INTRA-DAY VARIATION OF URINARY NUCLEAR MATRIX PROTEIN 22

YOSHIHISA FUKUI, SHOJI SAMMA, AKIRA YAMAGUCHI,
KATSUYA AOKI and KAZUHIRO SHIMIZU

Department of Urology, Nara Prefectural Nara Hospital
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Abstract : Nuclear Matrix Protein 22 (NMP22), a urinary tumor marker for urothelial cancers, is directly released into the urine from the nucleus after cell death. Accordingly, values of NMP22 do not require adjustment using other substances such as urinary creatinine. On the other hand, its values might vary according to urine concentration. This study investigated the intra-day variation in the urinary level of NMP22. NMP22 and urinary creatinine were measured in a 24-hour urine sample and 4 spot urine samples obtained from 20 inpatients (10 with bladder cancer, and 10 with non-urothelial cancer or benign tumors). The spot urine samples were collected at 6 a.m., 10 a.m., 2 p.m. and 9 p.m. There were no significant differences in NMP22 values between the 24-hour and spot samples in all patients. Out of 10 bladder cancer patients, 6 had positive 24-hour samples. Among these 6 patients, only 3 had 4 positive spot samples (>12.0 U/ml): one had 3 positive samples, and 2 had one positive sample. Among the controls, only one patient with renal cancer had a positive 24-hour sample. Only 3 controls, 2 with prostatic cancer and one with renal cancer, had a single positive spot sample. The highest margin between the maximum and minimum levels in the 4 spot samples was 237.8 U/ml in the bladder cancer patients and 16.6 U/ml in the controls. When the ratios of NMP22 and urinary creatinine values for the 24-hour to spot samples were calculated in each patient, a significant correlation was observed between the ratios of NMP22 and urinary creatinine ($r=0.575$, $p<0.001$). The urinary level of NMP22 shows intra-day variation and might be affected by the extent of the concentration of urine samples. The measurement results must be judged with this in mind, especially when judging the results around the cut-off value.

Key words : NMP22, urothelial cancer, urinary tumor marker, intra-day variation

INTRODUCTION

Nuclear matrix protein 22 (NMP22), a urinary marker for urothelial cancer, has recently been applied as a screening test. It has been reported that NMP22 was superior to urinary cytology in both sensitivity and specificity.[1-5] When the Bladder Tumor Antigen test,[6-8] another urinary marker for urothelial cancer, was performed simultaneously with NMP22,

Corresponding to: Shoji SAMMA, M.D. Department of Urology, Nara Prefectural Nara Hospital 1-30-1, Hiramatsu, Nara City, Nara 631-0846 JAPAN (Phone) 0742-46-6001 (Fax) 0742-46-6011 (E-mail) urodept@m3.kcn.ne.jp

NMP22 was the superior screening test.[9-12]

NMP22 is a component of nuclear mitotic apparatus (NuMA) protein, which becomes soluble and is released into the urine from the nucleus after cell death. NMP22 is recognized by 2 monoclonal antibodies, MAb 302-22 and MAb 302-18, which were raised against nuclear matrix protein.[13] Accordingly, the level of NMP22 is elevated in patients with urothelial cancer where the cell cycle is accelerated. However, a high NMP22 value was also observed in patients with active urinary tract infection or with an indwelling catheter.[2,3] In addition, variation of NMP22 values in the same patient has been reported.[14] The present study was conducted to investigate the intra-day variation of NMP22 values in 20 patients who had bladder cancer, non-urothelial cancer or benign urogenital tumors.

