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Surveillance for Recurrent Bladder Cancer Using a Point-of-Care Proteomic Assay

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BLADDER CANCER IS THE FIFTH most common malignancy in the United States. In 2005, there were an estimated 63 210 new cases and more than 13 000 deaths.¹ There are 500 000 patients in the United States with a history of bladder cancer, making its prevalence higher than that of cancer of the lung and bronchus.² The probability of recurrence ranges from 50% to 90%, depending on stage, grade, and number of primary tumors. Progression of stage and/or grade occurs in 10% to 50% of cases.³ Consequently, rigorous surveillance is advocated. A combination of methods is used to monitor patients at risk of recurrent bladder cancer because no single procedure is 100% sensitive.

Flexible cystoscopy is the primary diagnostic tool because it confers low risk and can be performed in the physician's office. However, visibility can be reduced by bleeding, and flat urothelial lesions like carcinoma in situ may be difficult to distinguish from normal bladder tissue.^{4,5} For this reason, cytologic analysis of voided urine frequently is used as an adjunctive test to aid in identifying occult cancers.

Cytology assesses morphological changes in intact cells. Sensitivity is largely dependent on the degree of differentiation of the tumor. High-grade tumors with marked pleomorphism and

Context At least 50% of patients with a history of bladder cancer have recurrences, so rigorous surveillance is necessary. Cystoscopy is standard but can fail to detect some bladder cancers, so a urine test is frequently part of the evaluation.

Objective To investigate whether a point-of-care proteomic test that measures the nuclear matrix protein NMP22 in voided urine could improve detection of recurrence during monitoring of patients with a history of bladder cancer.

Design, Setting, and Patients From September 2001 to February 2002, 23 academic, private practice, and hospital facilities in 9 US states prospectively enrolled 668 consecutive patients with a history of bladder cancer in this cross-sectional study. Patients provided a voided urine sample for analysis of NMP22 protein and cytology prior to cystoscopy.

Main Outcome Measures Diagnosis of bladder cancer recurrence, 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 detection. Testing for the NMP22 tumor marker was conducted in a blinded manner.

Results Bladder cancer was diagnosed in 103 patients. Cystoscopy alone identified 91.3% of the cancers (94/103; 95% confidence interval [CI], 84.1%-95.9%). The combination of cystoscopy with the NMP22 assay detected 99.0% of the malignancies (102/103; 95% CI, 94.7%-100%; $P=.005$). The NMP22 assay detected 8 of 9 cancers that were not visualized during initial cystoscopy, including 7 that were high-grade. The sensitivity and specificity of the NMP22 test alone were 49.5% (51/103; 95% CI, 39.5%-59.5%) and 87.3% (493/565; 95% CI, 84.2%-89.9%), respectively. Voided cytology detected only 3 of the malignancies missed during initial cystoscopy and did not significantly increase the sensitivity of cystoscopy (94.2%; 95% CI, 87.7%-97.8%; $P=.08$).

Conclusion The noninvasive point-of-care assay for elevated urinary NMP22 protein can increase the ability to detect recurrent bladder cancer, with test results available during the patient visit.

