



Online article and related content  
current as of July 12, 2009.

## Detection of Bladder Cancer Using a Point-of-Care Proteomic Assay

H. Barton Grossman; Edward Messing; Mark Soloway; et al.

*JAMA*. 2005;293(7):810-816 (doi:10.1001/jama.293.7.810)

<http://jama.ama-assn.org/cgi/content/full/293/7/810>

Correction	<a href="#">Contact me if this article is corrected.</a>
Citations	<a href="#">This article has been cited 75 times.</a> <a href="#">Contact me when this article is cited.</a>
Topic collections	Oncology; Oncology, Other <a href="#">Contact me when new articles are published in these topic areas.</a>
Related Articles published in the same issue	Bladder Cancer <a href="#">Janet M. Torpy et al. <i>JAMA</i>. 2005;293(7):890.</a>
Related Letters	Detection of Bladder Cancer Using a Proteomic Assay <a href="#">Donald B. Loria. <i>JAMA</i>. 2005;293(20):2466.</a> <a href="#">Stefano Ciatto. <i>JAMA</i>. 2005;293(20):2467.</a>  In Reply: <a href="#">H. Barton Grossman. <i>JAMA</i>. 2005;293(20):2467.</a>

Subscribe  
<http://jama.com/subscribe>

Permissions  
[permissions@ama-assn.org](mailto:permissions@ama-assn.org)  
<http://pubs.ama-assn.org/misc/permissions.dtl>

Email Alerts  
<http://jamaarchives.com/alerts>

Reprints/E-prints  
[reprints@ama-assn.org](mailto:reprints@ama-assn.org)

# Detection of Bladder Cancer Using a Point-of-Care Proteomic Assay

H. Barton Grossman, MD

Edward Messing, MD

Mark Soloway, MD

Kevin Tomera, MD

Giora Katz, MD

Yitzhak Berger, MD

Yu Shen, PhD

**B**LADDER CANCER IS THE FIFTH most common malignancy in the United States.<sup>1</sup> Early detection improves prognosis, treatment options, and quality of life. Although the 5-year survival rate is 95%<sup>1</sup> when tumors are detected while they are confined to the mucosa, up to 25% of the 60 240 bladder tumors predicted to be diagnosed this year will be detected after they have become invasive or metastatic, which lowers 5-year survival to approximately 48% and 10%, respectively.<sup>1</sup> As a result, 13 000 people in the United States will die of bladder cancer this year.<sup>1</sup> The incidence of bladder cancer is higher in men, individuals older than 60 years, and those exposed to carcinogens in their occupation or environment. Cigarette smoking is the most common risk factor and doubles the risk of bladder cancer, accounting for approximately 50% of the bladder cancer deaths in men and 30% in women.<sup>2</sup>

Hematuria and irritative voiding symptoms are the most common symptoms among patients with urinary tract malignancy. Asymptomatic individuals are frequently diagnosed after routine or screening analysis by their primary care physicians has demonstrated hematuria. Hematuria in bladder cancer can be intermittent, and its degree does not correlate with the severity of underlying disease. Consequently, it is recommended that patients with hematuria undergo an evaluation after even a single episode.<sup>3</sup>

**Context** A combination of methods is used for diagnosis of bladder cancer because no single procedure detects all malignancies. Urine tests are frequently part of an evaluation, but have either been nonspecific for cancer or required specialized analysis at a laboratory.

**Objective** To investigate whether a point-of-care proteomic test that measures the nuclear matrix protein NMP22 in voided urine could enhance detection of malignancy in patients with risk factors or symptoms of bladder cancer.

**Design, Setting, and Patients** Twenty-three academic, private practice, and veterans' facilities in 10 states prospectively enrolled consecutive patients from September 2001 to May 2002. Participants included 1331 patients at elevated risk for bladder cancer due to factors such as history of smoking or symptoms including hematuria and dysuria. Patients at risk for malignancy of the urinary tract provided a voided urine sample for analysis of NMP22 protein and cytology prior to cystoscopy.

**Main Outcome Measures** The diagnosis of bladder cancer, based on cystoscopy with biopsy, was accepted as the reference standard. The performance of the NMP22 test was compared with voided urine cytology as an aid to cancer detection. Testing for the NMP22 tumor marker was conducted in a blinded manner.

**Results** Bladder cancer was diagnosed in 79 patients. 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. The proteomic marker detected 4 cancers that were not visualized during initial endoscopy, including 3 that were muscle invasive and 1 carcinoma in situ.

**Conclusion** The noninvasive point-of-care assay for elevated urinary NMP22 protein can increase the accuracy of cystoscopy, with test results available during the patient visit.

JAMA. 2005;293:810-816

www.jama.com

cer can be intermittent, and its degree does not correlate with the severity of underlying disease. Consequently, it is recommended that patients with hematuria undergo an evaluation after even a single episode.<sup>3</sup>

A combination of methods is used to evaluate patients at risk for bladder cancer because no single procedure is 100% sensitive. Flexible cystoscopy is an excellent tool because it is low risk and generally can be done in the physician's office under local anesthesia.

