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ORIGINAL ARTICLE

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Usefulness of urinary NMP22 to detect tumor recurrence of superficial bladder cancer after transurethral resection

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Abstract
Background. In a prospective study we compared the usefulness of urinary nuclear matrix protein 22 (NMP22) with that of urine cytology and other urinary markers in the monitoring of superficial bladder cancer after transurethral resection (TURBT).
Methods. The subjects were 156 patients, comprising 99 patients with superficial bladder cancer in whom TURBT was planned (untreated group) and 57 patients without tumors in the bladder who had been followed up after TURBT (follow-up group).
Results. Among the 156 patients, who were monitored for 11–26 months (median, 21 months), recurrence was observed in 51 patients (33.0%). At the time of recurrence, the sensitivities of NMP22, basic fetoprotein (BFP), and bladder tumor antigen (BTA) tests, and urine cytology were 18.6%, 23.3%, 9.3%, and 7.0%, respectively. The factors affecting the sensitivity of NMP22 were tumor size and urinary WBC. The size of recurrent tumors was significantly smaller ($P < 0.05$) than that of the initial tumors. Based on receiver operating characteristic (ROC) curves calculated from the data of patients with recurrence, the ideal cutoff values at recurrence were recommended to be 5.0 U/ml for NMP22 and 6.0 ng/ml for BFP. Using these cutoff values, the sensitivities of NMP22 and BFP were 48.8% and 44.2%, respectively.
Conclusion. Because the size of recurrent bladder tumors is usually smaller than that of the initial tumors, the cutoff values of urinary markers should be reduced to detect these tumors. We recommend 5.0 U/ml as a cutoff value of NMP22 for detection of recurrence of bladder tumor.

Key words Superficial bladder cancer · NMP22 · Cytology · Tumor recurrence

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Introduction

Bladder cancer is the eleventh most frequent cancer worldwide. In Japan, the incidence of new cases is estimated to be 6000 in males and 2000 in females per year.[1] About 70% of patients with bladder cancer have superficial disease that is managed with transurethral resection (TURBT). The probability of recurrence or the development of a second primary cancer within 5 years of treatment is 50%. Regular surveillance is therefore necessary.[2] A standard method of assessing patients for recurrent disease consists of periodic cystoscopic examination and voided urine cytology (VUC). However, cystoscopy is invasive and VUC has inadequate sensitivity, particularly for superficial disease.[3]

Recently, the usefulness of many urinary tumor markers for bladder cancer has been reported. In Japan, three urinary tumor markers, nuclear matrix protein 22 (NMP22),[4] bladder tumor antigen (BTA) test,[5] and basic fetoprotein (BFP),[6] have been approved by the Ministry of Health, Labour, and Welfare. The NMP22 test is a quantitative enzyme immunoassay that detects complexed and fragmented forms of the nuclear mitotic apparatus (NuMA) protein associated with the mitotic spindle apparatus during mitosis.[7] The BTA test is an immunochromatographic assay that detects bladder tumor-associated antigens in voided urine.[8] BFP is a basic protein with a molecular weight of 55000 Da that was discovered in extracts of human fetal serum, intestine, and brain tissue in Japan.[6]

In general, bladder cancers detected during monitoring are smaller than those detected by screening. Our previous study revealed that the sensitivity of NMP22 was correlated with tumor size.[9] In the present multicenter prospective study, we performed monitoring, using urinary NMP22

after an operation for superficial bladder cancer, and compared its usefulness with that of other urinary tumor markers and VUC.

Patients and methods

Patients

The subjects were untreated patients with superficial bladder cancer in whom TURBT was planned (untreated group) and patients without tumors in the bladder who had been followed up after TURBT (follow-up group). Patients who had the possibility of false-positive results for urinary markers, due to the following factors, were excluded: presence of a tumor in the urinary tract other than in the bladder, urinary tract infection, radiation cystitis, interstitial cystitis, hemorrhagic cystitis, urinary diversion, indwelling catheter or stent, and a serum creatinine level of 3.0 mg/dl or more.[9]

Urine collection and measurement methods

In the untreated group, urine samples were collected before treatment, and 1, 2, and 3 months after treatment. After 3 months, urine collection and cystoscopy were performed simultaneously, at 3-month intervals or at the time of recurrence. Because intravesical administration affects urinary markers, data acquired during intravesical therapy were excluded.

In the follow-up group, after confirmation of the absence of tumors in the urinary tract, urine collection and cystoscopy were performed simultaneously at 3-month intervals or at the time of recurrence.

