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ORIGINAL PAPER

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Comparison of the nuclear matrix protein 22 with voided urine cytology in the diagnosis of transitional cell carcinoma of the bladder

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Abstract Several urinary markers for transitional cell carcinoma have been investigated, including urine cytology, bladder tumor antigen, autocrine motility factor receptor and fibrin degradation products. Unfortunately, they have poor overall sensitivity. The United States Food and Drug Administration have recently approved nuclear matrix protein (NMP 22) for the detection of occult or rapidly recurring disease after transurethral resection of bladder tumor. The objective of the current study was to assess the sensitivity of NMP 22 for the detection of bladder carcinoma, as well as to correlate the NMP 22 values with multiplicity of tumor, tumor size, configuration, stage and grade respectively. A total of 78 patients (38 with bladder cancer) provided a urine sample which was divided into appropriate aliquots for each of urine cytology and NMP 22. Comparative results demonstrate a clear superiority of NMP 22 in bladder cancer detection (52.6% vs 31.6% sensitivity), while specificity was in favor of urine cytology (100% vs 82.5%). For superficial tumors, sensitivity was 78.5% for NMP 22 and 41.6% for cytology and for invasive cancers, sensitivity was 90% for NMP 22 and 60% for cytology. Urinary NMP 22 levels were significantly correlated with tumor grade and were significantly higher in large tumors than small tumors. NMP 22 test results showed sufficient sensitivity in comparison with urine cytology for the detection of transitional cell carcinoma. However, we do not think that it is a useful tool as a substitute for endoscopic examination for the detection and surveillance in bladder cancer.

Keywords NMP 22 · Bladder cancer · Tumor marker

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Introduction

Bladder cancer is the fourth most common cancer in men and eight most common cancer in women in the United States [1]. Approximately 70–80% of bladder cancers are confined to epithelium or invade only the lamina propria. The probability of recurrence or development of a second primary cancer within 5 years of treatment is 50–70%, with the majority of tumors recurring within 1 year [2]. Cytology has been accepted as the most reliable technique for non-invasive monitoring. Unfortunately, cytology has poor sensitivity and is operator-dependent. Furthermore, there is variation between pathologists' assessments of specimens [3]. Several urinary markers for transitional cell carcinoma have been investigated, all of which have poor overall sensitivity [4, 5, 6]. Therefore, a non-invasive urine assay that is sensitive, objective and quantitative would be a useful adjunct for the urologist who treats patients with bladder cancer.

Nuclear matrix proteins make up the non-chromatin structure that confers nuclear shape, organizes the chromatin and regulates critical aspects of mitosis. Certain nuclear matrix proteins have been identified as cancer specific markers in human cancer of the colon, breast, bone and other tissues [7, 8, 9]. The United States Food and Drug Administration (FDA) recently approved nuclear matrix protein (NMP 22) for the detection of occult or rapidly recurring disease after transurethral resection of bladder tumor. The sensitivity and specificity of this assay for detecting bladder cancer has been described previously [10]. Tumor size, grade and stage have a strong impact on the sensitivity of the NMP 22 test. A recent study showed that sensitivity is poorer with small size tumors than expected in patients at follow-up [11]. We report a prospective study of the distribution of NMP 22 levels in three subject groups in comparison with voided urine cytology.

Our objective was to assess the sensitivity of NMP 22 for the detection of bladder carcinoma, as well as to

correlate its value with multiplicity of tumor, tumor size, configuration, stage and grade respectively.

Materials and methods

After local ethics committee approval, 78 patients were prospectively enrolled the study. Patients who entered were divided into three groups based on fulfilment criteria: group 1 newly diagnosed transitional cell carcinoma or transitional carcinoma recurrence, group 2 urinary tract stone disease, and group 3 healthy individuals.

Patients with transitional cell carcinoma had pathologically confirmed disease. Current cystoscopy and biopsy results including multiplicity, shape, grade and stage were obtained for cancer-positive specimens. Bladder cancer was staged according to the TNM system [12]. Grade was assessed using the World Health Organization grading system [13].

Patients who needed any endoscopic procedure for urinary tract stone disease were selectively enrolled the study.