However, accuracy can be reduced by poor visualization caused by inflammatory conditions or bleeding, and flat

urothelial lesions such as severe dysplasias and carcinoma in situ may be difficult to distinguish from normal bladder tissue.<sup>4,5</sup> For this reason, voided

**Author Affiliations:** Department of Urology (Dr Grossman) and Department of Biostatistics and Applied Math (Dr Shen), M.D. Anderson Cancer Center, Houston, Tex; Department of Urology, University of Rochester Medical Center, Rochester, NY (Dr Messing); Department of Urology, University of Miami School of Medicine, Miami, Fla (Dr Soloway); Alaska Clinical Research Center, Anchorage (Dr Tomera); Department of Surgery-Urology Service, Lake City Veterans Administration Hospital, and LakeShore Urology, Manitowoc, Wis (Dr Katz); and Associates in Urology, West Orange, NJ (Dr Berger).

**Corresponding Author:** H. Barton Grossman, MD, Department of Urology, M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Unit 446, Houston, TX 77030 (HBGrossman@mdanderson.org).

See also Patient Page.

urine cytology is frequently used as an adjunctive noninvasive test, but it is expensive, subjective, and has low sensitivity.

We investigated whether a new, non-invasive urine-based test for the nuclear matrix protein NMP22 proteomic marker, using monoclonal antibodies in a point-of-care format, has clinical utility as an aid in diagnosis of bladder cancer and compared its ability to detect cancer with that of voided urine cytology, which must be analyzed in a clinical laboratory.

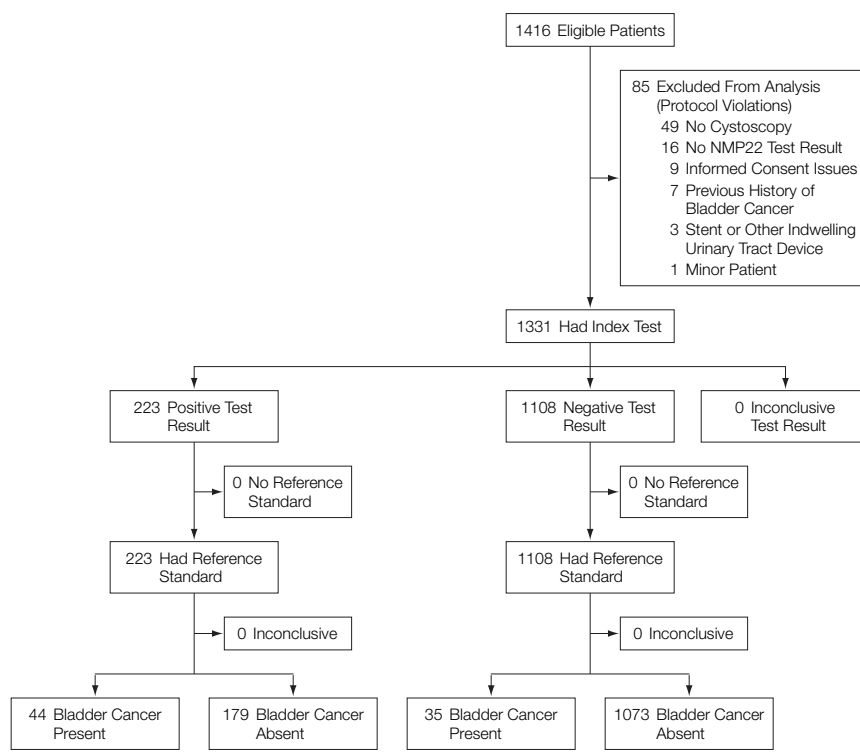
## METHODS

### Patients

Between September 2001 and May 2002, 22 geographically dispersed clinical sites, including academic, veterans', and private practice facilities, prospectively enrolled 1331 consecutive patients with bladder cancer risk factors or symptoms, such as smoking, hematuria, or dysuria (FIGURE). No individuals had a prior history of bladder malignancy. One additional site recruited 26 patients with active malignancies other than of the bladder to determine the specificity of the NMP22 test for bladder cancer. Information about race was obtained for Food and Drug Administration (FDA) submission purposes from patients by clinical staff at each site. Patients categorized themselves. Institutional review boards reviewed and approved the study protocol for each site, and all participants provided written informed consent.

Patients with cancers other than of the bladder provided a urine specimen for NMP22 protein analysis during a routine visit and did not have endoscopy or voided cytology evaluations. Each patient evaluated for bladder cancer provided a voided urine sample before undergoing cystoscopy. One portion of each sample was sent for routine cytological examination, either within the institution or at a reference laboratory, according to the standard practice at each participating facility. An aliquot of the remaining specimen was tested for the presence of NMP22 pro-

**Figure.** Flow Diagram of Study



tein by a member of the clinic staff. Each device was identified by study identification number so that the physicians who performed the subsequent cystoscopy were blinded to the NMP22 test results, and the staff members who performed the NMP22 assay were blinded to cystoscopy test results. Technicians who conducted the cytological examinations were physically distant from both the cystoscopy and NMP22 evaluations, and laboratory reports arrived after the cystoscopies had been completed and documented.

### NMP22 Assay

Staff members at each office performed the NMP22 assay per protocol by adding 4 drops of voided urine to the sample well of the point-of-care device. Positive or negative results were read 30 to 50 minutes later in the test window. A built-in control indicated that the assay was complete. There were no other procedural steps.