PATIENTS AND METHODS

From March to June 2001, a total of 20 inpatients (18 men and 2 women) were enrolled in this study. The subjects consisted of 10 patients with untreated bladder cancer, 5 with non-urothelial cancer, and 5 with benign urogenital tumors, who ranged from 58 to 76 years old with a mean age of 65.4 years. Among the 10 bladder cancer patients, one had bladder tumors that developed after a complete nephro-ureterectomy for ureteral cancer. The five patients with non-urothelial cancer consisted of 3 with renal cancer and 2 with prostatic cancer. The five patients with benign urogenital tumors consisted of 3 with benign prostatic hyperplasia, one with renal angiomyolipoma, and one with a benign adrenal tumor. Serum creatinine was within our standard range (0.8 to 1.1 mg/dl) in all subjects. All 10 bladder cancer patients were histologically confirmed to have transitional cell carcinoma: Six patients had a single bladder tumor, and 5 had a main tumor measuring more than 3.0 cm in diameter: The tumor grade was Grade 1 in 2 patients, Grade 2 in 5, and Grade 3 in 3. The clinical diagnosis of each control was confirmed histologically from the surgically removed specimens. Patients who showed more than 5 white blood cells per high power field in the urine sediment and patients with an indwelling catheter were excluded from the study.

The collection of urine samples was performed during hospitalization before surgical treatment. Subjects were not on intravenous therapy during 24-hour collection and they had no limitations on diet or water intake. Four voided urine samples were collected into paper cups at 6 a.m. (fasting), 10 a.m. (2 hours after breakfast), 2 p.m. (2 hours after lunch), and 9 p.m. (3 hours after dinner). Each sample was promptly transferred into special tubes for measurement of NMP22 and urinary creatinine (u-Cr) (5 ml each), and the rest was collected into a 24-hour urine sample bottle. The 24-hour samples were stirred after measuring volume. These 5 samples (4 spot samples and one 24-hour sample) were used for measuring NMP22 and u-Cr. All the samples were kept in a refrigerator at 4℃ until measurement. Collection of 24-hour urine samples was started at 2 p.m. after voiding and was finished with voiding at 2 p.m. on the following day. Then the first spot sample was taken at 9 p.m. and the final one was at 2 p.m. NMP22 was measured with a Matritech UNMP 22 test kit (Konica, Tokyo, Japan), and u-Cr was measured by the alkaline picrate method.

The objectives and methods were explained and informed consent was obtained from all the subjects. The kits for NMP22 were obtained from Konica. NMP22 and u-Cr were

measured by SRL (Tokyo, Japan).

For statistical analysis, the χ^2 test and McNear's test were used for inter-group comparisons, while Spearman's rank correlation and the Mann-Whitney U-test were used for assessing correlations. Calculations were done using StatView software (version 5.0, SAS Inc., Cary, NC, USA). The standard value of NMP22 employed in this study was 12.0 U/ml. The following analyses were done by judging samples with more than 12.1 U/ml as positive and those with less than 12.0 U/ml as negative. Samples with levels under the lower detection limit (2.0 U/ml) were handled as 2.0 U/ml.

RESULTS

NMP22 levels: The results of measuring NMP22 are shown in Table 1.

NMP22 values in 24-hour samples ranged from 2 to 330 U/ml, with a mean (± standard deviation) of 30.1 ± 78.5 U/ml: In the 10 bladder cancer patients, 4 samples had levels of less than 2 U/ml and the remaining 6 ranged from 9.8 to 330 U/ml, with a mean of 47.7 ± 100.6

Table 1. Results of measurement of NMP-22 in each patient

		Urine samples (U/ml)				
Case	**Disease**	**24-h**	**6:00**	**9:00**	**14:00**	**21:00**
1	**Bladder cancer**	**46.9**	**62.8**	**70.9**	**56.0**	**75.4**
2	**Bladder cancer**	**19.8**	**12.9**	**34.3**	8.3	**17.4**
3	**Bladder cancer**	**330**	**333**	**235**	**257**	**95.2**
4	**Bladder cancer**	2	2.4	4.2	3.7	2
5	**Bladder cancer**	2	2	2	2	2
6	**Bladder cancer**	**16.1**	7.3	3.7	**24.0**	7.3
7	**Bladder cancer**	**25.5**	5.3	9.1	11.3	**90.9**
8	**Bladder cancer**	**40.6**	**42.5**	**16.3**	**12.5**	**35.5**
9	**Bladder cancer**	2	2.2	2	2.4	2.2
10	**Bladder cancer**	2	3.4	3.6	2	2.4
11	**Adrenal tumor**	2.8	4.5	5.5	2.5	3.2
12	**BPH**	6	5.5	4.1	5.6	6.8
13	**Prostate cancer**	5.4	2.4	3.6	**12.2**	7.1
14	**Renal AML**	2	2.9	2	2	2.3
15	**Prostate cancer**	2	**13.1**	3.9	7.3	2
16	**RCC**	3.2	5.3	2.7	2	2.3
17	**RCC**	**12.9**	**18.6**	10.6	2	10.5
18	**RCC**	2.3	6.8	5.0	3.3	3.3
19	**BPH**	2	2	2	2	2
20	**BPH**	2	2	2	2	2

