



## Effect of retention time on NMP22 bladder check assay results in voided urine

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### ABSTRACT

**Background:** Bladder cancer health care costs are high, primarily due to the need for long-term follow-up. Several markers have been developed to detect the presence of urothelial cell carcinoma (UCC) in the urinary tract. These markers have differing sensitivity and specificity. The nuclear matrix protein-22 (NMP22) test increases the ability to detect recurrent bladder cancer.

**Objectives:** The aim of this study was to evaluate possible changes in the results of this test depending on the length of the retention time in the bladder of the collected urine.

**Patients and Methods:** Between January and June 2006 we prospectively evaluated voided urine specimens in 69 patients undergoing control cystoscopy or transurethral resection of primary or recurrent bladder cancer. We tested for NMP22 in the first morning urine sample and samples collected after 5 min, 30 min and 2 h of urine retention in the bladder. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined, and results were grouped according to tumor stage and grade.

**Results:** Global sensitivity was similar in all groups (first morning sample and 5-min, 30-min and 2-h urine retention); the best sensitivity was observed at the 30-min sample point (80%). Specificity varied from 75% to 100% and the best results were obtained in first morning urine and at 30 min and 2 h. PPV was 95.5-100% at the different urine retention periods, while NPV was in the range of 27.3-33.3%.

**Conclusion:** Although this was not a large series, it appears that there is a tendency towards better results with the 30-min urine retention time. It is not necessary to wait 2 h for test operability.

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#### ► Implication for health policy/practice/research/medical education:

This article suggests that the retention time of NMP22 test in detection of recurrent bladder cancer can be modified.

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## 1. Background

Bladder cancer health care costs are high, since non-muscle-invasive urothelial cell carcinoma (UCC) is a life-long disease that may recur and progress. Even though transurethral resection of a bladder tumor, intravesical

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chemotherapy and immunotherapy decrease the recurrence and time-to-progression rates (1), the likelihood of development of new tumors obliges the urologist to perform long-term follow-up. The gold standard for monitoring bladder cancer recurrence is cystoscopy. The general recommendation for cystoscopic surveillance has been every 3 months for the first year and every 6 months for the second year; however, this practice has changed to adapt to the risk of recurrence and progression of this heterogeneous disease through the adoption of EAU guidelines (1). Several markers have been developed as potential tests to detect the presence of UCC in the urinary tract. Globally, a sensitivity of 50–90% and a specificity of 60–90% (2) have been demonstrated. Nuclear matrix protein-22 (NMP22) is a nuclear mitotic apparatus protein involved in the distribution of the chromatin to offspring cells, and it is located in the nuclear matrix of all cell types. NMP22 is released from the nuclei of the tumor cells during apoptosis. Patients with bladder cancer have 25 times more NMP22 in their urine than normal individuals (3), and this has led to the development of the NMP22 BladderChek point-of-care assay (Inverness Medical Innovations Inc., Boston, USA). Furthermore, it has been demonstrated that the NMP22 BladderChek test increases the ability to detect recurrent bladder cancer (4), provides immediate results, is easy to perform and is not operator dependent.

In a series of 739 patients of whom 406 had bladder cancer, Poulakis *et al.* (5), obtained an overall sensitivity of 85% for NMP22, 70% for BTastat and 62% for voided urine cytology (VUC). For histological grades of UCC from 1 to 3, the sensitivity of NMP22 in detecting UCC was 82% (grade 1), 89% (grade 2) and 94% (grade 3). In patients followed up for bladder cancer, false-positive results from NMP22 and VUC, but not from BTastat, correlated with future recurrence. In patients with no apparent genitourinary disease on histology, the NMP22 test had a significantly higher specificity of 94%. Although the aforementioned results are encouraging, the effect of urine retention time in the bladder on the result of the NMP22 test has not been analysed.

## 2. Objectives

The purpose of the present study was to evaluate possible changes in the results of this test depending on the retention time in the bladder of the collected urine.