The NMP22 point-of-care device (NMP22 BladderChek Test, Matritech

Inc, Newton, Mass) is a lateral flow immunochromatographic qualitative assay. It detects elevated amounts of the nuclear mitotic apparatus protein, which is an abundant component of the nuclear matrix. Nuclear matrix proteins make up the internal structural framework of the nucleus<sup>6,7</sup> and are associated with such functions as DNA replication and RNA synthesis,<sup>8,9</sup> as well as regulation and coordination of gene expression.<sup>10-12</sup> In tumor cells, nuclear mitotic apparatus protein, which is present in the interphase nucleus and associated with the organization of mitotic spindles during cell division,<sup>13</sup> is elevated concordant with structural/morphological changes characteristic of malignant cell nuclei. Nuclear matrix protein expression varies with cell type of origin.<sup>14,15</sup> In individuals with bladder cancer, nuclear mitotic apparatus protein is released into the urine during cell death. Unlike cytological examination, its detection is not dependent on recovery of intact cells. A microtiter plate immunoassay was de-

veloped for this protein previously,<sup>16</sup> but unlike the new test in this investigation, it required that urine be stabilized and sent to a laboratory for analysis.

Two different monoclonal antibodies are used in the NMP22 point-of-care assay, one as a capture antibody, and one as a reporter. To perform the test, fresh unprocessed urine is added to the sample well of the device and allowed to react with the colloidal gold-conjugated reporter antibody. If NMP22 protein is present in the urine, it will interact with the reporter conjugate to form an immune complex. The reaction mixture flows through the membrane, which contains zones of immobilized antibodies. In the test zone, antigen-conjugate complexes are trapped by the capture antibody, forming a visible line if the concentration of NMP22 protein in the urine is greater than 10 U/mL. A procedural control zone contains an immobilized IgG-specific antibody that will capture the conjugated antibody independently of the presence or absence of the antigen, thereby always producing a visible control line in the window to demonstrate that each device is working properly.

A receiver operating characteristic (ROC) analysis, a plot of the true-positive rate vs the false-positive rate, is a tool for determination of an optimal decision point for sensitivity and specificity and requires quantitative data. An ROC analysis of quantitative data from the microtiter plate format of the NMP22 assay in an evaluation of patients at high risk for bladder cancer had an area under the curve (AUC) of 73%.<sup>17</sup> The 10-U/mL point of determination for the qualitative point-of-care test for NMP22 protein corresponds to the cutoff previously approved by the FDA for the quantitative measurement of NMP22 protein.<sup>18</sup>

### Diagnostic Criteria

All patients with risk factors or symptoms of bladder cancer underwent cystoscopy. They were considered positive for malignancy if 1 or more tumors were observed during initial cystos-

copy or within the subsequent 3 months. Nine patients with no malignancy found during their initial cystoscopy had a subsequent endoscopy due to continued suspicion, such as increased symptoms. Removed tumors were defined as malignant based on pathological examination. Tumors that were seen endoscopically but not removed were considered positive for malignancy and designated stage (TX) and grade (GX). Reasons that neoplasia were not removed included concurrent health problems that made patients poor candidates for surgery and advanced age. Patients were considered negative for cancer if no tumor(s) was seen endoscopically, or if tissue was biopsied and defined as nonmalignant on the basis of histopathological examination.

Pathological examination of biopsied tissue was done within each institution or at a reference laboratory, according to the standard practice at each participating facility. Staging criteria were those established by the American Joint Committee on Cancer.<sup>19</sup> Imaging information was not collected for comparison because standard practice at participating facilities varied widely on frequency and type.

### Statistical Analysis

Sample size estimate to determine the performance of the NMP22 test was based on a 1-sample test for binomial proportions using a 1-sided alternative and was derived from testing the null hypothesis that the observed proportion of detection is equal to the expected proportion of detection (newly diagnosed bladder cancers detected by cystoscopy) vs the alternative hypothesis that the observed proportion of detection is less than the expected proportion of detection. This was based on a type I error rate of 5% (ie,  $\alpha = .05$ ), 80% power, and finding a significant difference of 3% in the detection rate. Assuming 5% to 10% of patients with hematuria or other risk factors for bladder cancer could be expected to have a pathologically confirmed positive cystoscopic evaluation, an estimated sample size of 630 to 1300 patients was required.

Sensitivity of the NMP22 test to detect the presence of bladder cancer was calculated as the number of patients with true-positive test results (positive NMP22 test result and tumor) divided by the total number of patients with malignancy, as detected by endoscopy. Specificity was defined as the percentage of patients with a negative NMP22 test result who were not diagnosed with tumors. Corresponding 95% confidence intervals (CIs) were calculated for both sensitivity and specificity. The sensitivity and specificity of voided cytology were calculated for comparison. A positive cytology test result was defined as one in which malignant or dysplastic cells were present.

Statistical analysis was performed at the M.D. Anderson Cancer Center using S-PLUS version 6.1 (Insightful Corp, Seattle, Wash) and StatXact version 4.0 (Cytel Software Corp, Cambridge, Mass) statistical software.