Voided urine was collected before cystoscopy, and the NMP22 test, BTA test, BFP test, and VUC were performed using the same urine samples. Urine sampling was not performed within 5 days after cystoscopy or catheterization (including catheterization for intravesical administration). Urine samples were stored according to the requirements for each measurement item, and samples for urinary NMP22 were stored at 4°C. The objectives and methods were explained, and informed consent was obtained from all subjects.

The cutoff values were 12.0 U/ml (normal range, <12.0) for NMP22[10] and 10.0 ng/ml (normal range, ≦10.0 ng/ml) for BFP.[6] NMP22 and BTA tests were conducted as previously described.[4,5] We used the following test kits: NMP22 (Matritech, Newton, MA, USA), BFP (Eiken Chemical, Tokyo, Japan), and BTA (Bard, Murray Hill, NJ, USA). In this study, the values of the markers after TURBT and at the time of recurrence were used as endpoints. Classification of voided urine was carried out according to Papanicolaou's classification. Classes I, II, and III were regarded as negative, and classes IV and V were regarded as positive.[11]

Statistical analysis

The χ^2 test was used to analyze for differences between markers and urine cytology. For this analysis, data from patients who underwent examinations of the three markers and VUC simultaneously were included. Multivariate analysis (quantification method II) was used for factors affecting the sensitivity of each marker and for recurrence-associated factors. JUSE MA software (The Institute of the Japanese Union of Scientists and Engineers) was used for statistical analyses.

Results

Between January 2000 and March 2001, 156 patients (120 men, 36 women), with a median age of 69 years (range, 37 to 91 years) were enrolled in the present study. The patients comprised 99 patients who had untreated superficial bladder cancer (untreated group) and 57 patients who had been followed-up after operation for superficial bladder cancer (follow-up group). There was no significant difference in age or sex distribution between the untreated group and the follow-up group.

In the untreated group, all tumors were transitional cell carcinoma, and were graded as G1 in 34 patients, G2 in 56, and G3 in 9. The stage was Tis in 6 patients, Ta in 53, and T1 in 40. The untreated group was subclassified into primary and recurrent tumor groups (Table 1). The recurrent tumor group had significantly smaller tumors than the primary group ($P < 0.05$), and no patient in this group had a tumor greater than 3 cm in size.

In all patients, measurements of the three tumor markers were performed simultaneously with VUC, and the sensitivities for the NMP22, BFP, and BTA tests, and VUC were 33.3%, 41.3%, 33.3%, and 33.3%, respectively. Multivariate analysis of factors affecting the sensitivity of each marker showed tumor size and urinary WBC as significant factors for NMP22 (Table 2).

All 156 patients were monitored until March 2002. During monitoring for 11–26 months (median, 21 months), recurrence was observed in 51 patients (33%). Measurements of the three tumor markers were performed simultaneously with VUC in 137 of all the 156 monitored patients and in 43 of the 51 recurrent patients. The tumor size in these 43 patients was less than 10 mm in 28 patients (65%), 10–30 mm in 12 patients (28%), more than 30 mm in 1 patient (2%), and unknown in 2 patients (5%). When compared with the primary tumor group of untreated patients (Table 1), these 43 patients also had significantly smaller tumors than the primary tumor group ($P < 0.05$). In these patients, the sensitivities for NMP22, BFP, BTA test, and VUC were 18.6%, 23.3%, 9.3%, and 7.0%, respectively (Table 3).

Based on data from the patients with recurrence, receiver operating characteristic (ROC) curves were obtained, and the cutoff values during monitoring were determined to be 5.0 U/ml (normal range, <5.0 U/ml) for NMP22 and 6.0 ng/ml (normal range, <6.0 ng/ml) for BFP

Table 1. Characteristics of tumors in untreated patients ($n = 99$)

	Total ($n = 99$)		Primary tumors ($n = 73$)		Recurrent tumors ($n = 26$)		P value
Number							NS
1	44	45%	33	44%	11	42%	
2–4	36	36%	25	34%	11	42%	
>5	14	14%	11	15%	3	12%	
Unknown	5	5%	4	6%	1	4%	
Size (mm)							<0.05
<10	26	26%	15	21%	11	42%	
10–30	51	52%	39	53%	12	46%	
>30	14	14%	14	19%	0	0%	
Unknown	8	8%	5	7%	3	12%	
Stage							NS
Tis	6	6%	4	6%	2	8%	
Ta	53	54%	39	53%	14	54%	
T1	40	40%	30	41%	10	38%	
Grade							NS
G1	34	34%	24	33%	10	38%	
G2	56	57%	42	57%	14	54%	
G3	9	9%	7	10%	2	8%	