BPH: Benign prostatic hyperplasia
AML: Angiomyolipoma
RCC: Renal cell carcinoma

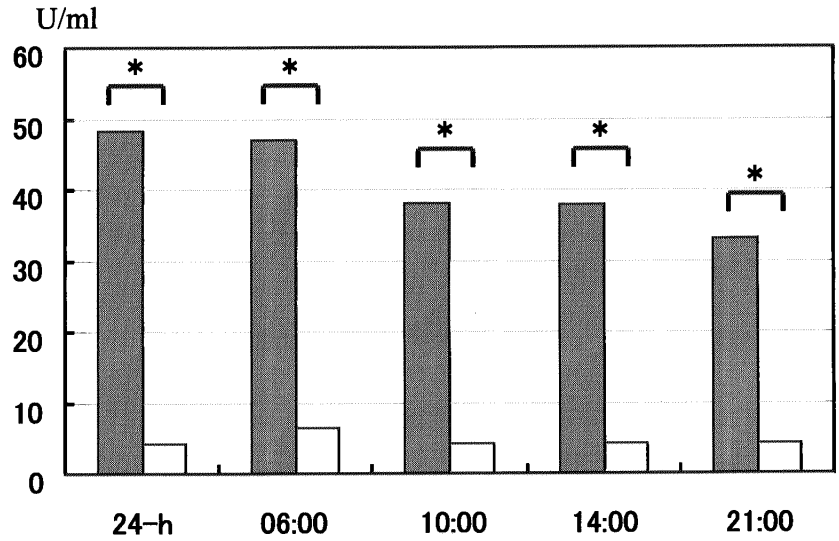

Fig. 1. Mean NMP22 value in spot samples and 24-hour samples from the bladder cancer patients and the controls.
*: $p < 0.05$, ■ bladder cancer patients, □ controls.

U/ml. In the 10 controls, the values ranged from less than 2 to 12.9 U/ml, with a mean of 4.9 ± 3.3 U/ml. There was a significant difference between the bladder cancer patients and the controls ($p<0.001$).

The values in a total of 80 spot samples ranged from 2 to 333 U/ml, with a mean of 25.4 ± 58.7 U/ml: In the bladder cancer patients, the values ranged from less than 2 to 333 U/ml, with a mean of 40.1 ± 74.5 U/ml. In the controls, the values ranged from less than 2 to 18.6 U/ml, with a mean of 5.7 ± 3.9 U/ml. A significant difference was observed between the 2 patient groups ($p<0.001$).

Comparison of the mean NMP22 values according to collection time: When the mean value of NMP22 in spot samples was compared according to the collection time, there were significant differences between the mean values for the bladder cancer patients and the controls ($p<0.05$). (Fig. 1) In the bladder cancer patients, no significant difference was observed between NMP22 levels in the 24-hour sample and the spot samples. Similarly, there was no significant difference in the controls.