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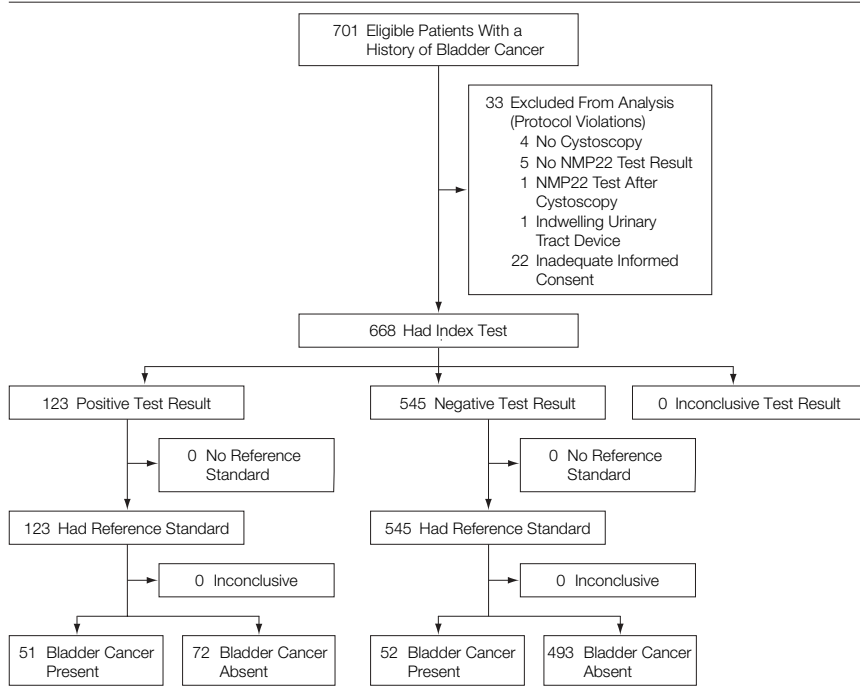
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distinctly abnormal nuclear features are identified more accurately. However, small and/or well-differentiated tumors are less likely to exfoliate cells because intercellular attachments are better preserved and the degree of morphologic departure from normal is smaller, making cytologic recognition difficult.⁶ This results in poor sensitivity in low-grade and early-stage cancers.^{7,8} In addition, instrumentation effect and some conditions cause reactive cellular changes, contributing to variability in interpretation. False-

positive reports of malignant cells are uncommon, but ambiguous reports of atypical cells are frequent. Bladder wash

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Figure. Study Flow Diagram

cytology yields more tumor cells in the sample and is more sensitive in identifying cancer, especially high-grade tumors,⁹⁻¹¹ but it also has a higher false-positive rate than voided urine cytology.

We investigated the clinical utility of a new, noninvasive urine test for the nuclear matrix protein NMP22, in a point-of-care format, as an aid in monitoring patients with a history of bladder cancer. This simple device can be used in the physician's office and involves no specialized equipment or training. We compared its usefulness with that of voided urine cytology, which must be analyzed in a clinical laboratory.

METHODS

Patients

Twenty-three clinical sites in 9 states, including academic, private practice, and veterans' facilities, prospectively enrolled 668 consecutive patients with a history of bladder cancer between September 2001 and February 2002 (FIGURE). Institutional review boards reviewed and approved the study protocol for each site, and all participants provided written informed consent.

In this cross-sectional study, each patient provided a voided urine sample before undergoing cystoscopy. One portion was sent for routine cytologic examination, either within the institution or at a reference laboratory, according to the standard practice at each facility. Clinic staff tested an aliquot of the remaining specimen for the presence of NMP22 protein. Devices were identified by study identification numbers so that physicians who performed the subsequent cystoscopies were blinded to the NMP22 results and the staff who performed the NMP22 assays were blinded to cystoscopy results. Cytology 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 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 functioning properly. 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 nuclear mitotic apparatus protein, a component of the nuclear matrix essential for cell division that is released into urine during cell death.¹²⁻¹⁶ We have described the assay mechanism in detail previously.¹⁷ Unlike cytologic examination or fluorescence in situ hybridization (FISH)-based tests, detection of the NMP22 protein is not dependent on recovery of intact cells. The 10-U/mL threshold of determination for the qualitative point-of-care test for NMP22 protein corresponds to the cutoff previously approved by the US Food and Drug Administration (FDA) for quantitative measurement of the marker.¹⁸

In this study, each patient had a single NMP22 assay performed; serial NMP22 testing was not routinely performed.