Healthy volunteers were those whose self-reported current health status was confirmed by medical examination and urinalysis.

All 78 subjects provided urine samples for analysis before an urological procedure such as cystoscopy. A total of 10 ml of urine per patient was stabilized according to the package insert for the NMP 22 test kit. Another 100 ml of urine was collected for the cytological examination.

NMP 22 assay

Calibrators, controls and stabilized urine samples were added to an antibody-coated microplate. The captured NMP 22 antigen was washed and then reacted with a second antibody labeled with digoxigenin. The digoxigenin labeled antibody was washed again, reacted with an anti-digoxigenin antibody coupled to horseradish peroxidase and detected using O-phenylenediamine as substrate. The reaction was terminated by the addition of 2 M sulfuric acid. The concentration of antigen in the urine was proportional to the intensity of color development, and concentration of digoxigenin labeled NMP 22 in the urine was calculated from a standard curve as determined by the concurrent testing of calibrators. The cut-off value of urine NMP 22 was set at 10 U/ml, based on the value of the healthy controls (mean ± 3SD).

Cytology

The voided-urine cytology (classes I, II, III, IV, V) was evaluated according to Papanicolaou's classification [14].

Nonparametric statistical methods were used for analyses because the assumption of a normal distribution was violated. The Mann-Whitney U-test was used to compare two groups, and the Kruskall-Wallis test for differences among multiple groups. A P value < 0.05 was considered statistically significant. The coefficient of correlation was determined by Spearman's rank correlation.

The tests used for detecting bladder cancer were defined with sensitivity, specificity, positive predictive value, negative predictive value and accuracy formulas. Sensitivity was defined as the number of truly positive bladder cancer cases classified as positive by a test. Specificity was defined as the number of truly negative cases classified as negative by a test. The positive predictive value of a test was defined as the probability that the patient had bladder cancer, given that the test was positive. The negative predictive value of a test was defined as the probability that the patient was bladder cancer free, given that the test was negative. Accuracy was defined as the true positives and true negatives in the total sample.

Results

Comparative analysis was done to evaluate the diagnostic and predictive capabilities of cytology and NMP 22 in relation to sensitivity, specifity, positive predictive value, negative predictive value and overall accuracy within the sample groups.

In the groups with a benign condition, all cytological results were benign. A total of 31.6% of the patients with bladder cancer had positive cytology. On the other hand, some patients with a benign condition had a positive NMP 22 test (six patients in group 2 and one patient in group 3). These cases were considered false positives (17.5%). While all cytologically positive patients had positive NMP 22 tests, eight patients with cytologically benign tests also tested positive. They were histologically proven to have transitional cell carcinoma. Six had low grade and low stage tumors, but two had high grade and high stage tumors. The pathologist indicated that these tumors had very undifferentiated areas.

Comparative analysis demonstrated that patients with malign cytology had significantly higher NMP 22 levels (177.5 ± 181.40 U/ml) than the patients with benign urinary cytology (33.92 ± 101.89 U/ml) ($P = 0.0001$).

The subjects who were diagnosed as having bladder cancer were distributed almost equally according to stage and grade (stage Ta = 12, T1 = 16, > T2 = 10, grade 1–2 = 24, grade 3 = 14).

Comparative results demonstrated a clear superiority of NMP 22 in bladder cancer detection (Table 1), while specificity was in favor of urine cytology (100% vs 82.5%). In 78 patients, the overall accuracy was similar, with 67.9% for NMP 22 and 66.6% for cytology ($P > 0.05$) (Table 2). The highest value of NMP 22 was determined in the transitional cell cancer group. It was statistically different from those in other groups ($P = 0.022$).

There was no significant difference between NMP 22 and cytology in terms of ability to predict the absence of bladder cancer in non-bladder cancer patients ($P > 0.05$).

