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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

MARK S. SOLOWAY, JOSEPH V. BRIGGMAN, GENNARO A. CARPINITO, GERALD W. CHODAK, PAUL A. CHURCH, DONALD L. LAMM, PAUL LANGE, EDWARD MESSING, ROBERT M. PASCIAK, GEORGE B. RESERVITZ, DANIEL B. RUKSTALIS, MICHAEL F. SAROSDY, WALTER M. STADLER, ROBERT P. THIEL AND CHERYL L. HAYDEN

From the University of Miami, Miami, Florida; Matritech, Inc., Newton, Boston City Hospital and New England Deaconess Hospital, Boston, and Cambridge Urological Associates, Cambridge, Massachusetts; Weiss Memorial Hospital and University of Chicago, Chicago, and Dupage Urological Associates, Naperville, Illinois; West Virginia University, Morgantown, West Virginia; University of Washington, Seattle, Washington; University of Rochester, Rochester, New York; Medical College of Pennsylvania and Hahnemann University, Philadelphia, Pennsylvania; University of Texas at San Antonio, San Antonio, Texas; and Dianon Systems, Inc., Stratford, Connecticut

ABSTRACT

Purpose: We evaluated the ability of an immunoassay for nuclear matrix protein 22 (NMP22* test kit) to predict the subsequent disease status of patients with transitional cell carcinoma of the urinary tract at approximately 10 days after transurethral resection of bladder tumor.

Materials and Methods: A total of 90 patients with transitional cell carcinoma provided voided urine samples at least 5 days postoperatively. NMP22 was determined using a commercial test kit. At initial cystoscopic examination 3 to 6 months later the disease status was recorded, and the NMP22 values before and after transurethral resection of bladder tumor were compared.

Results: Of 125 followup cystoscopic examinations (60 patients had 1, 26 had 2, 3 had 3 and 1 had 4 recurrences) transitional cell carcinoma was pathologically confirmed in 33. No malignancy was present at 79 examinations (if tumor was seen endoscopically, pathological evaluation indicated atypia, dysplasia or no abnormality). NMP22 values in these 2 populations were significantly different (malignancy median 20.81 units per ml. and no malignancy median 5.72 units per ml., Mann-Whitney U test for differences between 2 medians $p = 0.00005$). Of the 33 recurrences 23 (70%) had NMP22 values greater than the reference range (10 units per ml.). Additionally, NMP22 identified all 6 subjects (100%) who had invasive disease 3 to 6 months later. Of 72 patients with NMP22 less than 10 units per ml. 62 (86%) had no malignancy at subsequent cystoscopy.

Conclusions: NMP22 was highly predictive of tumor status at followup cystoscopy. This quantitative, noninvasive assay, with high negative predictive value (86%) and sensitivity to detect malignancy (100% for invasive disease and 70% overall), may be a helpful adjunct to cytology and endoscopy for monitoring disease status after endoscopic tumor resection.

KEY WORDS: immunoassay; carcinoma, transitional cell; bladder neoplasms; tumor markers, biological

Due to the multifocal nature of bladder cancer, either as multiple primary tumors at diagnosis or new tumors at different sites in the bladder after initial diagnosis, current practice is to consider all patients with newly diagnosed transitional cell carcinoma at high risk for recurrence. Those who present with early stage bladder cancer are usually treated by transurethral resection or fulguration. The probability of recurrence or development of a second primary cancer within 5 years of treatment is 50 to 70%, with the majority of tumors recurring within 1 year.[1]

Standard practice for evaluating a patient after surgery for early stage bladder cancer includes cystoscopic examination and cytopathological analysis of a urine (voided or catheterized) or bladder wash specimen. Because of the relatively hostile environment of urine for cellular preservation, and the comparatively low probability of observing low grade malignant cells, urine cytology is insensitive in cases of low grade papillary and flat lesions. Only approximately 40% of well differentiated tumors are diagnosed by voided urine cytology.[2] Additionally, evaluation of disease status with urine cytology is subjective. Accuracy of the analysis depends on the skill of the cytopathologist. Furthermore, inconsistencies have been observed among pathologists grading bladder tumors and for the same pathologist grading the same bladder tumors at different times.[3] Also, intravesical chemotherapy, radiotherapy, repeated catheterization, urinary tract infections and stone disease can cause changes in the exfoliated cells in voided urine, which may interfere with diagnosis of bladder cancer.[4]

Consequently, a noninvasive urine assay that is sensitive (especially in the detection of low grade tumors), objective and quantitative might be a useful adjunct for the urologist who treats patients with transitional cell carcinoma, and

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* Matritech, Inc., Newton, Massachusetts.