Diagnostic Criteria

All patients underwent cystoscopy, but biopsy was performed only for suspicious areas. Patients were considered positive for malignancy if 1 or more tumors were observed during cystoscopy and, if removed, were defined as malignant on pathological examination. Tumors seen endoscopically but not removed were considered positive for malignancy and designated as cancer stage TX. Patients were considered negative for cancer if no tumor was seen endoscopically, or if tissue underwent biopsy and was defined as non-malignant on histopathological examination. Pathological examination of biopsied tissue was performed within each institution or at a reference laboratory according to the standard practice at each facility. Staging criteria were those established by the American Joint Committee on Cancer.¹⁹

Statistical Analysis

A 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. It was derived from testing the null hypothesis that the observed proportion of detection is equal to the expected proportion of detection (recurrent bladder cancers detected by cystoscopy) vs the alternative hypothesis that the observed proportion of detection is greater than the expected proportion of detection. This was based on a type I error rate (α level) of .05, with 80% power to find a significant difference of 3% in the detection rate. Assuming that 10% to 20% of patients with a history of bladder cancer could be expected to have a positive cystoscopic evaluation, an estimated sample size of 550 to 1000 patients was required.

All comparative analyses and reported *P* values are 2-sided. Sensitivity of the NMP22 test to detect bladder cancer was calculated as the number of patients with true test-positive results (positive NMP22 assay results and presence of tumor) divided by the total number of patients with malignancy, as detected by cystoscopy. Specificity was defined as the percentage of patients with a negative NMP22 assay result who were not diagnosed as having tumors. Corresponding 95% confidence intervals (CIs) were calculated for both sensitivity and specificity.

The sensitivity and specificity of cytologic test results of voided urine were calculated for comparison. A positive cytologic result was defined as one in which malignant or dysplastic cells were present. Sensitivity and specificity of the NMP22 test and cytologic analysis were compared using a McNemar χ^2 test. To take into account the inherent variability among the investigational sites, an adjusted McNemar χ^2 test²⁰ was also used with site as the adjustment variable. For the analyses using this adjusted test, clinical sites with the same principal investigators were merged, resulting in 15 sites.

Statistical analysis was performed at the University of Texas M. D. Anderson Cancer Center, Houston, using S-PLUS, version 6.1 (Insightful Corporation, Seattle, Wash) and StatXact,

Table 1. Baseline Patient Characteristics

Characteristics	No Urinary Tract Disease (n = 264)	Benign Disease (n = 301)	Urinary Tract Cancer (n = 103)	Overall (N = 668)
Age, y				
Mean (SD)	68.1 (12.2)	73.4 (10.1)	73.2 (9.8)	71.3 (11.2)
Range	30-90	39-95	46-91	30-95
No. (%) of patients				
≤40	6 (2.3)	1 (0.3)	0	7 (1.1)
41-50	16 (6.1)	8 (2.7)	1 (1.0)	25 (3.7)
51-60	45 (17.1)	27 (9.0)	11 (10.7)	83 (12.4)
61-70	71 (26.9)	77 (25.6)	25 (24.3)	173 (25.9)
71-80	82 (31.1)	110 (36.5)	39 (37.9)	231 (34.6)
≥81	44 (16.7)	78 (25.9)	27 (26.2)	149 (22.3)
Sex, No. (%) of patients				
Male	175 (66.3)	255 (84.7)	73 (70.9)	503 (75.3)
Female	89 (33.7)	46 (15.3)	30 (29.1)	165 (24.7)

version 4.0 (Cytel Software Corporation, Cambridge, Mass).

RESULTS

Patient Characteristics

Demographic and baseline characteristics are summarized in TABLE 1. Among the 668 patients who had cystoscopies, 103 (15.4%) had tumors, 301 (45.1%) were diagnosed as having benign urological conditions (281 cystoscopically and 20 by cystoscopy with biopsy), and 264 (39.5%) had no cystoscopic evidence of urinary tract disease. The mean age of the patients with bladder tumors was 73.2 years (range, 46-91 years), and they comprised more than twice as many men as women.

Eighty-six of the 103 patients with tumors had resections and 17 did not undergo surgery. Of these 17 (designated as cancer stage TX), 8 had fulguration, 3 died of nonurological causes before surgery, 3 were treated without surgery because of concurrent health issues, and 3 underwent treatment from a nonstudy physician and were lost to follow-up.