Intra-day variation of NMP22: Among the bladder cancer patients, 3 were positive for all 4 spot samples. Another 3 patients had both positive and negative samples, including 2 patients with one positive spot sample and one patient with 3 positive spot samples. All of these 6 patients had a positive 24-hour sample. In the remaining 4 patients, all of the spot samples and the 24-hour samples were negative. Among the controls, only one patient with renal cancer showed a positive 24-hour sample, with a value of 12.9 U/ml. Seven patients also had completely negative spot samples, while 3 patients (2 with prostatic cancer and one with renal cancer) had one positive spot sample each, the values of which were 12.2, 13.1, and 18.6 U/ml. Out of the 80 spot samples collected from the bladder cancer patients and the controls, the number of positive samples was 17 and 3, respectively. A significant difference

Table 2. Number of positive and negative samples

Group	Positive*	Negative	Total
Bladder cancer	17 (6)	23 (4)	40 (10)
Control	3 (1)	37 (9)	40 (10)
Total	20 (7)	60 (13)	80 (20)

* Positive: more than 12.1 U/ml; Negative: less than 12.0 U/ml.
( ): number of 24-hour samples.
P < 0.005, bladder cancer group vs control group.

was observed between the bladder cancer patients and the controls ($p<0.005$). (Table 2)

Collection times, yielding the maximum and minimum NMP22 levels: The sample collection time that yielded the maximum NMP22 level in each patient was analyzed. Out of 9 bladder cancer patients (excluding one with NMP22 levels of less than 2 U/ml in every spot sample), 2 had a maximum value at 6 a.m., 3 at 10 a.m., 2 at 2 p.m., and 2 at 9 p.m. The minimum value was observed at 6 a.m. in one patient, at 10 a.m. in 2, at 2 p.m. in 4, and at 9 p.m. in 2. Out of 8 controls (excluding 2 with NMP22 values of less than 2 U/ml in every sample), the maximum value was observed at 6 a.m. in 5, and at 10 a.m., 2 p.m. and 9 p.m. in one each. Among 3 patients who had a positive spot sample, 2 showed the maximum value at 6 a.m. and one showed it at 2 p.m. The most common time for the minimum value was 2 p.m. observed in 3 patients.

Margin of NMP22 values: Margins between the maximum and minimum values in spot samples from each patient were analyzed. In the bladder cancer patients, the margin ranged from 0 U/ml (all samples were less than 2 U/ml) to 237.8 U/ml, with a mean of 42.3 ± 73.3 U/ml and a median of 19.9 U/ml. In the controls, the margin ranged from 0 U/ml (all samples were less than 2 U/ml) to 16.6 U/ml, with a mean of 5.1 ± 5.5 U/ml and a median of 3.2 U/ml.

Correlation between NMP22 and u-Cr: Because there was a possibility that NMP22 values were affected by urine volume or concentration, the correlation between NMP22 values and u-Cr values was analyzed. No significant correlation was observed between the values of NMP22 and u-Cr in the spot samples. Then, the change rates of the NMP22 value of each spot sample were calculated by setting the NMP22 value of the 24-hour sample as 1 (NMP22 value of spot sample / NMP22 value of 24-hour sample). The change rates of the u-Cr values were obtained in the same manner as the NMP22 values. The correlation of the respective rates for NMP22 and u-Cr was investigated. There was a significant correlation between the change rates of NMP22 and those of u-Cr ($r=0.575$, $p<0.001$), suggesting that the variation of NMP22 was associated with urine concentration. (Fig. 2)