In this study, the sensitivity of both tests increased as the stage and grade of the tumor increased. When the superficial category (Ta and T1) and invasive category (> T2) were compared, urinary NMP 22 levels

Table 1 Sensitivity of diagnostic tests for each group

Group	*n*	Sensitivity of cytology (%)	Sensitivity of the NMP 22 test (%)	Urinary NMP 22 levels (U/ml)
Transitional cell carcinoma	38	31.6	52.6	79.26 ± 146
Urinary tract stone disease	20	0	30	19.40 ± 30.52
Healthy	20	0	5	7.06 ± 6.38

Table 2 The validity of NMP 22 and urine cytology in bladder cancer

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Overall accuracy (%)	Kappa (κ)
NMP 22	52.6	82.5	74.1	64.7	67.9	0.35
Cytology	31.6	100	100	60.6	66.6	0.32

(34.98 ± 43.43 U/ml in Ta, 31.59 ± 63.51 U/ml in T1 and 208.67 ± 231.46 U/ml in T2 tumors) and cytology were significantly higher in patients with invasive tumors than in those with superficial ones ($P = 0.006$ for NMP 22 and $P = 0.007$ for cytology). A significant correlation occurred between tumor stage and urinary NMP 22 levels ($r = 0.43$, $P < 0.05$). There were only five patients with grade I bladder tumor. This was too few for statistical analysis. Therefore grade I and II tumors were pooled in the same category (low grade). Urinary NMP 22 values (26.26 ± 37.84 U/ml in grades I and II and 170.12 ± 210.28 U/ml in grade II tumors) and cytological characteristics of the tumors showed significant differences between low grade (grades I and II) and high-grade (grade III) tumors ($P = 0.005$ for NMP 22 and $P = 0.013$ for cytology). Urinary NMP 22 levels were significantly correlated with tumor grade ($r = 0.48$, $P < 0.05$).

However, NMP 22 proved to be more sensitive than cytology in patients with high stage and high-grade tumors (Table 3). The sensitivity of the NMP 22 test in high stage tumors was statistically better than in low grade tumors, and also it was better than the sensitivity of cytology ($P < 0.05$).

Multiplicity of tumors showed no relation with urinary NMP 22 levels or cytology. However, in patients with a tumor size above 20 mm, the positive rates of NMP 22 (98.8%) and cytology (50%) was significantly higher than in patients with small tumors (< 20 mm) (Table 3). There was a significant difference in terms of the positivity of cytology between papillary and solid tumors ($P < 0.05$), while NMP 22 levels were not different ($P > 0.05$).

Table 3 Comparison of NMP 22 and urine cytology according to tumor properties. An asterisk indicates statistically different: $P < 0.05$

Properties of tumors	Number of patients	NMP 22 sensitivity (%)	Cytology sensitivity (%)
Stage			
Ta	12	41	16.6
T1	16	37.5	25
T2 and over	10	*90	*60
Grade			
I and II	24	37.5	16.7
III	14	*78.6	*57.1
Number of tumors			
Single	15	43.6	26.7
Multiple	23	44.9	34.8
Diameter			
< 2 cm	22	24.4	18.2
> 2 cm	16	*98.8	*50
Shape			
Papillary	33	32.5	24.2
Solid	5	67.8	*80
Total	38	*52.6	*31.6

Discussion

Bladder cancer is a common disease that causes significant morbidity and mortality worldwide. Because the progression and recurrence rates are high, close follow-up is mandatory in the management of this chronic disease. The standard of method for the early detection of recurrent bladder cancer is cytology and cystoscopic examination at 3 monthly intervals for 2 years after the initial diagnosis. However, cystoscopy is invasive. Patients frequently experience severe discomfort during the examination and they may develop urinary tract infections as a result of the procedure, which is also costly and operator dependent.

Although urinary cytology has been accepted as the most reliable technique for the non-invasive monitoring the bladder cancer, because of its inability to distinguish between inflammation and malignant conditions as well as variation between the results of pathologists examining the specimens, its effectiveness is limited. Bladder washing cytology is only moderately better and specificity is poor when borderline, dysplastic and suspicious lesions are categorized as positive [15].

The development of new non-invasive tumor markers that can reliably predict the presence of tumor would be clinically useful in terms of disease management, quality of life and cost of care. Several urinary markers for transitional cell carcinoma have been investigated, including autocrine motility factor receptor, fibrinogen degradation products, bladder tumor antigen (BTA) and (NMP 22 [4, 5, 6, 16]. Miyanaga et al. demonstrated that urinary NMP 22 is a useful tool as a substitute for voided-urine cytology in the surveillance of urothelial cancer [17]. The overall sensitivity for the detection of bladder cancer for NMP 22 in another study population was 73% [18].