TABLE 1. *Days between surgery and urine sample or cystoscopy*

	Mean Days ± SE	Median Days (range)
Surgery to urine sample	35.24 ± 3.07	22 (5–166)
Urine sample to cystoscopy	90.34 ± 4.6	85 (1–302)
Surgery to cystoscopy	125 ± 4.26	119 (39–315)

TABLE 2. *Day of postoperative sample collection according to outcome and NMP22*

Days Sample Collected Postop.	No. Cases (%)						Total No. Cases
	Neg. Outcome*		Pos. Outcome†		Unknown Outcome‡		
	NMP22 Less Than 10 Units/Ml.	NMP22 More Than 10 Units/Ml.	NMP22 Less Than 10 Units/Ml.	NMP22 More Than 10 Units/Ml.	NMP22 Less Than 10 Units/Ml.	NMP22 More Than 10 Units/Ml.	
5–10	13 (36.0)	6 (16.7)	1 (2.8)	12 (33.3)	4 (11.1)	0 (0)	36
11–20	13 (50.0)	4 (15.4)	1 (3.8)	4 (15.4)	3 (11.5)	1 (3.8)	26
21–30	9 (69.2)	0 (0)	1 (7.7)	2 (15.4)	1 (7.7)	0 (0)	13
31–60	16 (55.2)	3 (10.3)	5 (17.2)	4 (13.8)	1 (3.4)	0 (0)	29
More than 60	12 (57.1)	3 (14.3)	2 (9.5)	1 (4.8)	2 (9.5)	1 (4.8)	21
Totals	63 (50.4)	16 (12.8)	10 (8.0)	23 (18.4)	11 (8.8)	2 (1.6)	125

Chi-square analysis—2-tailed p = 0.3492, contingency table randomization test, 100 shuffles—2-tailed p = 0.3960 ± 0.05.
* Episodes with no tumor, or with tumor classified pathologically as showing atypia, dysplasia or no abnormality.
† Episodes with pathological malignancy.
‡ Episodes with tumor noted but no pathological information was available, or with suspicious lesion but no tissue was collected for pathological review.

TABLE 3. *Status of subjects at subsequent cystoscopy*

	No. Cases
No tumor at followup (9 of 9 biopsy neg.)	71
Tumor on subsequent cystoscopy:	50
No tissue submitted, 9	
No pathological abnormality, 1	
Pathological atypia or dysplasia, 7	
Pathological malignancy, 33	
Other outcome	4*

* Suspicious area not biopsied in 3 patients, and cystectomy was performed in 1.

may significantly improve ability to make clinical decisions regarding disease progress and effectiveness of treatment, especially with low grade tumors. Nuclear matrix proteins make up the nonchromatin 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,[5] breast,[6] bone[7] and other tissues. An immunoassay that measures nuclear matrix protein 22 (NMP22) has been developed. Normal subjects have low levels of NMP22 in the urine, while patients with active transitional cell carcinoma may have high levels of urinary NMP22. We demonstrate that NMP22 from a urine sample collected 5 to 60 days postoperatively can provide information about the risk of occult or rapidly recurring disease as noted on the initial followup cystoscopic examination.