## 3. Patients and Methods

Between January and July 2006 we performed a prospective study on voided urine specimens in patients undergoing a control cystoscopy or transurethral resection of a primary or recurrent bladder cancer (TURBT). Every patient underwent a urine analysis the day before in order to rule out the presence of a urinary tract infection (UTI). Urine for cytology was also collected together with the second sample. The following samples were collected

after voiding and tested for NMP22: first morning urine and samples after 5 min, 30 min and 2 h of retention in the bladder. Exclusion criteria were: actual or recent presence of UTI, presence or history of foreign body, renal/bladder calculi, bowel interposition segment, recent TURBT (within the past 3 months), intravesical bacillus Calmette-Guerin or chemotherapy, and other genitourinary cancer.

The Bladderchek test procedure was carried out in accordance with standard practice, which is as follows: immediately (within 30 min) after receiving the sample from the patient, four full drops of fresh urine are placed into the sample field of the NMP22 BladderChek test device (proteolytic enzymes can destroy the NMP22 protein). Room temperature adjustment is not necessary. The result is read in the test field after 30–50 min. The test is considered positive if a pink-coloured band appears (even a very small faint band is judged as positive) and negative if the band is absent. Statistical analysis comprised of determination of the sensitivity, specificity, and positive predictive value (PPV) and negative predictive value (NPV). The results were grouped according to tumor stage and grade. Sensitivity was compared between groups using the McNemar test.

## 4. Results

A total of 69 patients (84.1% males) were enrolled in the study with a mean age of 70 years (34–90 years). Sixty-one (88.4%) of the patients had bladder urothelial carcinoma and the remaining eight (11.6%) were negative for genitourinary cancer (i.e. T0). Non-muscle-invasive bladder cancer (Ta–T1b) was found in 75.8% of patients and 7.2% had muscle-invasive tumors. Three patients (4.3%) did not have their primary tumor assessed (i.e. Tx). WHO cancer grade was grade 1 in 8.7% of patients, grade 2 in 40.6% and grade 3 in 34.8% (WHO 1973) (Table 1). The cytology was positive in 39.1% of all patients and in 25% with grade 1 tumors, 28.2% with grade 2 tumors and 87.5% with grade 3 tumors. Global sensitivity was similar in all

**Table 1.** Details of the patient population (n=69)

<b>Age (yr)</b>	<b>70.0</b> (range 34.2–90.3)
<b>Sex [No.(%)]</b>	
<b>Male</b>	58 (84.1)
<b>Female</b>	11 (15.9)
<b>Cancer stage [No.(%)]</b>	
<b>No tumor</b>	8 (11.6)
<b>Ta</b>	40 (58.0)
<b>Tis</b>	4 (5.8)
<b>T1</b>	9 (13.2)
<b>T2</b>	5 (7.3)
<b>Tx</b>	3 (4.4)
<b>Cancer grade<sup>a</sup> [No.(%)]</b>	
<b>No tumor</b>	8 (11.6)
<b>G1</b>	6 (8.7)
<b>G2</b>	28 (40.6)
<b>G3</b>	24 (34.8)
<b>Gx</b>	3 (4.4)

<sup>a</sup> WHO 1973 classification

**Table 2.** Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for NMP22 Bladder Check according to urine collection time

	Sensitivity	Specificity	PPV	NPV
<b>1<sup>st</sup> morning urine</b>	71.7% (43/60)	100% (8/8)	100% (43/43)	32.0% (8/25)
<b>5-min urine <sup>a</sup></b>	72.4% (42/58)	75.0% (6/8)	95.5% (42/44)	27.3% (6/22)
<b>30-min urine</b>	80.3% (49/61)	85.7% (6/7)	98.0% (49/50)	33.3% (6/18)
<b>2-h urine</b>	72.1% (44/61)	87.5% (7/8)	97.8% (44/45)	29.2% (7/24)