NS, not significant

Table 2. Factors affecting the sensitivity of each marker

	NMP22	BFP	BTA	VUC
Age	0.68	1.10	0.38	0.96
Sex	0.07	1.07	0.91	0.00
Size	**2.49**	1.59	0.89	1.12
Number	1.76	0.96	0.25	0.87
Stage	0.48	0.14	0.30	0.01
Grade	0.36	0.46	0.02	**3.29**
Hb	1.05	1.03	**2.63**	0.13
RBC	0.97	**2.28**	**2.89**	0.27
WBC	**2.17**	1.35	1.77	**3.34**

Quantification method II (multivariate analysis)
Numbers in bold indicate significant factors
NMP22, nuclear matrix protein 22; BFP, basic fetoprotein; BTA, bladder tumor antigen; VUC, voided urine cytology

(Fig. 1). The sensitivities using these cutoff values were 48.8% for NMP22 and 44.2% for BFP (Table 4). NMP22 and BFP had superiority over the BTA test and VUC in the detection of recurrent bladder cancer ($P < 0.001$). The sensitivity and specificity of the NMP cutoff value (5.0 U/ml) were 48.8% and 66.0%, respectively, and those of the BFP cutoff value (6.0 ng/ml) were 44.2% and 53.2%, respectively. No significant differences were observed between NMP22 and the other markers using an NMP22 cutoff value of 12.0 U/ml, but a significant difference was observed between NMP22 and the BTA test ($P < 0.01$) in addition to VUC ($P < 0.001$) using a cutoff value of 5.0 U/ml.

As recurrence-associated factors, multiple tumors were significant, but there were no associations between the pretreatment values of the urinary markers and recurrence (Table 5).

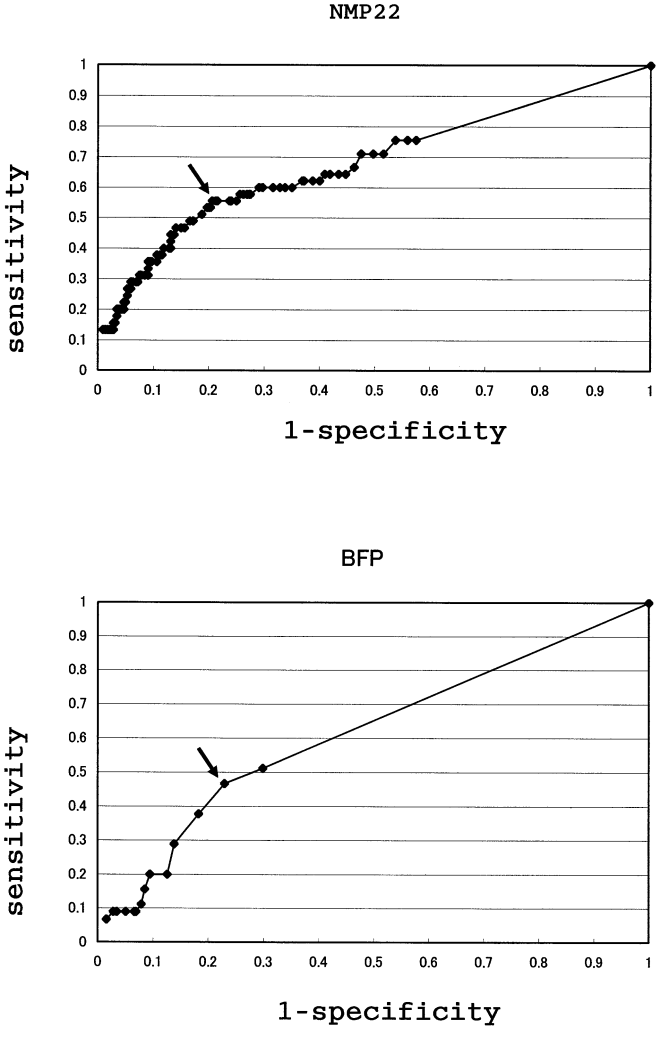

Fig. 1. Receiver operating characteristic (ROC) curves. From the ROC curves, the cutoff values during monitoring were determined to be 5.0 U/ml for nuclear matrix protein 22 (*NMP22*) and 6.0 ng/ml for basic fetoprotein (*BFP*)

Table 3. Overall sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) at recurrence (NMP22 cutoff, ≧12.0 U/ml; BFP cutoff, >10 ng/ml)

	NMP22	BFP	BTA	VUC
Sensitivity	18.6 (8/43)[a]	23.3 (10/43)[a]	9.3 (4/43)[a]	7.0 (3/43)[a]
Specificity	85.1 (80/94)	69.1 (65/94)	86.2 (81/94)	97.9 (92/94)
Accuracy	64.2 (88/137)	54.7 (75/137)	62.0 (85/137)	69.3 (95/137)
PPV	36.4 (8/22)	25.6 (10/39)	23.5 (4/17)	60.0 (3/5)
NPV	69.6 (80/115)	66.3 (65/98)	67.5 (81/120)	69.7 (92/132)

[a] Percentage (No. samples/total no.)