MATERIALS AND METHODS

Subjects were recruited from the regular clinical populations of our participating medical centers and clinics. Subjects with transitional cell carcinoma must have had pathologically confirmed transitional cell carcinoma within 18 months before entry into the trial. To be included in the analysis a subject had to have at least 1 disease episode as defined during the time he or she was enrolled in the trial. The 3 events required for a disease episode were surgery for transitional cell carcinoma, assay of a urine sample for NMP22 at 5 to 60 days postoperatively and cystoscopic examination 30 to 180 days postoperatively. Subjects were excluded from the trial if they had a history of or current malignancy other than transitional cell carcinoma, except for nonmelanomatous skin cancer. All tumors were staged according to the system established by the American Joint Committee on Cancer,[8] using depth of invasion by tumor as the criterion. Patients were recruited from academic and private practices so as to include those with a wide range of disease characteristics. Demographic and medical history data were collected at enrollment of the patient in the trial. Followup information was collected each time the patient was seen at the participating institution.

Each subject was given a home NMP22 urine collection kit after surgical treatment and requested to collect a sample no sooner than 5 days postoperatively. One kit contained materials to collect 3 separate voiding specimens within 1, 24-hour period. After collection of the 3 specimens the kit was shipped to the manufacturer by overnight courier for NMP22 analysis according to the test kit package insert. The results of the 3 voids were averaged to yield 1 NMP22 result for each sample. Laboratory technicians performing the NMP22 assay were blinded to the status of all subjects throughout the study.

Nonparametric statistical methods were used for analyses because the assumption of a normal distribution is violated. Statistical inferences were evaluated with the Mann-Whitney U test for differences between 2 medians, and the Kruskal-Wallis nonparametric 1-way analysis of variance for differences among 3 or more groups. All patients participating in the study signed an informed consent form approved by an institutional review board.

RESULTS

Median time between surgery and urine sampling was 22 days, and median time between surgery and subsequent cystoscopy was 119 days (table 1). NMP22 results by time of sample collection postoperatively were analyzed to determine whether trauma resulting from the procedure causes elevations in NMP22 (table 2). The results indicated no effect of time of collection after surgery on NMP22 values. Samples collected 5 days or more postoperatively showed results that reflect malignancy at subsequent examination.

The outcome of subjects at subsequent cystoscopic examination is shown in table 3. NMP22 was determined for 125 disease episodes in 90 subjects for whom clinical information could be collected at operation and cystoscopy, and from whom an appropriate urine sample was collected (60 patients had 1, 26 had 2, 3 had 3 and 1 had 4 recurrences). No tumor was noted at followup for 71 disease episodes (9 cases were examined pathologically by random biopsies and no abnormality was found). Of 50 disease episodes in which tumor was noted on subsequent cystoscopy 1 was associated with visible tumor but pathological findings indicated no abnormality, while 7 showed pathological atypia or dysplasia and 33 showed malignancy. Tissue was not submitted for pathological examination in 9 cases.

There was a strong relationship between the urinary NMP22 assay result and clinical finding on subsequent cystoscopy. The 71 cases with no evidence of tumor at followup showed a median urinary NMP22 of 5.45 units per ml., while those with a malignant tumor had a median urinary NMP22 of 20.81 units per ml. Urinary NMP22 assay values for disease episodes with pathological evidence of atypia or dysplasia (median 9.39 units per ml.) were midway between those of subjects with no evidence of tumor and with malignant disease (table 4 and fig. 1).

Since intravesical therapy would be expected to have an

TABLE 4. *Clinical outcome and results of urinary NMP22 assay*

Outcome	No. Cases	NMP22 (units/ml.) Mean ± SE	Median (95% confidence interval)
No tumor	71	9.01 ± 1.55	5.45 (3.46–6.29)
Tumor:	54		
No pathological abnormality or no tissue submitted	10	12.44 ± 5.75	3.66 (2.20–9.88)
Pathological atypia or dysplasia	7	13.50 ± 4.22	9.39 (4.58–16.65)
Pathological malignancy	33	36.13 ± 10.76	20.81 (10.34–29.97)
Other outcome	4*	5.67 ± 0.74	5.00 (4.81–7.87)

* Suspicious area not biopsied in 3 patients and cystectomy was performed in 1.

effect on outcome as determined by followup cystoscopy, NMP22 was analyzed according to outcome and administration of intravesical therapy. Some patients received bacillus Calmette-Guerin, mitomycin C or thiotepa postoperatively and before subsequent cystoscopy. Table 5 shows NMP22 results by disease episode outcome and administration of intravesical therapy.