## RESULTS

### Characteristics of the Patients

Demographic and baseline characteristics of the individuals with risk factors or symptoms of bladder cancer are summarized in TABLE 1. Among the 1331 patients who had cystoscopies, 79 (6%) had cancer, 685 (51%) were diagnosed with 1 or more benign urological conditions, and 567 (43%) had no cystoscopic evidence of urinary tract disease. The mean age of the patients with bladder tumors was 65.8 years (range, 21-86 years), and they comprised 3 times as many men as women.

Staging information (TABLE 2) was available for the 72 cancers that were surgically removed. The 7 tumors seen during cystoscopy but not excised were categorized as TX. Of the cancers with pathological staging data, 62 were superficial (stages Ta, Tis, or T1), and 10 were muscle invasive (T2-T3). Pathological determination of grade was available for 70 of the 72 removed tumors (Table 2). Of these, 27 were well differentiated (low grade), 18 were moderately differentiated (medium grade), and 25 were poorly differentiated (high grade). A total of 27 cancers were

muscle invasive (T2 or T3) and/or poorly differentiated (high grade). No patients had detectable metastases or involvement of regional lymph nodes. The NMP22 test results were available for all patients with risk factors (1331), and cytology test results for 1287 of the patients with risk factors, including 76 of the 79 diagnosed with cancer.

### Detection

Initial cystoscopy alone detected 88.6% (70/79) of the cancers. The remaining 9 malignancies were identified during subsequent cystoscopies conducted due to continued suspicion, such as increased symptoms, within 3 months of the initial evaluation. The NMP22 assay was positive in 55.7% (44/79), and cytology test results of malignant or dysplastic cells were found in 15.8% (12/76).

The NMP22 test was significantly more sensitive than voided urine cytology when compared using the McNemar  $\chi^2$  test ( $\chi^2=24.7$ ,  $P<.001$ ). This difference remains significant after taking into account the inherent variability among the investigational sites using an adjusted McNemar  $\chi^2$  test ( $\chi^2=7.0$ ,  $P=.008$ ).<sup>20</sup> This significant difference is also reflected by the CIs for the sensitivity proportions since they do not overlap, at 55.7% (95% CI, 44.1%-66.7%) for the NMP22 test vs 15.8% (95% CI, 7.6%-24.0%) for cytology. The positive predictive values of the NMP22 assay and cytology were 19.7% (95% CI, 14.5%-25.0%) and 54.6% (95% CI, 32.2%-75.4%), respectively.

The same methods were used to compare the specificity proportions and demonstrated that cytology was significantly more specific than the proteomic assay ( $\chi^2=149.6$ ,  $P<.001$ ), at 99.2% (95% CI, 98.7%-99.7%) vs 85.7% (95% CI, 83.8%-87.6%), respectively. The difference remains significant after taking variability among the sites into account (adjusted McNemar test  $\chi^2=9.0$ ,  $P=.003$ ). The negative predictive values of the NMP22 assay and cytology were 96.8% (95% CI, 95.6%-97.8%) and 94.9% (95% CI, 93.6%-96.1%), respectively.

Ten of the 79 malignancies were muscle invasive. Initial cystoscopy visualized 6 (60%) of these, compared with the NMP22 test, which identified 9 (90%) with elevated protein marker. By comparison, voided cytology was positive in only 2 (22%) of the 9 patients with muscle-invasive disease for whom test results were available. The

NMP22 assay was also positive for a patient diagnosed with carcinoma in situ after an initial cystoscopic report of benign disease. Thus, a total of 4 potentially life-threatening tumors (T2 G2 of the ureter; T2 G3, Tis G3, and T3 G2 of the bladder) were detected by the NMP22 test but not visualized in the first cystoscopy. One of the 4 tumors

**Table 1.** Patient Demographics and Baseline Characteristics

Variable	No Urinary Tract Disease (n = 567)	Benign Disease (n = 685)	Urinary Tract Cancer (n = 79)	Overall (N = 1331)
Age, y				
Mean (SD)	54.1 (13.8)	61.7 (13.7)	65.8 (13.3)	58.7 (14.3)
Range	18-91	27-96	21-86	18-96
No. (%) of patients				
≤40	90 (15.9)	50 (7.3)	4 (5.1)	144 (10.8)
41-50	153 (27.0)	95 (13.9)	5 (6.3)	253 (19.0)
51-60	146 (25.8)	171 (25.0)	14 (17.7)	331 (24.9)
61-70	94 (16.6)	167 (24.4)	23 (29.1)	284 (21.3)
71-80	73 (12.9)	153 (22.3)	26 (32.9)	252 (18.9)
≥81	11 (1.9)	49 (7.2)	7 (8.9)	67 (5.0)
Sex, No. (%) of patients				
Male	225 (39.7)	472 (68.9)	62 (78.5)	759 (57.0)
Female	342 (60.3)	213 (31.1)	17 (21.5)	572 (43.0)
Race, No. (%) of patients				
Black, non-Hispanic	54 (9.5)	62 (9.1)	4 (5.1)	120 (9.0)
White, non-Hispanic	447 (78.8)	572 (83.5)	70 (88.6)	1089 (81.8)
Hispanic	43 (7.6)	36 (5.3)	5 (6.3)	84 (6.3)
Asian	15 (2.7)	11 (1.6)	0	26 (2.0)
Other	5 (0.9)	1 (0.2)	0	6 (0.5)
Unknown	3 (0.5)	3 (0.4)	0	6 (0.5)