Table 4. Overall sensitivity, specificity, accuracy, PPV, and NPV at recurrence (NMP22 cutoff, ≧5.0 U/ml; BFP cutoff, ≧6 ng/ml)

	NMP22	BFP	BTA	VUC
Sensitivity	48.8 (21/43)[a]	44.2 (19/43)[a]	9.3 (4/43)[a]	7.0 (3/43)[a]
Specificity	66.0 (62/94)	53.2 (50/94)	86.2 (81/94)	97.9 (92/94)
Accuracy	60.6 (83/137)	50.4 (69/137)	62.0 (85/137)	69.3 (95/137)
PPV	39.6 (21/53)	30.2 (19/63)	23.5 (4/17)	60.0 (3/5)
NPV	73.8 (62/84)	67.6 (50/74)	67.5 (81/120)	69.7 (92/132)

[a] Percentage (No. samples/total no.)

Table 5. Recurrence-associated factors

Factor	F
Size	0.677
Number	2.163
Stage	0.034
Grade	0.287
Hb	0.694
RBC	0.869
WBC	0.412
NMP22	0.287
BFP	0.025
BTA	0.025
VUC	0.025

Quantification method II (multivariate analysis)

Discussion

Monitoring after treatment of superficial bladder cancer should be performed for a long period. Therefore, minimally invasive tests that allow the detection of recurrence as early as possible are desired, and in this respect, urinary tumor markers have an important role to play. However, most previous studies of urinary markers have evaluated sensitivity at the time of diagnosis, and there have been few studies of their usefulness for the monitoring of superficial bladder cancer. We evaluated the usefulness of NMP22 for the monitoring only of superficial bladder cancer.

The sensitivity of NMP22 in bladder cancer has been reported to be closely associated with tumor size.[9] However, on detection, recurrent tumors are generally smaller than initial tumors. In the present study, when the untreated group was classified into primary tumor and recurrent tumor groups, the tumor size was significantly smaller in the recurrent tumor group ($P < 0.05$), and no patient in this group had a tumor greater than 3 cm in size. Therefore, the establishment of an NMP22 cutoff value for monitoring was necessary.

Based on the data of the patients with recurrence, we determined the cutoff values of NMP22 and BFP to be 5.0 U/ml and 6.0 ng/ml, respectively. Using these values, the sensitivities of NMP22 and BFP at the time of recurrence improved from 18.6% to 48.8% and from 23.3% to 44.2%, respectively. NMP22 and BFP were significantly more sensitive than the BTA test and VUC, while the difference between NMP22 and BFP was not significant. Stampfer et al.[12] reported an NMP22 cutoff value of 6.4 U/ml (sensitivity, 68%) in patients during monitoring after treatment of bladder cancer. However, this is the first time a new cutoff value of NMP22 has been established based on the results of prospective monitoring for recurrence, considering the low sensitivity of NMP22 at recurrence during monitoring. In this study, NMP22 was primarily evaluated, but the present results also suggested the usefulness of a reduction in the BFP cutoff value from 10.0 to 6.0 ng/ml. We also recommend the establishment of new cutoff values for other markers during monitoring.

In the evaluation of tumor markers, their usefulness as prognostic factors is expected. Soloway et al.[13] reported a high incidence of recurrence in patients with a high NMP22 value within 3 months after operation. Poulakis et al.[14] also reported the prognostic value of NMP22, when the cutoff value was set at 8.25 U/ml. However, in the present study, we could not find any associations between pretreatment values of the urinary markers and recurrence.

Conclusion

Recurrent tumors are generally small on detection. For the early detection of such tumors, the cutoff values of urinary markers should be reduced. The findings in the present

study suggested that the appropriate cutoff value of NMP22 during monitoring was 5.0 U/ml. As a result of the study to determine the optimal cutoff value of each urinary tumor marker during monitoring, it seems that NMP22 may be superior to other tumor markers or VUC in detecting recurrent tumors, and therefore, NMP22 may be useful as a tumor marker for the postoperative monitoring of superficial bladder cancer. The frequency of cystoscopy could be reduced by using NMP22 measurements.

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