Of the 86 cancers that were resected, 75 (87.2%) were non-muscle invasive (stages Ta, Tis, T1) and 11 (12.8%) were muscle invasive (stages T2, T3, T4) (TABLE 2). There were 38 (44.2%) well-differentiated, 16 (18.6%) moderately differentiated, and 32 (37.2%) poorly differentiated tumors

(TABLE 3). A total of 33 malignancies (38.4%) were muscle invasive and/or poorly differentiated. The NMP22 assay results were available for all patients, and voided urine cytologic results were available for 98 of the 103 with cancer and 552 of the 565 without cancer.

Detection

Initial cystoscopy alone detected 94 (91.3%) of the 103 cancers. Nine additional malignancies, 8 of which were high-grade, were identified during repeat evaluations conducted because of continued suspicion or close follow-up. Three of the 9 were found at 1 month (T2 G3, Tis G3, Tis G3), 4 at 3 months (T2 G3, extensive Tis G3, Ta G1, T4 G3), 1 at 4 months (T4 G3), and 1 at 5 months (T1 G3).

The NMP22 assay was positive in 43 (45.7%) of the 94 tumors identified by initial cystoscopy, and malignant or dysplastic cells were seen in 9 (10.1%) of 89. Of the 9 patients diagnosed as having bladder cancer in repeat evaluations (4 because of increased symptoms and 5 because of close monitoring), 8 had positive NMP22 test results, and 7 of the 8 NMP22-positive patients had high-grade disease. Cytologic results were positive in 3 of 9 occult cancers, all stage Tis G3. Thus, the NMP22 assay detected 49.5% of total cancers (51/103), and in cytologic analysis, malignant or dysplastic cells were found in

12.2% (12/98). Among only cancers with stage and grade information (no TX), the point-of-care test was positive in 50.0% (43/86) and cytologic analysis in 9.8% (8/82).

The NMP22 test was significantly more sensitive than cytologic analysis of voided urine when compared using a McNemar χ^2 test ($\chi^2=27.0$; $P<.001$). This difference remained significant after taking into account the inherent variability among the investigational sites using an adjusted McNemar test ($\chi^2=6.5$; $P=.01$). This significant difference is also confirmed by the CIs for the sensitivity proportions, since they do not overlap, at 49.5% (95% CI, 39.5%-59.5%) for the NMP22 test vs 12.2% (95% CI, 6.5%-20.4%) for cytologic analysis. The positive predictive values of the NMP22 assay and cytologic analysis were similar at 41.5% (95% CI, 32.7%-50.7%) and 41.4% (95% CI, 23.5%-61.1%), respectively.

The combination of the NMP22 test with cystoscopy increased overall sensitivity to 99.0% (102/103; 95% CI, 94.7%-100%) compared with 91.3% (94/103; 95% CI, 84.1%-95.9%) for cystoscopy alone. This difference was statistically significant (McNemar $\chi^2=8.0$; $P=.005$). The use of voided urine cytology with cystoscopy resulted in a sensitivity of 94.2% (97/103; 95% CI, 87.7%-97.8%), which was not statistically significantly different from cystoscopy alone ($\chi^2=3.0$; $P=.08$).

The sensitivity of cystoscopy plus the NMP22 test appears to be better than cystoscopy plus cytologic analysis ($\chi^2=3.6$; $P=.06$).

The same methods were used to compare the specificities and demonstrated that cytologic analysis was significantly more specific than the proteomic assay (McNemar $\chi^2=33.8$; $P<.001$) at 96.9% (95% CI, 95.1%-98.2%) vs 87.3% (95% CI, 84.2%-89.9%). The difference remained sig-

nificant after taking variability among the sites into account (adjusted McNemar $\chi^2=6.7$; $P<.001$).