<sup>a</sup> The times shown refer to retention time in the bladder

**Table 3.** Sensitivity of the NMP22 test according to gender, WHO grade and disease stage

	First morning sample	5-min <sup>a</sup> sample	30-min sample	2-h sample
<b>Gender</b>				
Male	70.6%	68.8%	78.4%	70.6%
Female	77.8%	90.0%	90.0%	80.0%
<b>Grade</b>				
1	50.0%	60.0%	83.3%	66.7%
2	70.4%	70.3%	82.1%	71.4%
3	79.2%	78.3%	79.2%	75.0%
<b>Stage</b>				
Non-muscle-invasive	71.2%	72.0%	81.1%	71.7%
Muscle-invasive	80.0%	80.0%	80.0%	80.0%

<sup>a</sup> The times shown refer to retention time in the bladder

groups (first morning urine sample and samples after 5 min, 30 min and 2 h of urine retention in the bladder). The best sensitivity was observed at the 30-min sample time point (80%), even though the result was not statistically significant. The specificity varied from 75% to 100%, with the best results obtained in the first morning urine sample and the 30-min and 2-h samples. The PPV was very high (95.5-100%) and similar at the different urine retention periods. The NPV was low, in the range of 27.3-33.3%. Three patients had invalid results at the 5-min sample time point (Table 2).

Sensitivity did not differ markedly according to gender, though it was higher in females, especially at the 5- and 30- minutes sample time points (Table 3). There was variability in the sensitivity at different time points according to the WHO grade. In grade 1 and 2 tumors, sensitivity was markedly higher for the 30-min sample (82.1-83.3 %) than for the first morning sample and also higher than that for the 5-min and 2-h samples (60-71.4 %). Values for grade 3 tumors were similar for the four samples. Muscle-invasive disease samples showed similar sensitivity at each of the four sampling periods, while in non-muscle-invasive disease samples the sensitivity was higher at 30 min (81.1 %).

### 5. Discussion

The bladder is the fourth most frequent site of cancer for males and the eighth for females. Bladder cancer has an incidence of 44.6 per 100,000 in men and 4.4 per 100,000 in women, and 50,000 new cases are diagnosed every year in the United States (4). Non-muscle-invasive tumors have a high recurrence rate (30-85%), particularly during the first 3 years of follow-up. This high recurrence

rate necessitates exhaustive follow-up with cystoscopy and cytology every 3-6 months, depending on tumor grade and staging, which increases the costs and also the discomfort to patients. Cytology is a non-invasive method for detecting recurrence of bladder cancer and has good specificity for this purpose; however, it has a low sensitivity (39% in our study), is operator dependent and entails a considerable cost. In recent years, multiple tumor markers have been developed with the aim of reducing the number of cystoscopic procedures performed each year, decreasing illness-related costs and enhancing patients' quality of life (6). An ideal tumor marker has to be easy to use and easy to interpret; moreover, a marker should preferably be cheap and have good sensitivity and specificity. One of the new tumor markers to have been proposed is NMP22. The NMP22 test has obvious benefits: it provides almost immediate results, thereby avoiding further unnecessary visits, and costs are less than half those of cytology. It is the only test approved by the Food and Drug Administration in the United States for the diagnosis and monitoring of bladder cancer patients.

Various studies have addressed the value of the NMP22 test. Saad *et al.* (7) reported an 81% overall sensitivity for the NMP22 test, with a 13% false-positive rate. Accuracy was determined to be 84%, indicating this assay to be very promising for the follow-up of bladder cancer and early detection of recurrence. In a different study, Kumar *et al.* (8) compared NMP22 with cytology using cystoscopy as the gold standard. The authors reported sensitivities of 85% and 41% for NMP22 and cytology, respectively, and concluded that NMP22 could replace cytology but not cystoscopy. In a prospective trial of 668 patients, Grossman *et al.* (4) found a sensitivity of 49.5% for NMP22. The sensitivity of cystoscopy rose from 91% to 99% when it

was used in addition to NMP22. The combination of both procedures detected seven high-grade tumors that were not diagnosed through the invasive work-up alone. The authors concluded that NMP22 is an important advancement in the early detection and follow-up of bladder cancer.