**Table 2.** Sensitivity of NMP22 Assay and Voided Cytology by Stage and Grade of Cancer (n = 72)

	NMP22 Assay		Voided Cytology	
	No. With Positive Test Result/Total No. With Bladder Cancer	Sensitivity, % (95% CI)	No. With Positive Test Result/Total No. With Bladder Cancer	Sensitivity, % (95% CI)
Stage				
Ta	14/30	46.7 (28.3-65.7)	2/28	7.1 (1.0-23.5)
Tis	4/5	80.0 (28.4-99.5)	3/5	60.0 (14.7-94.7)
T1	13/27	48.2 (28.7-68.1)	5/27	18.5 (6.3-38.1)
T2, T2a	6/6	100 (54.1-100)	2/6	33.3 (4.3-77.7)
T3a, T3b	3/4	75.0 (19.4-99.4)	0/3	0 (0-70.8)
TX	4/7	57.1 (18.4-90.1)	0/7	0 (0-41.0)
Noninvasive: Ta-T1	31/62	50.0 (37.0-63.0)	10/60	16.7 (8.3-28.5)
Muscle invasive: T2-T3	9/10	90.0 (55.5-99.8)	2/9	22.2 (2.8-60.0)
Grade				
Well differentiated	13/27	48.2 (28.7-68.1)	0/25	0 (0-13.7)
Moderately differentiated	9/18	50.0 (26.0-74.0)	3/18	16.7 (3.6-41.4)
Poorly differentiated	18/25	72.0 (50.6-87.9)	9/24	37.5 (18.8-59.4)
GX (grade unknown)	4/9	44.4 (13.7-78.8)	0/9	0 (0-33.6)

Abbreviation: CI, confidence interval.

**Table 3.** Specificity of NMP22 Assay

Patients With Risk Factors for Bladder Cancer*	No. With Negative Test Result/Total No. Without Bladder Cancer	Specificity, % (95% Confidence Interval)
No urinary tract disease (with risk factor)	512/567	90.3 (87.6-92.6)
Benign prostatic hypertrophy/prostatitis	231/280	82.5 (77.5-86.8)
Cystitis/inflammation/trigonitis/urinary tract infection	97/125	77.6 (69.3-84.6)
Erythema	42/51	82.4 (69.1-91.6)
Hyperplasia/squamous metaplasia/cysts and polyps	41/53	77.4 (63.8-87.7)
Calculi	29/40	72.5 (56.1-85.4)
Trabeculations	175/217	80.7 (74.7-85.7)
Other benign diseases, kidney and genitourinary	179/220	81.4 (75.6-86.3)
Other cancer history, nonbladder†	7/8	87.5 (47.3-99.7)
Other active cancer, nonbladder‡	33/38	86.8 (71.9-95.6)

\*Patients may have more than 1 benign disease.  
†Other cancer history: lung cancer (n = 1), prostate cancer (n = 7).  
‡Other active cancer: breast cancer (n = 14), kidney/renal cancer (n = 5), leukemia/lymphoma (n = 3), lung cancer (n = 1), prostate cancer (n = 12), other types of cancer (n = 3, tongue, testes, spindle-cell [flank]).

was located in the ureter and therefore outside the viewing area of the cystoscope. Urine tests are often added to an evaluation to identify urinary tract tumors such as this. The combination of the NMP22 test and cystoscopy detected 93.7% of malignancies vs 88.6% for initial cystoscopy alone ( $P = .26$ ). Cytology detected 2 of the 4 cancers not seen in the initial endoscopy, but which were positive by the NMP22 assay.

Among the most aggressive malignancies, those that were poorly differentiated (high grade) and/or muscle invasive (stage T2 or T3), the NMP22 test result was positive in 74% (20/27) compared with cytology, which was positive in 39% (10/26). Of the superficial cancers (Ta, Tis, T1) that were moderately or well differentiated (medium or low grade), the NMP22 assay identified 47% (20/43), compared with 5% (2/41) for cytology. Overall, the point-of-care assay detected 32 malignancies missed by cytology: 11 Ta, 10 T1, 4 T2, 2 T3, 1 Cis, and 4 TX. Voided cytology was positive in only 2 cancer patients for whom the NMP22 test result was negative, both T1 G3.

The specificity of the NMP22 assay was 90.3% among individuals with symptoms but with no evidence of urinary tract disease seen during cystoscopy, and 85.7% overall (TABLE 3). All risk patients in the study were under-

going an evaluation for bladder cancer that included cystoscopy, so false-positive test results did not require any additional procedures. Cytology demonstrated a specificity of 99.2% among patients with symptoms and was not performed for individuals with nonbladder cancer. Of the 38 patients with active cancers other than bladder, the NMP22 assay was negative in 86.8% (33/38) and positive in 13.2% (5/38).