The specificity of the NMP22 assay for monitored patients with no evidence of urological disease was 89.4% (236/264) and ranged from 83.3% to 91.3% among those with benign urinary tract conditions, except for those with urinary tract infection (0/3) (TABLE 4). There were only 3 patients in the study with active urinary tract infections, but the consistent positive results suggest that the NMP22 test should be used after infections have been treated. All monitored patients were undergoing an evaluation that included cystoscopy, so discordant results did not result in any additional procedures in this study. The negative predictive value of the NMP22 test (90.5%; 95% CI, 87.7%-92.8%) was better than that of cytologic analysis (86.2%; 95% CI, 83.2%-88.8%).

Eleven of the 86 cancers (12.8%) with pathologic staging information were muscle invasive. The NMP22 test results were positive in 90.9% (10/11), including 4 malignancies that were not detected by either initial cystoscopy or voided urine cytologic analysis. Voided urine cytologic analysis did not identify any of the muscle-invasive tumors (0/10). Of the 32 high-grade cancers, the NMP22 assay results were positive in 75.0% (24/32) compared with voided urine cytologic analysis results, which were positive in 19.4% (6/31). Initial cystoscopy alone visualized 75.0% (24/32) of the high-grade cancers, whereas the combination of the NMP22 test with cystoscopy identified 96.9% (31/32), a statistically significant difference ($\chi^2=7.0$; $P=.008$).

Among the most aggressive malignancies, those that were poorly differentiated and/or muscle invasive, the NMP22 assay results were positive in 72.7% (24/33) compared with cytologic analysis results, which were positive in 18.8% (6/32). Of the non-muscle-invasive cancers (Ta, Tis, T1) that were moderately or well differentiated, the NMP22 test identified 35.9% (19/53)

Table 2. Sensitivity of NMP22 Assay and Cytologic Analysis of Voided Urine by Stage of Bladder Cancer

Cancer Stage	NMP22 Assay		Cytologic Analysis of Voided Urine	
	No. Detected/Total Cancers	Sensitivity, % (95% CI)	No. Detected/Total Cancers	Sensitivity, % (95% CI)
Ta	18/50	36.0 (22.9-50.8)	3/48	6.3 (1.3-17.2)
Tis	4/8	50.0 (15.7-84.3)	3/8	37.5 (8.5-75.5)
T1	11/17	64.7 (38.3-85.8)	2/16	12.5 (1.6-38.4)
T2	7/8	87.5 (47.3-99.7)	0/7	0 (0-41.0)
T3	1/1	100 (2.5-100)	0/1	0 (0-97.5)
T4	2/2	100 (15.8-100)	0/2	0 (0-84.2)
TX	8/17	47.1 (23.0-72.2)	4/16	25.0 (7.3-52.4)
Non-muscle invasive: Ta, Tis, T1	33/75	44.0 (32.5-56.0)	8/72	11.1 (4.9-20.7)
Muscle invasive: T2-T4	10/11	90.9 (58.7-99.8)	0/10	0 (0-30.9)
Overall	51/103	49.5 (39.5-59.5)	12/98	12.2 (6.5-20.4)

Abbreviation: CI, confidence interval.

Table 3. Sensitivity of NMP22 Assay and Cytologic Analysis of Voided Urine by Grade of Bladder Cancer

Cancer Grade	NMP22 Assay		Cytologic Analysis of Voided Urine	
	No. Detected/Total Cancers	Sensitivity, % (95% CI)	No. Detected/Total Cancers	Sensitivity, % (95% CI)
Well differentiated	12/38	31.6 (17.5-48.7)	2/37	5.4 (0.7-18.2)
Moderately differentiated	7/16	43.8 (19.8-70.1)	0/14	0 (0-23.2)
Poorly differentiated	24/32	75.0 (56.6-88.5)	6/31	19.4 (7.4-37.5)

Abbreviation: CI, confidence interval.

compared with 4.0% (2/50) for cytologic analysis. Overall, the point-of-care assay detected 42 malignancies missed by cytologic analysis: 9 muscle invasive, 27 non-muscle invasive, and 6 TX (no surgery). Cytologic analysis results were positive in 6 patients with cancer for whom the NMP22 result was negative (4 noninvasive and 2 TX).

