

NMP22: A Sensitive, Cost-Effective Test in Patients at Risk for Bladder Cancer

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Abstract. *Background:* This study was designed to determine the clinical utility of NMP22 as a urinary marker for the early detection of transitional cell carcinoma (TCC) of the bladder in patients with hematuria or other indications for risk of malignancy. Its utility will be measured by sensitivity and specificity estimates as compared to cystoscopy. Since urine cytology is normally collected in this population of patients, it will also be analyzed and compared to cystoscopy. *Materials and Methods:* Each patient submitted a single voided urine which was stabilized with the NMP22 urine collection kit or preserved in the appropriate cytology medium for cytopathologic testing. All patients provided the urine samples before cystoscopic exam. Of the 146 patients, there were 43 patients with microscopic hematuria and 13 with gross hematuria. Other indications for cystoscopy included unexplained or medically refractory voiding. There were 8 patients with biopsy confirmed bladder cancer and 138 patients with benign conditions of the bladder. *Results:* The median NMP22 value for the bladder cancer malignancies was 27.8 U/mL (95% Confidence interval: 10.5-32.1 U/mL). The median NMP22 value for the benign conditions of the bladder was 3.25 U/mL (95% Confidence interval: 2.5-3.8 U/mL). The urinary NMP22 values from the bladder cancer group was statistically different ($p < .000001$ Mann-Whitney U test) than the NMP22 values in the benign conditions group. Using a reference value of 10.0 U/mL, the sensitivity of NMP22 was 100% with a specificity of 90%, while cytology had a sensitivity of 25% and a specificity of 100%. Due to its high negative predictive value, using NMP22 alone could have eliminated 124 cystoscopies with total savings ranging from \$24,824 to \$63,264 depending on the type of insurance carrier. *Conclusions:* This study indicates that urinary NMP22 is a useful, cost-effective marker for the early detection of bladder cancer.

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Hematuria, either gross or microscopic, is the most common presenting symptom of bladder cancer(1). Hematuria requires a thorough investigation. The degree of hematuria is not related to the gravity of the disease and microscopic bleeding must be taken as seriously as frank hematuria(2,3). Initially, urine cytology is used in conjunction with cystoscopy to determine the cause of hematuria. The sensitivity of voided urine cytology is, on average, less than 50%(4,5,6) and as low as 30%(7) in low stage, low grade disease.

Recently, the NMP22 Test Kit, an EIA for nuclear mitotic apparatus protein NuMA3 in voided urine, has been used for the detection of occult or rapidly recurring disease following TURBT. Using an NMP22 reference value of 10.0 U/mL, Soloway *et al* demonstrated sensitivities of 71% and 100% to predict recurrence following TURBT for non-invasive and invasive tumors respectively.⁸ For patients with NMP22 ≤ 10 U/mL, the negative predictive value was 86%. Further studies have shown it to be a promising tumor marker in other aspects of patient care(9,10,11,12,13)

This article describes a comparison of two non-invasive techniques for detecting tumors of the urinary tract in patients who present with risk for bladder cancer. The two methods are NMP22, a quantitative tumor marker and voided urine cytology.

Materials and Methods

One hundred and forty-six patients were referred to the urology clinic due to microscopic or gross hematuria or another indication of risk for bladder cancer. They provided a urine sample before the performance of a cystoscopy. Each patient provided a urine sample for voided urine NMP22 test and cytology and underwent a cystoscopy. All patients had a negative upper tract study within 6 months of the date of the cystoscopy.

The sample was collected and divided into two aliquots, one for transport to the andrology laboratory for NMP22 analysis and the other to the cytopathology laboratory. Both were performed at the Cleveland Clinic Foundation. The urologist and the pathologist were blinded to the results.

All test results, NMP22, cytology and cystoscopy, were recorded. A diagnosis of bladder malignancy was determined when a bladder tumor was observed during cystoscopic evaluation and confirmed by positive histology result from the biopsy sample. The sensitivity estimates are defined as the number of biopsy-proved bladder tumors which were

Table I. NMP22 values in patient groups.

Patient group	N	Median NMP22 value (U/mL)	95% Confidence interval for the median	Mean	Range
Benign conditions	138	3.20	2.5-3.8	5.0	0-64.7
Malignancy	8	27.8	10.5-32.1	256	10.5-1894

Table II. NMP22 and voided urine cytology: numbers used for estimation of sensitivity and specificity.

Patient group	N	NMP22 > 10.0 U/mL	Cytology positive	Cytology negative or atypical
Benign conditions	138	14	0	138
Malignancy	8	8	2	6

accurately classified as positive by the NMP22 test (>10 U/mL) or cytology (positive for malignancy). The specificity estimates are defined as the number of "no tumor seen", negative or biopsy-negative cystoscopies which were accurately classified as negative by the NMP22 test (<10 U/mL) or cytology (atypical or negative for malignancy).

The medians and means for the NMP22 values were calculated. The medians were compared to each other (non-normal distribution of values). The statistical test used to compare two medians was the Mann-Whitney U test, non-parametric one-way analysis of variance. The methodology used included computations of proportions and their confidence intervals, CI, (exact 95% CI base on binomial distribution).