Moonen *et al.* (9) compared cytology and NMP22 in patients with suspicion of bladder cancer and found that NMP22 detected 40% of non-invasive papillary carcinoma tumors (Ta) and 83.3% of tumors that had invaded the subepithelial connective tissue (T1). Detection rates with cytology were 33.3% and 66.6%, respectively. The single patient with carcinoma in situ (CIS) was diagnosed by cytology but not by NMP22, indicating that NMP22 cannot replace cytology in the detection or follow-up of CIS. As NMP22 is a novel test, the optimal technique for sampling urine is not totally clear. Moonen *et al.* recommended that patients retain urine in the bladder for 2 h in order to prevent false-negative results. This empirical time was considered necessary for the detachment and lysis of the cells and for the subsequent release of NMP22. In our study, even though no clear and statistically significant difference was found between any of the four groups, there was a tendency towards an advantage for the 30-min urine sample group, as evidenced by 80.3% sensitivity versus 72.1% in the 2-h group. Our study shows that it is not necessary to wait for 2 h in order to perform this test in primary or follow-up studies.

Even though the differences in our study did not reach statistical significance, it can be stated that the sensitivity of the 30-min sample was globally better than that of the other three samples in detecting low-grade tumors and non-muscle-invasive bladder carcinoma. It should be noted that a lower level of nuclear matrix proteins is detected in urine collected after only a short period of retention in the bladder. The lower initial levels of free NMP22 suggest that at sample time points earlier than 30 min there will be insufficient apoptotic cells and released NMP22 for ideal protein detection. The NMP22 BladderChek test is an easy-to-use test for the diagnosis and follow-up of bladder cancer that has a higher sensitivity than cytology. Even though our series was not

large, it appears that there is a tendency to achieve better NMP22 test results after a 30-min urine retention time. We conclude that it is not necessary to wait 2 h for test operability.

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### Conflict of interest

First author of this article, J. Palou, is consultant of Sanofi Pastor, General Electric.

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### References

1. Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Böhle A, Palou-Redorta J, et al. EAU Guidelines on Non-Muscle-Invasive Urothelial Carcinoma of the Bladder, the 2011 Update. *Eur Urol.* 2011.
2. Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA. *Campbell-Walsh UROLOGY*. 9th ed: Saunders/Elsevier; 2007.
3. Keese SK, Briggman JV, Thill G, Wu YJ. Utilization of nuclear matrix proteins for cancer diagnosis. *Crit Rev Eukaryot Gene Expr.* 1996;**6**(2-3):189-214.
4. Grossman HB, Soloway M, Messing E, Katz G, Stein B, Kassabian V, et al. Surveillance for recurrent bladder cancer using a point-of-care proteomic assay. *JAMA.* 2006;**295**(3):299-305.
5. Poulakis V, Witzsch U, De Vries R, Altmannsberger HM, Manyak MJ, Becht E. A comparison of urinary nuclear matrix protein-22 and bladder tumour antigen tests with voided urinary cytology in detecting and following bladder cancer: the prognostic value of false-positive results. *BJU Int.* 2001;**88**(7):692-701.
6. Shirodkar SP, Lokeshwar VB. Potential new urinary markers in the early detection of bladder cancer. *Curr Opin Urol.* 2009;**19**(5):488-93.
7. Saad A, Hanbury DC, McNicholas TA, Boustead GB, Morgan S, Woodman AC. A study comparing various noninvasive methods of detecting bladder cancer in urine. *BJU Int.* 2002;**89**(4):369-73.
8. Kumar A, Kumar R, Gupta NP. Comparison of NMP22 BladderChek test and urine cytology for the detection of recurrent bladder cancer. *Jpn J Clin Oncol.* 2006;**36**(3):172-5.
9. Moonen PM, Kiemeny LA, Witjes JA. Urinary NMP22 BladderChek test in the diagnosis of superficial bladder cancer. *Eur Urol.* 2005;**48**(6):951-6; discussion 6.