COMMENT

Tumors treated while still confined to the urothelium have lower recurrence rates and progress to higher stages and grades less often than those invading the lamina propria, but even patients with noninvasive cancers have about a 50% recurrence rate.^{21,22} For this reason, patients are monitored throughout their lifetimes.

Cystoscopy is integral to the management of bladder cancer, allowing physicians to visualize the bladder wall directly. The sensitivity of this method is very good, but carcinoma in situ as well as other cancers can escape detection. As seen in this study, even muscle-invasive cancers sometimes are not visualized during cystoscopy, and upper-tract malignancies are outside the viewing area of the cystoscope. In this study, 8.7% of the cancers (9/103) were not visible during initial cystoscopy. The 9 occult cancers occurred in patients at 7 different sites throughout the United States, so it was not an isolated phenomenon. In another multisite investigation of the NMP22 assay evaluating patients without a history of bladder cancer who had symptoms of or risk factors for bladder malignancy, 10% of the tumors were not found by initial cystoscopy.¹⁷ Other studies have found similar or higher incidences of occult cancers.²³⁻²⁸ The combination of cystoscopy with the point-of-care NMP22 assay in this investigation increased detection of bladder cancer recurrences to 99.0%, compared with 91.3% for cystoscopy alone.

The sensitivity of cytology in this investigation was low at 12.2%, but, unlike studies that report results from a single site, this performance reflects the variability across multiple facilities. Cy-

Table 4. Specificity of NMP22 Assay*

	Negative/Total	Specificity (95% CI)
No evidence of urinary tract disease	236/264	89.4 (85.0-92.8)
Benign prostatic hyperplasia/prostatitis	103/120	85.8 (78.3-91.5)
Erythema/cystitis/inflammation	70/82	85.4 (75.8-92.2)
Urinary tract infection	0/3	0 (0-70.8)
Hyperplasia/squamous metaplasia/cyst/polyp/caruncle	21/23	91.3 (72.0-98.9)
Calculi	5/6	83.3 (35.9-99.6)
Trabeculations	43/49	87.8 (75.2-95.4)
Diverticulum/pouch/cellule	15/18	83.3 (58.6-96.4)
Overall	493/565	87.3 (84.2-89.9)

Abbreviation: CI, confidence interval.

*Patients with more than 1 benign disease were grouped according to their primary condition, so each patient with a benign diagnosis is represented once in the table.

tologic examinations were conducted either within the participating institutions or at reference laboratories, according to the standard practice at each facility. The combination of cytologic analysis with cystoscopy did not significantly increase detection of cancers ($P=.08$). Voided urine cytologic analysis was more specific than the proteomic assay (99.6% vs 87.3%, respectively). However, the negative predictive value of the NMP22 test (90.5%) was better than that of cytologic analysis (86.2%) because of fewer false-negative results among patients with cancer by the point-of-care test.

The specificity of 87.3% for the NMP22 assay suggests that false-positive results may occur and lead to further diagnostic testing in patients without recurrent bladder cancer. In this study, all patients underwent cystoscopy, so additional procedures were not required. However, it is possible that some of these patients may have had recurrent cancer not detected by cystoscopy, but lack of prospective follow-up data precludes definitely ruling out recurrence.

Typical surveillance includes evaluation for new tumors every 3 months for the first 2 years after surgery, twice a year for the next 2 years, then yearly thereafter, unless a recurrence is diagnosed, at which point monitoring resumes at 3-month intervals.³

Despite intensive monitoring of patients with a history of cancer, progression of stage and/or grade occurs in 10% to 50% of cases.³ In this investigation,

12.8% of all recurrences with pathological information (11/86) were muscle invasive. Multiple studies have demonstrated that in patients with muscle-invasive disease, a delay in surgery is associated with a more advanced pathological state and poorer prognosis, especially if the delay is greater than 12 weeks.²⁹⁻³¹ The sensitivity of the NMP22 assay alone for muscle-invasive disease is high at 90.9%, which could make it a useful noninvasive tool between cystoscopies to detect dangerous lesions earlier.