#### COMMENT

Prognosis and survival of individuals with bladder cancer are related to the stage of the malignancy at the time of detection. Approximately 50% of patients with muscle-invasive disease at first diagnosis demonstrate a recurrence within 2 years of surgery, despite apparently adequate surgical resection. The majority of these patients will experience a cancer-related death within 5 years of diagnosis.<sup>21</sup> By comparison, tumors treated while still confined to the epithelium have lower recurrence rates and progress to higher stages and grades less often, thereby improving patients' long-term outcome.<sup>22,23</sup> In addition, early stage disease can be treated by bladder-sparing therapy rather than cystectomy, the standard for advanced disease, which impacts quality of life as well as survival.

The direct cost of treatment for patients with metastatic genitourinary

cancer has been estimated to be more than 6 times greater than for those patients with localized disease for the same period of time.<sup>24</sup> The challenge, therefore, is to improve detection of bladder cancer without adding increased risk or discomfort to the patient.

Cystoscopy is integral to the diagnosis of bladder cancer, allowing the physician to visualize the bladder wall directly. The sensitivity of cystoscopy is very good, but hematuria and other conditions can obscure lesions, and flat neoplasia can be confused with erythema. As seen in this study, even later-stage cancers are sometimes missed during endoscopy. The precise rate of false-negative cystoscopy test results is difficult to determine, but estimates range from 10% to 40%.<sup>25-27</sup> In this study it was 11.4%. For this reason, physicians frequently use multiple tools to aid in diagnosis of bladder cancer, including urinalyses and imaging of the upper tract.

Voided cytology has been a widely accepted adjunctive test to cystoscopy because it is noninvasive. This method involves visual assessment of morphological changes and therefore requires intact cells. Small tumors, well-differentiated (low-grade) tumors, or both are less likely to exfoliate cells spontaneously because the strong intercellular attachments are better preserved, and the degree of morphological departure from normal is less, making recognition difficult.<sup>28</sup> This results in low sensitivity, approximately 15% to 30% in early stage cancers.<sup>29,30</sup> In addition, some inflammatory conditions cause cellular changes that can be confused with neoplastic process, contributing to subjective interpretation. False-positive reports of malignant cells are rare, but ambiguous reports of atypical cells are common. Collecting and analyzing 3 serial first-morning specimens for voided cytology is used by some physicians to improve the detection rate of cancer,<sup>31</sup> but it significantly increases cost, and patient compliance is difficult. Because specially trained technicians are required for the analysis, samples must be stabilized and sent to a laboratory, and test results are not avail-

able immediately. The high specificity of cytology is offset by low sensitivity, ambiguous test results, expense, and time lag to obtain reports.

We found that the NMP22 test is a useful adjunctive tool in the evaluation of patients at risk for bladder cancer and that it identified several malignancies missed by initial cystoscopy. Specificity of the NMP22 test was lower than for cytology (85.7% vs 99.2%), but sensitivity was significantly greater (55.7% vs 15.8%), with test results available during the patient visit. The NMP22 protein is the only tumor marker approved by the FDA as an aid in the initial diagnosis of bladder cancer, and the test has been waived under the Clinical Laboratory Improvement Act so it can be performed in any physician's office. The cost of urine tests varies by location. The average Medicare reimbursement for voided cytology is approximately \$56, compared with \$24 for the NMP22 point-of-care assay.<sup>32</sup>

In the general US population, bladder cancer occurs in approximately 39 of 100 000 men and 10 of 100 000 women.<sup>1</sup> Patients enrolled in this investigation were at elevated risk for urological cancer due to behaviors such as smoking or symptoms including hematuria and dysuria. Consequently, the incidence of malignancy in this group was 6%. Currently, 20% or more of symptomatic patients present with disease that is already invasive at the time of first diagnosis. Identifying these malignancies earlier could improve prognosis and reduce the cost of treatment.

Hematuria is the most common symptom of bladder cancer, but it is often intermittent.<sup>33</sup> Repetitive home testing of high-risk populations has shown good detection results,<sup>34</sup> and the cost of urinary dipstick testing is minimal. However, because hematuria is not specific to cancer, it is estimated that only 1 in 20 patients with hematuria will have bladder cancer.<sup>34-36</sup> Positive predictive value, the percentage of times that a positive test result corresponds to the presence of the disease in question, is about 5% for hematuria testing

in men for bladder cancer<sup>34,35</sup> and even lower in women.<sup>36</sup> Nevertheless, because the amount of blood in urine is unrelated to stage and grade of cancer, the American Urological Association Best Practice Policy Panel on Asymptomatic Microscopic Hematuria and others have concluded that there is no safe lower limit for hematuria, and high-risk patients should be considered for a urological evaluation after even a single episode.<sup>3,37</sup> Among study patients with the highest risk for bladder cancer, men older than 60 years with a history of smoking, the positive predictive value of the NMP22 test was 37%. This is higher than the 20% to 30% predictive value typically reported for prostate-specific antigen in men who have an elevated risk of prostate cancer, those with levels between 4 to 10 ng/mL.<sup>38-41</sup>

In conclusion, the NMP22 assay may be a useful adjunct to cystoscopy for diagnosing bladder cancer. Studies in different patient populations are necessary to further define the role of this assay in patients with risk factors and symptoms suggestive of possible bladder cancer.

**Author Contributions:** As principal investigator, Dr Grossman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Grossman, Messing, Soloway, Katz.