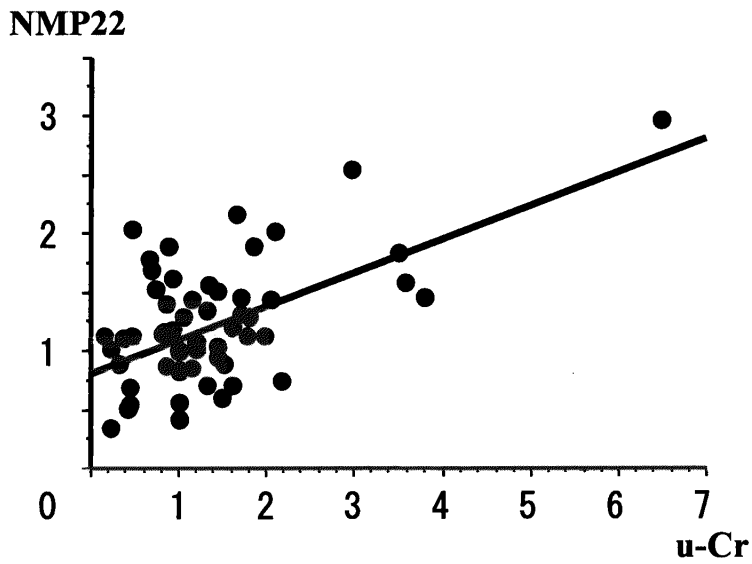

Fig. 2. Correlation between change rates of NMP22 and those of urinary creatinine. Change rate = NMP22 (or u-Cr) of spot sample / NMP22 (or u-Cr) of 24-hour sample.
u-Cr: urinary creatinine, n = 80, r = 0.575, $p < 0.001$.

DISCUSSION

NMP22 not only shows a high sensitivity as a screening method for urothelial cancers but also a low false-negative rate compared to urinary cytology that depends on the skill of the examiner. NMP22 levels were significantly higher in bladder cancer patients than in controls for both the 24-hour and spot samples, suggesting the usefulness of NMP22 as a screening method.

Since cell cycle acceleration would result in an elevated NMP22 level, urinary tract infection and mechanical stimulation of the urothelium such as catheterization or transurethral surgery might cause false-positive results. Moreover, it was reported that the NMP22 value was affected by hematuria,[15] while Chen et al.[14] mentioned that urine collection should be done between midnight and noon. Thus, NMP22 values may vary according to the conditions of urine sampling. The present study excluded patients with an indwelling catheter, surgical treatment, or obvious urinary tract infection, but the variation of NMP22 values was still observed depending on the sampling time, with the highest margins of 237.8 U/ml in the bladder cancer patients and 16.6 U/ml in the controls. In addition, 3 bladder cancer patients and 3 controls showed the intra-day changes of NMP22 that crossed the cut-off value.

Because the NMP22 level is not affected by renal function, adjustment using other substances such as u-Cr may not be required. There is a possibility, however, that the value can be affected by concentration of the urine, e.g., assuming that NMP22 is released constantly into urine, the value would be lower after a high water intake and would be raised in concentrated urine. In the present study, the maximum value was most frequently

detected in the samples voided at 6 a.m. (7 out of 17 patients), and the minimum was most often at 2 p.m. (7 out of 15 patients). This suggested that urine concentration is one of the factors leading to variation. To investigate the influence of urine concentration, correlation between NMP22 and u-Cr was then analyzed, because creatinine is constantly excreted into the urine and is commonly employed for adjusting the urinary level of glomerular-filtered substances. There was a significant correlation between the changes of NMP22 and u-Cr when the ratio of each spot sample to the 24-hour value was assessed in each subject. Thus, bigger changes of u-Cr led to larger changes of NMP22. Although these data do not reduce the clinical usefulness of NMP22, one must keep in mind that water or food intake during sample collection might affect NMP22 values, especially when judging results around the cut-off value.

CONCLUSIONS

There was wide intra-day variation of NMP22 values in some patients. The changes of NMP22 were strongly correlated with those of u-Cr, suggesting that the NMP22 level was affected by the urine concentration. When NMP22 is employed as a screening method for urothelial cancer, these points should be taken into consideration.

REFERENCES

1) **Soloway, M. S., Briggman, J. V., Carpinito, G. W., Chodak, G. W., Church, P.A., Lamm, D. L., Lange, P., Messing, E., Pasciak, R. M., Reservitz, G. B., Rukstalis, D. B., Sarosdy, M. F., Stadler, W. M., Thiel, R. P.** and **Hayden, C. L.**: Use of a new tumor marker, urinary NMP-22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. J. Urol, **156**: 363-367, 1996.