Lifelong follow-up makes the expense of bladder cancer from diagnosis to death the highest of all cancers in the United States, ranging in cost from \$96 000 to \$187 000 per patient.³² Although the price of surveillance for early bladder cancer recurrence is high, it is less costly in terms of patient survival, quality of life, and treatment expenses than detection of malignancy at a later stage. The 5-year survival rates for patients with regional and metastatic disease are 49% and 6%, respectively, compared with 94% for noninvasive disease,¹ and typical treatment for advanced tumors is cystectomy rather than bladder-sparing therapy. The direct cost of treatment for patients with metastatic genitourinary cancer has been estimated to be more than 6 times greater than for those with localized disease.³³ Finding tumors early therefore improves both prognosis and treatment expense.

There are other tumor marker tests available as adjuncts to cystoscopy. One

involves a combination of 3 antibodies labeled with fluorescent markers that bind to a mucin glycoprotein and a carcinoembryonic antigen that are expressed by bladder tumor cells (ImmunoCyt, DiagnoCure Inc, Sainte-Foy, Quebec). Another use of FISH technology is a 4-color, 4-probe mixture of DNA probe sequences homologous to specific regions on chromosomes 3, 7, 9, and 17 (UroVysion, Vysis Inc, Downers Grove, Ill). These assays also use voided urine as the test sample but, like traditional voided urine cytology, are dependent on intact exfoliated urothelial cells and must be performed in high-complexity laboratories.

Published results of both tests report higher sensitivity and lower specificity than voided urine cytologic analysis alone.³⁴⁻³⁹ A direct performance comparison with the NMP22 assay is problematic because the immunocytofluorescence method is FDA-approved for surveillance only in conjunction with traditional urine cytology.³⁴⁻³⁶ The FISH-based test is the only assay other than the NMP22 marker that is FDA-approved for use in diagnosis (in patients with hematuria only) as well as surveillance, but published sensitivity calculations include cancers detected up to 16 months after study evaluation, and specificity is calculated not from the target population but from a cohort of healthy volunteers and patients without a history of bladder cancer.³⁶⁻³⁹ Unlike the NMP22 assay, costs for both tests are equivalent to or higher than voided urine cytology, thereby increasing the cost of cancer detection. In addition, neither test is suitable for a point-of-care format.

Other candidate markers, like telomerase, have shown good results in early trials⁴⁰ but are still in an investigational stage and are not approved for clinical use.

CONCLUSIONS

When combined with cystoscopy, the NMP22 test improves the detection of recurrence in patients with a history of bladder cancer. Unlike cytologic analy-

sis, the proteomic test does not require expert analysis or laboratory time, is not dependent on intact cells, and provides unambiguous results. In addition, the NMP22 test provides results during the patient visit and its cost is less than half that of cytology. The combination of cystoscopy and NMP22 testing increased the detection of cancers to 99.0%, compared with cystoscopy alone at 91.3% ($P = .005$), and it has clinical utility in routine monitoring of bladder cancer patients. The NMP22 assay is approved by the FDA as both an aid in the initial diagnosis of bladder cancer⁴¹ and its surveillance,⁴² and has been Clinical Laboratory Improvement Act-waived so it can be performed in any physician's office. These results will need to be validated in a prospective randomized study comparing cystoscopy and urine cytology with cystoscopy and the NMP22 test with respect to outcome and overall costs of medical management.

Author Contributions: Dr Shen 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.

Acquisition of data: Grossman, Soloway, Messing, Katz, Stein, Kassabian.

Analysis and interpretation of data: Grossman, Katz, Stein, Shen.

Drafting of the manuscript: Grossman.

Critical revision of the manuscript for important intellectual content: Grossman, Soloway, Messing, Katz, Stein, Kassabian, Shen.

Statistical analysis: Shen.

Study supervision: Soloway, Messing, Katz, Stein, Kassabian.

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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.

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