**Acquisition of data:** Grossman, Messing, Soloway, Tomera, Katz, Berger, Shen.

**Analysis and interpretation of data:** Grossman, Messing, Soloway, Shen.

**Drafting of the manuscript:** Grossman, Messing.

**Critical revision of the manuscript for important intellectual content:** Grossman, Messing, Soloway, Tomera, Katz, Berger, Shen.

**Statistical analysis:** Grossman, Shen.

**Obtained funding:** Grossman, Messing, Soloway, Tomera, Katz, Berger.

**Administrative, technical, or material support:** Grossman, Messing, Soloway, Tomera, Katz.

**Study supervision:** Grossman, Messing, Soloway, Katz, Berger.

**Financial Disclosures:** None reported.

**Funding/Support:** Matritech Inc supplied the experimental assay to the investigators at no cost and reimbursed clinical sites for the time involved in collection of data related to FDA submission. This included risk factors, demographic information, and test results.

**Role of the Sponsor:** Matritech Inc designed the study, monitored the conduct and collection of data, and reviewed the manuscript for factual accuracy and approved it.

**Independent Statistical Analysis:** Independent statistical analysis was performed by Yu Shen, PhD, at the University of Texas M. D. Anderson Cancer Center, Department of Biostatistics and Applied Math.

**Acknowledgment:** We thank statistician Heather E. Kelley, BA (West Roxbury, Mass), for her efforts analyzing the study data, as well as the following physicians who contributed to the performance of the clinical trial: Anthony Abner, MD (Mt. Auburn Hospital, Cambridge, Mass); David Bock, MD (Kansas City Urology Care, Kansas City, Mo); Jeffrey Brady, MD (Winter Park Urology Associates, Orlando, Fla); M. Patrick Collini, MD (Urology Associates of North Texas, Fort Worth); Martin Dineen, MD (Atlantic Urological Associates, Daytona Beach, Fla); Vahan Kassabian, MD (Georgia Urology, Atlanta); Shiva Maralani, MD (St Clair Shores, Mich); Raoul Salup, MD (James A. Haley Veterans Administration Hospital, Tampa, Fla); Barry Stein, MD (Rhode Island Hospital, Providence); Alan Treiman, MD (Urology Treatment Center, Sarasota, Fla).

## REFERENCES

1. American Cancer Society. Overview: bladder cancer. Available at: [http://www.cancer.org/docroot/CRI/CRI\\_2\\_1x.asp?dt=44](http://www.cancer.org/docroot/CRI/CRI_2_1x.asp?dt=44). Accessibility verified January 11, 2005.
2. National Cancer Institute. General information about bladder cancer. Available at: [www.cancer.gov/cancertopics/pdq/treatment/bladder/patient](http://www.cancer.gov/cancertopics/pdq/treatment/bladder/patient). Accessibility verified January 11, 2005.
3. Grossfeld GD, Wolf JS Jr, Litwin MS, et al. Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy, II: patient evaluation, cytology, voided markers, imaging, cystoscopy, nephrology evaluation, and follow-up. *Urology*. 2001;57:604-610.
4. Frimberger D, Zaak D, Hofstetter A. Endoscopic fluorescence diagnosis and laser treatment of transitional cell carcinoma of the bladder. *Semin Urol Oncol*. 2000;18:264-272.
5. Hudson MA, Herr HW. Carcinoma in situ of the bladder. *J Urol*. 1995;153:564-572.
6. Berezney R, Coffey DS. Identification of a nuclear matrix protein. *Biochem Biophys Res Commun*. 1974;60:1410-1417.
7. Fey EG, Krochmalnic G, Penman S. The non-chromatin substructures of the nucleus: the ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. *J Cell Biol*. 1986;102:1654-1665.
8. Kumara-Siri MH, Shapiro LE, Surks MI. Association of the 3,5,3e-triiodo-L-thyronine nuclear receptor with the nuclear matrix of cultured growth hormone-producing rat pituitary tumor cells (GC cells). *J Biol Chem*. 1986;261:2844-2852.
9. Pardoll DM, Vogelstein B, Coffey DS. A fixed site of DNA replication in eukaryotic cells. *Cell*. 1980;19:527-536.
10. Zeitlin S, Parent A, Silverstein S, Efstratiadis A. Pre-mRNA splicing and the nuclear matrix. *Mol Cell Biol*. 1987;7:111-120.
11. Nakayasu H, Berezney R. Mapping replicational sites in the eukaryotic nucleus. *J Cell Biol*. 1989;108:1-11.
12. Berrios M, Osheroff N, Fisher PA. In situ localization of topoisomerase II, a major polypeptide component of the Drosophila nuclear matrix fraction. *Proc Natl Acad Sci U S A*. 1985;82:4142-4146.
13. Lydersen BK, Pettijohn DE. Human-specific nuclear protein that associates with the polar region of the mitotic apparatus: distribution in a human/hamster hybrid cell. *Cell*. 1980;22:489-499.
14. Miller TE, Beausang LA, Winchell LF, Lidgard GP. Detection of nuclear matrix proteins in serum from cancer patients. *Cancer Res*. 1992;52:422-427.
15. Partin AW, Getzenberg RH, Carmichael MJ, et al. Nuclear matrix protein patterns in human benign prostatic hyperplasia and prostate cancer. *Cancer Res*. 1993;53:744-746.
16. Soloway MS, Briggman JV, Carpinito GA, et al. Use of a new tumor marker, Urinary NMP22, in the

- detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. *J Urol*. 1996;156:363-367.
17. US Food and Drug Administration Premarket Approval (PMA) Database: MA 940035 S002. Available at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/PMA.cfm?ID=7413>. Accessed August 18, 2004.
  18. US Food and Drug Administration Premarket Approval (PMA) Database: MA 940035. Available at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/PMA.cfm?ID=7411>. Accessed August 18, 2004.
  19. American Joint Committee on Cancer. *Cancer Staging Manual, 5th Edition*. Philadelphia, Pa: American Joint Committee on Cancer; 1997.
  20. Durkalski V, Palesch Y, Lipsitz S, Rust P. The analysis of clustered matched-pair data. *Stat Med*. 2003;22:2417-2428.
  21. Lerner SP, Skinner DG. Radical cystectomy for bladder cancer. In: Vogelzang WJ, Scardino PT, Shipley WV, Coffey DS, eds. *Comprehensive Textbook Of Genitourinary Oncology*. 2nd ed. New York, NY: Lippincott Williams & Wilkins; 2000:425-447.
  22. Holmang S, Hedelin H, Anderstron C, Johansson SL. The relationship among multiple recurrences, progression, and prognosis of patients with stages Ta and T1 transitional cell cancer of the bladder followed for at least 20 years. *J Urol*. 1995;153:1823-1826.
  23. Heney NM, Ahmed S, Flanagan MJ, et al. Superficial bladder cancer: progression and recurrence. *J Urol*. 1983;130:1083-1086.
  24. Mariani AJ, Mariani MC, Macchioni C, Stams UK, Harihan A, Moriera A. The significance of adult hematuria: 1,000 hematuria evaluations including a risk-benefit and cost-effectiveness analysis. *J Urol*. 1989;141:350-355.
  25. Zaak D, Kriegmair M, Stepp H, et al. Endoscopic detection of transitional cell carcinoma with 5-aminolevulinic acid: results of 1012 fluorescence endoscopies. *Urology*. 2001;57:690-694.
  26. Schneeweiss S, Kriegmair M, Stepp H. Is everything all right if nothing seems wrong? a simple method of assessing the diagnostic value of endoscopic procedures when a gold standard is absent. *J Urol*. 1999;161:1116-1119.
  27. Kriegmair M, Baumgartner R, Knuechel R, Stepp H, Hofstadter F, Hofstetter A. Detection of early bladder cancer by 5-aminolevulinic acid induced porphyrin fluorescence. *J Urol*. 1996;155:105-110.
  28. Farrow GM. Urine cytology in the detection of bladder cancer: a critical approach. *J Occup Med*. 1990;32:817-821.
  29. Badalament RA, Hermansen DK, Kimmel M, et al. The sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. *Cancer*. 1987;60:1423-1427.
  30. Brown FM. Urine cytology: is it still the gold standard for screening? *Urol Clin North Am*. 2000;27:25-37.
  31. Cohen RA, Brown RS. Microscopic hematuria. *N Engl J Med*. 2003;348:2330-2338.
  32. Centers for Medicare & Medicaid Services. Physician fee schedule payment amount file national/carrier. Available at: [www.cms.hhs.gov/providers/pufdownload/#pfpayment](http://www.cms.hhs.gov/providers/pufdownload/#pfpayment). Accessed August 18, 2004.
  33. Messing EM, Young TB, Hunt VB, Wehbie JM, Rust P. Urinary tract cancers found by home screening with hematuria dipsticks in healthy men over 50 years of age. *Cancer*. 1989;64:2361-2367.
  34. Messing EM, Vaillancourt A. Hematuria screening for bladder cancer. *J Occup Med*. 1990;32:838-845.
  35. Britton JP, Dowell AC, Whelan P, Harris CM. A community study of bladder cancer screening by the detection of occult urinary bleeding. *J Urol*. 1992;148:788-790.
  36. Khadra MH, Pickard RS, Charlton M, Powell PH, Neal DE. A prospective analysis of 1,930 patients with hematuria to evaluate current diagnostic practice. *J Urol*. 2000;163:524-527.
  37. Grossfeld GD, Litwin MS, Wolf JS, et al. Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy, part 1: definition, detection, prevalence, and etiology. *Urology*. 2001;57:599-603.
  38. Andriole GL, Catalona WJ. Using PSA to screen for prostate cancer: the Washington University experience. *Urol Clin North Am*. 1993;20:647-651.
  39. Brawer MK, Chetner MP, Beatie J, et al. Screening for prostatic carcinoma with prostate-specific antigen. *J Urol*. 1992;147:841-845.
  40. Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate specific antigen in serum as a screening test for prostate cancer. *N Engl J Med*. 1991;324:1156-1161.
  41. Arcangeli CG, Ornstein DK, Keetch DW, et al. Prostate-specific antigen as a screening test for prostate cancer. *Urol Clin North Am*. 1997;24:299-305.

All adventures, especially into new territory, are scary.  
—Sally Ride (1951- )