 6.0U/ml	48.8	66.0
	Combined cytology/ NMP22 10U/ml	78.6	84.7
Shariat SF (2004)[17]	NMP22 10U/ml	54	85
	NMP22 6.5U/ml	66	73
	Combined cytology/ NMP22 10U/ml	70	82
	Combined cytology/ NMP22 6.5U/ml	77	70
Moonen (2005)[18]	NMP22 10U/ml	57.1	89.8
Grossman (2006)[19]	NMP22 10U/ml	49.5	87.3

The sensitivity of NMP22 in bladder cancer has been reported to be related to size,[19] and as recurrent tumours on surveillance are generally smaller than primary tumours, many authors have suggested lower thresholds of determination for the NMP22 for the detection of recurrent bladder cancer. [12-16]

For the detection of recurrent disease, a test with 100% sensitivity and negative predictive value would be ideal, as this will detect all recurrent disease. Since the specificity of

any diagnostic test has an inverse relationship to its sensitivity, there will be more cases of false positive readings. In this context, it is far better to have a false positive result resulting in additional investigations rather than a false negative result with the attendant morbidity of a missed or delayed diagnosis of tumour recurrence. Based on analysis with a receiver operating characteristic(ROC) curve, the ideal threshold of determinance of the NMP22 for the detection of recurrences with maximal specificity and 100% sensitivity is 8.5U/ml. At this level, the NMP22 would have 73.9% specificity. Giannopoulos[13] and Poulakis[14] have also proposed lowering the cutoff to 8 and 8.25U/ml respectively. We propose that the performance of the BladderChek test as a surveillance tool will be improved if the threshold of determinance is lowered to 8.5U/ml.

In this population with superficial transitional cell carcinoma on surveillance for recurrent bladder cancer, the most important test parameter is the negative predictive value, which is largely dependent on the test's sensitivity. We feel that the high sensitivity and negative predictive value of the NMP22 at the revised cutoff of 8.5U/ml makes it viable to be alternated with cystoscopy examination, thereby reducing the frequency of cystoscopy and its attendant morbidity and costs. Shariat et al [20] developed and internally validated nomograms incorporating urinary NMP22, cytology, age and gender to predict the probability of disease recurrence and progression in patients with superficial transitional cell carcinoma of the bladder, with an accuracy of 84%. The nomograms may help individualise follow-up in these patients.

Patients with false positive results will undergo cystoscopy to confirm the result. Following this, they will revert to the current surveillance schedule of combined cystoscopies and cytology.

Superficial transitional cell carcinomas have a protracted natural history, which coupled with its high incidence rate results in a high overall prevalence of disease. This high disease prevalence together with the need for lifelong surveillance makes the cost of bladder cancer the highest of all cancers in the United States, ranging in cost from US$96000 to US$187000 per patient.[21] Although the price of surveillance is high, it is less costly in terms of patient survival, quality of life and treatment expenses than detection of recurrence at a later stage.

In our centre, the current cost of surveillance using cystoscopy and urine cytology over 5 years at 3 monthly

intervals for the first year, then 6 monthly for the next 2 years, followed by annually for the last 2 years is USD$2000 per patient. If we use the NMP22 to replace cystoscopy examination at 3,9,18 and 30 months, the cost is USD$1260, which is a cost saving of 37% per patient over 5 years. In the event of false positives, the patient will revert to the current surveillance schedule, with no increased costs incurred.

We acknowledge that the main weakness of our study is insufficient numbers to make firm conclusions on the optimal cutoff for NMP22 , but we hope that it can be the starting point for further study into how optimizing the performance of NMP22 can reduce the need for cystoscopy and its attendant morbidity in the surveillance of superficial bladder cancer.

CONCLUSION

Tumours found on surveillance are generally small. For the early detection of such tumours, the cutoff value of the NMP22 should be reduced to improve the sensitivity of the test. We propose that the optimal value would be 8.5U/ml.

The combination of the NMP22 BladderChek test and voided urine cytology is a sensitive non-invasive alternative to cystoscopy in the surveillance of patients who have had superficial low grade bladder cancer. Its use can reduce the frequency of cystoscopy, reducing patient morbidity and discomfort, and the overall cost of management.

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Author Information

Wei Jin Chua, FAMS Urology
Department of Urology, National University Hospital

Lincoln GL Tan, MRCS(Edin)
Department of Urology, National University Hospital

Revathi Kamaraj, MBBS
Department of Urology, National University Hospital

Edmund Chiong, FAMS Urology
Department of Urology, National University Hospital

Shen Liang, Ph.D (Statistics)
Yong Loo Lin School of Medicine, National University of Singapore

Kesavan Esuvaranathan, FAMS Urology
Department of Urology, National University Hospital