2) **Miyanaga, N., Akaza, H., Ishikawa, S., Ohtani, M., Noguchi, R., Kawai, K., Koiso, K., Kobayashi, M., Koyama, A.** and **Takahashi, T.**: Clinical evaluation of nuclear matrix protein 22 (NMP22) in urine as a novel marker for urothelial cancer. Eur. Urol, **31**: 163-168, 1997.

3) **Stampfer, D. S., Carpinito, G. A., Rodriguez-Villanueva, J., Willsey, L. W., Dinney, C. P., Grossman, H. B., Fritsche, H. A.** and **McDougal W. S.**: Evaluation of NMP22 in the detection of transitional cell carcinoma of the bladder. J. Urol, **159**: 394-398, 1998.

4) **Sawczuk, I. S.** and **Lee, B.**: The mechanism and clinical application of the NMP22 tumor marker immunoassay: a review. Am. Clin. Lab, **18**: 24-26, 1999.

5) **Chahal, R., Darshane, A., Browning, A. J.** and **Sundaram, S. K.**: Evaluation of the clinical value of urinary NMP22 as a marker in the screening and surveillance of transitional cell carcinoma of the urinary bladder. Eur. Urol, **40**: 415-20, 2001.

6) **Sarosdy, M. F., De Vere White, R. W., Soloway, M. S., Sheinfeld, J., Hudson, M. A., Schellhammer, P. F., Jarowenko, M. V., Adams, G.** and **Blumenstein, B. A.**: Results of a multicenter trial using BTA test to monitor for and diagnose recurrent bladder cancer. J. Urol, **154**: 379-384, 1995.

7) **D'Hallewin, M.A.** and **Baert, L.**: Initial evaluation of the bladder tumor antigen test in superficial bladder cancer. J. Urol, **155**: 475-476, 1996.

8) **Miyanaga, N., Akaza, H., Kameyama, S., Hachiya, T., Ozono, S., Kuroda, M., Koga, H.** and **Koiso, K.**: Significance of the BTA test in bladder cancer. A multicenter trial. Int. J. Urol, **4**: 557-560, 1997.

9) **Ramakumar, S., Bhuiyan, J., Besse, J. A., Roberts, S. G., Wollan, P. C., Blute, M. L.** and **O'kane, D. J.**: Comparison of screening methods in the detection of bladder cancer. J. Urol, **161**: 388–394, 1999.

10) **Fukui, Y., Samma, S., Fujimoto, K., Akiyama, T., Yamaguchi, A.** and **Hirayama, A.**: Screening methods in the detection of bladder cancer: comparison of nuclear matrix protein–22, bladder tumor antigen and cytological examinations. Hinyokika Kiyo **47**: 311–314, 2001. (in Japanese)

11) **Poulakis, V., Witzsch, U., Vries, R., Altmannsberger, H. M., Manyak, M. J.** and **Becht, E.**: A comparison of nuclear matrix protein–22 and bladder tumour antigen tests with voided urinary cytology in detecting and following bladder cancer: the prognostic value of false–positive results. BJU Int, **88**: 692–701, 2001.

12) **Gutierrez Banos, J. L., Rebollo Rodrigo, M. H., Antolin Juarez, F. M.** and **Martin Garcia, B.**: NMP 22, BTA stat test and cytology in the diagnosis of bladder cancer: a comparative study. Urol. Int, **66**: 185–190, 2001.

13) **Miller, T. E., Beausang, L. A., Winchell, L. F.** and **Lidgard, G. P.**: Detection of nuclear matrix protein in serum from cancer patients. Cancer Res. **52**: 422–427, 1992.

14) **Chen, Y–T., Hayden, C. L., Marchand, K. J.** and **Makuch, R. W.**: Comparison of urine collection methods for evaluating urinary nuclear matrix protein, NMP22, as a tumor marker. J. Urol, 158: 1899–1901, 1997.

15) **Atsu, N., Ekici, S., Oge, O., Ergen, A., Hascelik, G.** and **Ozen, H.**: False–positive results of the NMP22 test due to hematuria. J. Urol, **167**: 555–558, 2002.