The report by Soloway et al. showed that postoperative NMP 22 levels can predict the cystoscopy outcome 2–3 months later. In that study, there was an encouraging result for skipping check cystoscopies in patients with low urinary NMP 22 levels [19].

In five studies of more than 50 patients with bladder cancer, the reported sensitivity for the NMP 22 test ranged from 48% to 92% [20, 21, 22, 23, 24]

In the present study, comparative results demonstrated a clear superiority of NMP 22 in bladder cancer

detection (52.6% vs 31.6% sensitivity), while specificity was in favor of urine cytology (100% vs 82.5%). Overall accuracy was not significantly different.

The highest value of NMP 22 was determined in the transitional cell cancer group. The mean NMP 22 level was 79.26 ± 146 U/ml in the tumor group. Urinary NMP 22 was greater in men with transitional cell carcinoma than in those with no evidence of disease or benign conditions, as well as in healthy individuals.

Comparative results demonstrated a clear superiority of NMP 22 in every stage and grade. Sensitivity of NMP 22 was significantly higher than cytology even in Ta tumors. Although the sensitivity of both tests increased with stage, NMP 22 sensitivity was significantly higher than cytology in low stage tumors. Other studies have also shown an increasing NMP 22 sensitivity parallel to increasing tumor stage [20, 22, 23, 24, 25]. However, Wiener et al. did not find any significant difference between NMP 22 sensitivity and tumor stages [21]. Contrasting data were found when NMP 22 sensitivity was correlated with histological tumor grade [20, 21, 22, 23, 24, 25]. Our results show a similar sensitivity with increasing tumor grade. Although the sensitivity of cytology increased with grade as well, NMP 22 sensitivity was significantly better in low grade tumors than cytology in our study. However, other studies failed to find a direct correlation between these variables [25].

According to a Swedish group from Göteborg, tumor size is more important than grade and stage for NMP 22 [11]. They found a better sensitivity with increasing tumor size. Patients with recurrent tumors tended to have small tumors and the sensitivity of NMP 22 was poor. In contrast, sensitivity of NMP 22 was better in newly diagnosed large tumors in his study. Similarly, we observed that NMP 22 levels in larger tumors were significantly higher than in small tumors ($P < 0.024$).

The number and shape of tumor had no effect on NMP 22 levels (Table 3), but the pattern of growth had a strong impact on sensitivity for urinary cytology.

The major finding of this study is that while all cytologically positive patients had a positive NMP 22 test, eight patients who were cytologically benign also had a positive NMP 22 test. Although the number of cytologically false negative patients with bladder cancer was rather small, we think that this finding is the major advantage of NMP 22 in this study.

The overall low sensitivity of urine cytology is explained almost exclusively by its unreliable detection of well-differentiated, low-grade tumors. The cells of such tumors closely resemble normal epithelium [26] and urinary cytology is more operator-dependent than quantitative tests. Inconsistencies have been observed among pathologists evaluating bladder tumors and for the same pathologist grading the same bladder tumors at different times [27]. This would not be expected in quantitative tests such as NMP 22.

In addition, the changes in the exfoliated cells in the urine due to intravesical drug administration, catheterization, urinary tract infection and stone disease may also interfere with the diagnosis of bladder cancer [28]. In contrast, the NMP 22 test has the advantage of being useful in the presence of microscopic or gross hematuria [3]. In the presented study, eight of 38 patients with bladder tumor remained undiagnosed cytologically, but were diagnosed with the NMP 22 test.

We conclude that the urinary NMP 22 value shows considerable promise as a tumor marker in comparison with classical urinary cytology. But because of low sensitivity rates in low grade, low stage and small tumors, NMP 22 cannot replace check cystoscopies. In addition, the contradictory sensitivity results of the NMP 22 test which have been reported have possibly prevented general acceptance of this test in clinical practice to date.

Although it is invasive and costly, we still think that cystoscopy should be considered the most valuable diagnostic technique in detecting the bladder cancer.

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