Since separate Mann-Whitney U tests were performed in our analysis, a probability value of 0.01 was considered statistically significant. When all disease episodes were considered regardless of outcome median NMP22 was greater for patients who received intravesical therapy than for those who did not, with a difference that approached statistical significance. This trend extended through all of the outcome groups except the small group with unknown pathological disease or a lesion on cystoscopy that was pathologically described as "other."

For further analysis, disease episodes were combined to yield 2 groups: 33 cases with malignancy versus 79 with no tumor or nonmalignant tumor at subsequent cystoscopy. A significant difference was observed between urinary NMP22 assay values for disease episodes with (median 20.81 units per ml., 95% confidence interval on the median 10.34 to 29.97 units per ml.) and without (median 5.72 units per ml., 95% confidence interval on the median 4.19 to 6.50 units per ml.) malignancy (table 6). There was no overlap between the confidence intervals, indicating that these groups represent different populations. A value of $p = 0.00005$ was obtained with the Mann-Whitney U test for difference between 2 medians, indicating that NMP22 levels for these 2 groups are significantly different.

A receiver operator characteristic curve was developed to determine the optimal reference value to differentiate patients at risk and not at risk for malignant disease on subsequent cystoscopic examination (fig. 2). A reference value of 10.0 units per ml. is close to the inflection point on the curve, thereby maximizing sensitivity and specificity. In this group a reference value of 10 units per ml. yields a sensitivity of

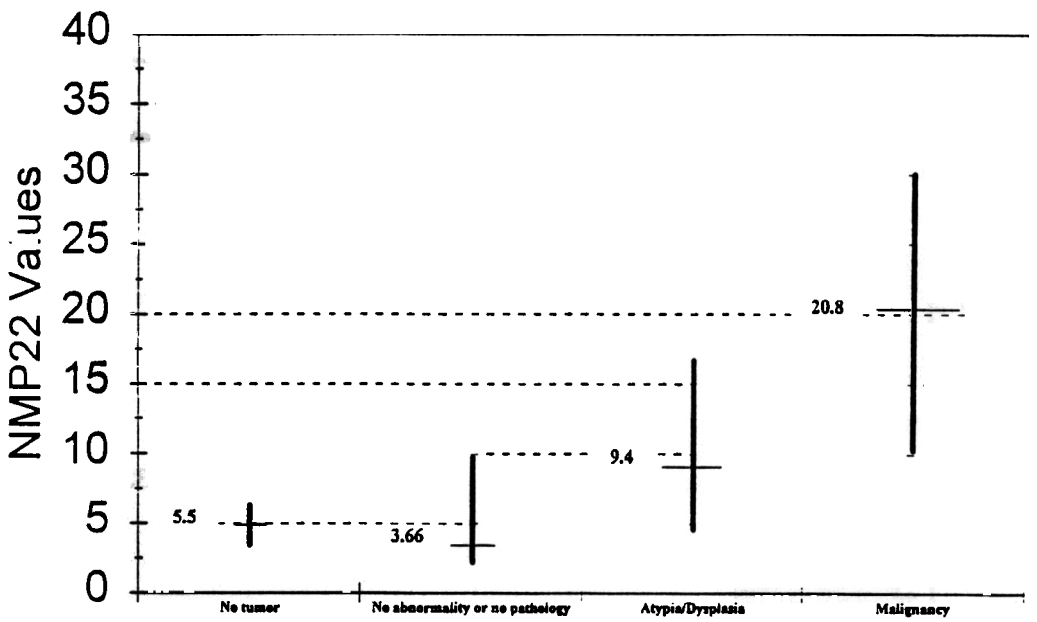

FIG. 1. Comparison of clinical outcome 3 to 6 months postoperatively to NMP22 results