Results

A total of 146 patients were evaluated. There were eight histologically confirmed bladder cancer patients and 138 patients in which the cystoscopy was negative; no tumor seen or histologically negative by biopsy. There were 113 males and 33 females. The average age was 63.5 years. A summary of descriptive statistics was done for the two different groups: those with malignancy and those with benign conditions (Table I). There was a significant difference between the NMP22 values from the benign conditions as compared to the NMP22 values from the malignancy group ($p < .000001$ Mann-Whitney U test). The benign conditions were chronic cystitis, stones, BPH, urethral strictures, epididymitis, prostatitis, or urinary tract infections.

There is a description of the calculation for sensitivity and specificity in Tables II and III. There were too few bladder cancer patients to determine an association between NMP22 value and degree of invasion (Table IV)

Table III. Sensitivity and Specificity: NMP22 and cytology.

Patient group	N	NMP22 > 10.0 U/mL	Cytology
Sensitivity	8	100% (8/8)	25% (2/8)
95% Confidence interval	---	63-100%	3-65%
Patient group	N	NMP22 > 10.0 U/mL	Cytology
Specificity	138	90% (124/138)	100%
95% Confidence interval	---	84-94%	97-100%

Table IV. NMP22 values in 8 newly diagnosed bladder cancers.

Patient number	NMP22 value (U/mL)	Stage	Grade
1	15.0	Ta	I
2	32.1	Ta	II
3	31.1	Ta	II
4	1894	Ta/T1	I/II
5	24.5	T1	I
6	31.1	CIS	III
7	10.5	T2	II/III
8	14	T2	III

Discussion

It is standard practice to examine patients with hematuria or other indication for risk of bladder cancer by cystoscopy. Since the overall prevalence of bladder cancer in the subset of patients with hematuria ranges from 4-10% (1,2,3) the need for a sensitive, cost effective tumor marker is evident. The limitations of urinary cytology are a) the suboptimal sensitivity and b) the professional costs. The professional costs for a voided urine cytology at the Cleveland Clinic Foundation costs \$100.00. Considering the sensitivity of urine cytology is, at best 50%, and the prevalence of disease being less than 10%, urine cytology is not cost-effective. The need for an automated, objective test to evaluate the many patients who present with hematuria or who are at risk for bladder cancer is evident. The ideal test would not only have a better sensitivity than urine cytology but would also have a high negative predictive value to eliminate the numerous negative cystoscopies currently done to evaluate patients with hematuria.

In the present study, 8 cancers were found in 146 cystoscopies performed. All 8 cancers were found in patients

with microscopic or gross hematuria. The NMP22 test did not miss any bladder cancers and the sensitivity was four times that of cytology. Although there were 14 false positive NMP22 results, using the reference value of 10U/mL, we wanted to optimize the sensitivity at 100%, and we felt comfortable sacrificing the specificity. Equally as important, in the 124 patients (48 with hematuria), with a negative NMP22 test, there was not one cancer detected upon cystoscopic examination. Thus, if NMP22 was used to indicate whether a cystoscopy was done or not, we would have done 14 unnecessary cystoscopies for the 14 false positive results, but could have eliminated 124 cystoscopies currently done for bladder cancer checks.

At our institution, the reimbursement is \$106.00 (Medicare) versus \$416.00 (private insurance carrier) per cystoscopy. Eliminating 124 cystoscopies would carry a cost savings ranging from \$13,144 to \$51,584. For 146 patients requiring evaluation, an additional cost saving is seen when we compare the price of an NMP22 test, \$20.00 per sample, to the cost of urinary cytology, \$ 100.00 per sample. This is a cost savings of \$11,680. Using the NMP22 test vs. urinary cytology to determine whether cystoscopy is required to eliminate the risk of bladder cancer would have saved total cost ranging from \$24,824 to \$63,264. This is a savings of, at least, \$3,103 per diagnosis of bladder cancer.

Whether the NMP22 test allows earlier detection of bladder cancer (at a lower stage) remains to be seen as we collect data on the stage and grade of the tumors. The predicted advantage of the NMP22 test over urinary cytology is that it is equally effective in diagnosing Ta/T1 tumors as well as muscle invasive, T2 or greater cancers. Published data on NMP22 vs. cytology on recurrent or prevalent bladder cancers shows similar sensitivity with high grade/ muscle invasive cancers, and NMP22 was 2-3X more sensitive in detecting recurrent low stage/ low grade cancers(10). As indicated in Table III, there is not enough data to determine the answer to this question. However, of the 8 cancers, the urinary cytology was positive in only patients 7 and 8, which had T2 or muscle invasive cancers. We will continue to collect data to determine if there is an association between NMP22 values and the degree of invasion.

This study indicates that urinary NMP22 is a sensitive, cost effective tool for the screening of urothelial cancer in patients with hematuria and symptoms at risk for malignancy. It may prove to be an excellent test for the diagnosis of low stage, low grade bladder cancer.

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