TABLE 5. *Effects of intravesical therapy on urinary NMP22*

Outcome*	Intravesical Therapy	No. Cases	Median Units/Ml. NMP22 (range)	2-Tailed p Value†
No tumor on cystoscopy	No*		3.94 (0.14–75.29)	
	Yes		7.04 (0.79–58.41)	
Tumor:				
Pathologically no abnormality, or atypia/dysplasia	No		7.42 (4.04–16.65)	0.14
	Yes		9.92 (9.39–37.42)	
Pathological malignancy	No		17.85 (0.28–58.97)	0.44
	Yes		21.43 (2.09–334.67)	
Pathology unknown, or other lesion noted on cystoscopy	No*		9.82 (2.1–58.46)	0.41
	Yes		4.81 (2.3–5.14)	
All pts.	No		5.55 (0.14–75.29)	0.02
	Yes		8.52 (0.79–344.67)	

* One subject on intravesical therapy, status unknown, was not included in this analysis.
† Calculated with Mann-Whitney U test for difference between subjects receiving and not receiving intravesical therapy for each outcome group.

TABLE 6. *Clinical outcome and results of urinary NMP22 excluding patients without pathological results*

Outcome	No. Cases	NMP22 (units/ml.) Mean ± SE	Median (95% confidence interval)
No tumor noted endoscopically or tumor pathologically classified as atypia, dysplasia or no abnormality	79	9.35 ± 1.44	5.72 (4.19–6.50)
Malignant tumor	33*	36.13 ± 10.76	20.81 (10.34–29.97)

* Effect of invasion on NMP22 levels in episodes with malignant disease outcomes is shown in table 8.

70%, specificity 79%, accuracy 76%, positive predictive value 58% and negative predictive value 86%. With a reference value of 20 units per ml., the positive predictive value increases to 71%, specificity 91% and accuracy 80% (table 7). A Tukey jackknife analysis[9] was conducted to estimate the parameters in the underlying population. Estimates of sensitivity and specificity of the underlying population at the reference value of 10.0 units per ml. are similar to those of the sample reported.

Of particular interest is the further analysis of 33 patients with malignancy at subsequent cystoscopic examination. Table 8 shows the NMP22 values in these patients according to pathological findings. NMP22 identified 100% of patients who subsequently had invasive disease. The range in this group was significantly greater than the reference value of 10 units per ml.

DISCUSSION

While cystoscopy is sensitive in detecting disease, it is an invasive and expensive procedure. Urine cytology is subject to variation among cytopathologists and is relatively insensitive for low grade disease. A noninvasive tool that could assist the urologist in planning treatment of patients with transitional cell carcinoma would improve patient care, prevent unnecessary procedures and decrease medical costs. Attempts have been made to increase the sensitivity and specificity of urine cytology for diagnosis of bladder cancer. The Lewis X antigen and other antibodies, such as M344, G4+E7, P12 and DueABC3, have yielded impressive results but they have yet to be transferred to the clinic.[10] We evaluated the use of NMP22 to detect occult or rapidly recurring disease postoperatively.

NMP22 in urine collected at least 5 days postoperatively was evaluated with regularly scheduled followup cystoscopy as the end point. There were 79 episodes in which no tumor was seen endoscopically or, if tumor was noted, the pathological result was atypia, dysplasia or no abnormality. There were 33 episodes in which tumor was pathologically confirmed as malignant. NMP22 in these 2 distinct groups of patients was statistically significantly different at a $p = 0.00005$ level. A receiver operator characteristic curve was

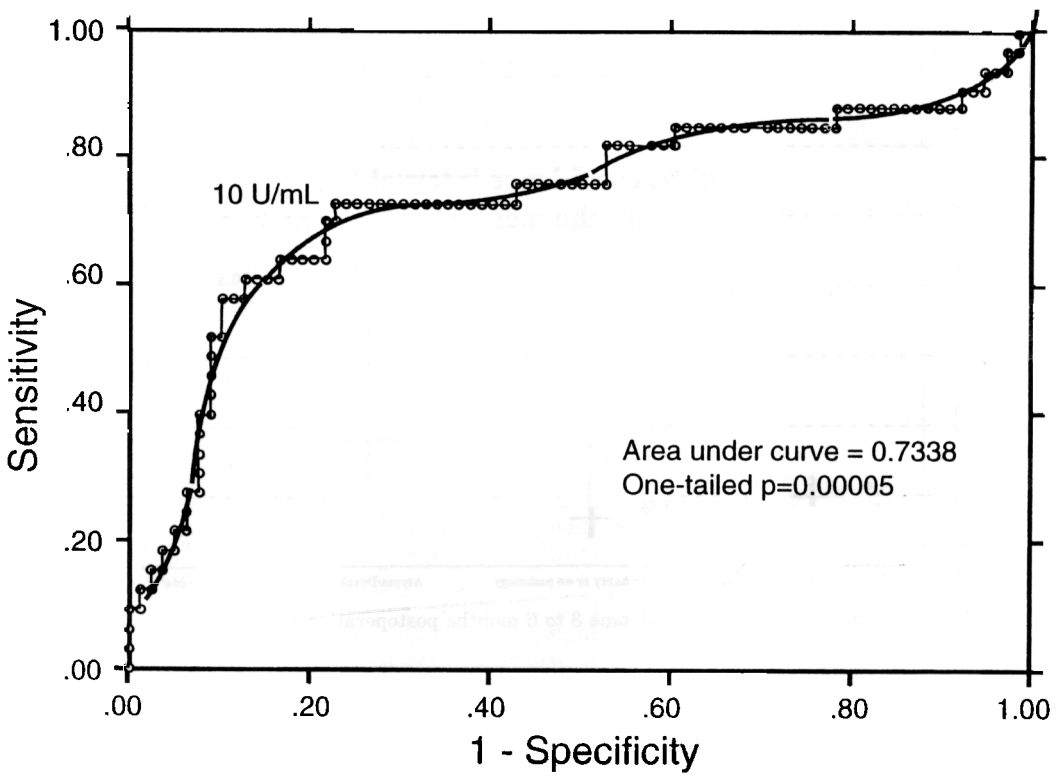

FIG. 2. Receiver operator characteristic curve for subjects with transitional cell carcinoma

TABLE 7. *Sensitivity, specificity, and positive and negative predictive values for 112 episodes*

Reference Value (units/ml.)	% (No. episodes/total No.) Sensitivity	Specificity	Accuracy	Pos. Predictive Value	Neg. Predictive Value
10.0	69.7 (23/33)	78.5 (62/79)	75.9 (85/112)	57.5 (23/40)	86.1 (62/72)
20.0	51.5 (17/33)	91.1 (72/79)	79.5 (89/112)	70.8 (17/24)	81.8 (72/88)

TABLE 8. *Effect of invasion on NMP22 levels in episodes with malignant disease outcomes*

Pathological Result	No. Cases	NMP22 (units/ml.)		No. Cases With More Than 10 Units/Ml. (%)
		Mean (range)	Median (95% confidence interval)	
Malignant, not otherwise specified	3	51.33 (1.12–128.26)	24.62 (0–128.26)	1 (33)
Malignant, papillary, noninvasive	20	22.14 (0.28–133.77)	13.09 (4.96–20.81)	12 (60)
Malignant, invasive	6	83.86 (15.99–334.67)	35.00 (15.99–58.97)	6 (100)
Ca in situ	4	23.15 (6.01–33.79)	26.40 (0–33.8)	3 (75)

developed and validated by the Tukey jackknife analysis in these 2 groups. At a reference value of 10 units per ml. the maximum sensitivity was 70% and specificity was 79%.

The interval between surgery and followup cystoscopy was 39 to 315 days. Although the commonly accepted interval is 90 days, there are several reasons why a patient may return after 90 days. The urologist may believe the patient to be at low risk for invasive disease within a short time postoperatively considering the history of the disease, pathological determinants from the most recent operations and assessment of adequacy of tumor resection. The patient may be noncompliant with the scheduled cystoscopic examination. Other conditions may cause the urologist to choose a less rigorous management schedule, or unforeseen circumstances may cause the long followup.

Since NMP22 is collected postoperatively the time between surgery and subsequent cystoscopy does not affect the accuracy of this test in detecting malignancy. NMP22 results are reported as 1 value, which is the average of 3 separate values (from 3 separate voids within 1, 24-hour period).

The effect of intravesical therapy on the predictive value of NMP22 in our patients was examined. When all patients were considered regardless of outcome a difference in median NMP22 approaching statistical significance was noted, with those receiving intravesical therapy having greater values than those who did not receive intravesical therapy. This finding may reflect the judgment of the urologists in recommending that patients with more aggressive disease receive intravesical therapy. This trend also was noted in most of the outcome subgroups.

The assay provides the urologist an additional quantitative, noninvasive tool for evaluation of the patient with transitional cell carcinoma shortly after surgery, which might allow the urologist to treat the patient better according to the risk profile. The high negative predictive value (86%) of NMP22 combined with its sensitivity to detect patients with invasive disease (6 of 6 or 100%) might give the clinician confidence in extending the time between followup cystoscopic examinations in patients with values less than 10 units per ml. NMP22 more than 20 units per ml. increases the positive predictive value to 71%, which indicates a relatively high probability of malignant disease at initial followup cystoscopy. These patients may benefit from immediate medical intervention, such as transurethral resection of bladder tumors or intravesical therapy.

CONCLUSIONS

NMP22 is a noninvasive, quantitative test performed on voided urine with good accuracy in predicting disease status following cystoscopy. If NMP22 is less than 10.0 units per ml. (negative predictive value 86%) the risk of malignant disease at subsequent cystoscopy is low. NMP22 greater than 20 units per ml. indicates with high probability (positive predictive value 71%) that there will be malignant disease on subsequent cystoscopic examination, which may prompt the urologist to intensify followup of the patient, perhaps not waiting as long to perform cystoscopy, or perhaps skipping followup cystoscopy and performing a procedure that would allow tissue to be collected for pathological analysis. Although cystoscopy is considered the gold standard, it is invasive and costly. Serious consideration should be given to decreasing the frequency of endoscopic procedures.

REFERENCES

1. Abel, P. D.: Prognostic indices in transitional cell carcinoma of the bladder. Brit. J. Urol., **62:** 103, 1988.
2. Badalament, R. A., Hermansen, D. K., Kimmel, M., Gay, H., Herr, H. W., Fair, W. R., Whitmore, W. F., Jr. and Melamed, M. R.: The sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. Cancer, **60:** 1423, 1987.
3. Ooms, E. C., Anderson, W. A., Alons, C. L., Boon, M. E. and Veldhuizen, R. W.: Analysis of the performance of pathologists in the grading of bladder tumors. Hum. Path., **14:** 140, 1983.
4. Wiener, H. G., Vooijs, G. P. and van't Hof-Grootenboer, B.: Accuracy of urinary cytology in the diagnosis of primary and recurrent bladder cancer. Acta Cytol., **37:** 163, 1993.
5. Keesee, S. K., Meneghini, M. D., Szaro, R. P. and Wu, Y. J.: Nuclear matrix proteins in human colon cancer. Proc. Natl. Acad. Sci., **91:** 1913, 1994.
6. Khanuja, P. S., Lehr, J. E., Soule, H. D., Gehani, S. K., Noto, A. C., Choudhury, S., Chen, R. and Pienta, K. J.: Nuclear matrix proteins in normal and breast cancer cells. Cancer Res., **53:** 3394, 1993.
7. Bidwell, J. P., Fey, E. G., van Wijnen, A. J., Penman, S., Stein, J. L., Lian, J. B. and Stein, G. S.: Nuclear matrix proteins distinguish normal diploid osteoblasts from osteosarcoma cells. Cancer Res., **54:** 28, 1994.
8. American Joint Committee on Cancer: Manual for Staging of Cancer, 3rd ed. Philadelphia: J. B. Lippincott Co., pp. 171–176, 1988.
9. Sokal, R. R. and Rohlf, F. J.: Biometry: The Principles and Practices of Statistics in Biological Research. New York: W. H. Freeman & Co., p. 821, 1995.
10. Golijanin, D., Sherman, Y., Shapiro, A. and Pode, D.: Detection of bladder tumors by immunostaining of the Lewis X antigen in cells from voided urine. Urology, **46:** 173, 1995.