Diagnostic Value of the Nuclear Matrix Protein 22 Test and Urine Cytology in Upper Tract Urothelial Tumors

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Key Words

Nuclear matrix protein 22 · Cytology · Upper urothelial tumor

Abstract

Objective: To investigate the diagnostic value of the nuclear matrix protein 22 (NMP22) test in comparison to urine cytology for the detection of upper tract urothelial carcinoma.

Patients and Methods: Patients with transitional cell carcinoma of the upper urinary tract (n = 34) and patients with renal calculosis (n = 25) were included in this study. Voided urine samples and separated catheter urine specimens were assayed for NMP22 and cytological examination. **Results:** The sensitivity of the NMP22 test in separated and voided urine was 73.2 and 70.5%, respectively, compared to 64.7 and 58.8% of urine cytology. The specificity of the NMP22 test in separated and voided urine was 88 and 92%, respectively, compared to 96 and 96% of urine cytology. The combination of separated and voided urine is the best method because the sensitivity is 79.41% and specificity 88%. There is a high agreement of the NMP22 test in voided and separated urine ($\kappa = 0.795$, $p < 0.01$), indicating that the voided urine is adequate for diagnosis. **Conclusions:** The NMP22 test has higher sensitivity but lower specificity than cytology. The combination of these two tests could be a very useful diagnostic method for detection of upper urothelial tumors.

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Introduction

The nuclear matrix protein 22 (NMP22) test is a non-invasive method for the detection of protein level of nucleus mitotic apparatus (nuclear mitotic apparatus protein, NuMA) in urine sample. Healthy people usually have a very small amount of NMP in their urine, while it is specific to the transitional epithelial cells in the urinary tract. Transitional cell carcinoma (TCC) cells contain up to 80 times higher concentration of NMP than normal cells, and it is released in the urine after cell death. It has been reported that patients with TCC of the bladder have a higher concentration of NMP22 in the urine compared to healthy controls. The studies show test specificity of over 90% with the sensitivity values even up to 90% for T2 stage of bladder carcinoma, whereas combining the NMP22 test with cystoscopic examination increases the overall rate of bladder cancer detection up to 96% [1–4]. Based on the previous studies, the result of the NMP22 tests with protein level above 10 U/ml in urine is associated with high probability of the presence of TCC. Bladder check NMP22 test is a rapid immunochromatography analysis that is designed to identify urine samples with NMP22 concentration greater than 10 U/ml. Since the same epithelial cells line the bladder and the upper urinary tract (UUT), it could be hypothesized that the increased concentration of the NMP occurs in the urine of patients with upper tract urothelial carcinoma. There-

fore, the diagnostic and prognostic value of the NMP22 test in UUT carcinoma remains to be assessed.

The purpose of this trial was to evaluate the diagnostic value of the NMP22 test in separated and voided urine of patients with diagnosed UUT carcinoma and to compare the NMP22 test with the diagnostic value of urine cytology. Also, the association of the NMP22 test with clinical and pathohistological characteristics of UUT carcinoma was analyzed.

Patients and Methods

Study Patients

The study included 34 patients with upper tract urothelial carcinoma in various disease stages treated at the Urology Clinic of the Clinical Center of Serbia from 2005 to 2009. All patients underwent complete diagnostic procedures which included laboratory analyses, ultrasound examination, X-ray, IVP, retrograde pyelography, ureterorenoscopy and CT if needed to establish extracapsular extensions. Two samples of urine were taken from each patient, the first total voided urine and the other separated urine obtained by ureteral catheterization of the diseased UUTs. Both samples were assayed by the NMP22 test and urine cytology. The control group consisted of 25 patients with the diagnosis of renal calculus who were planned to be treated by extracorporeal shock wave lithotripsy. Controls have been examined by ultrasonography, X-ray and IVP to exclude the presence of malignant disease. Urine samples from the control subjects were collected and analyzed in the same way as the samples from TCC patients.

Statistical Analyses

All computations were performed with the Statistical Package for Social Sciences release 12.0. Sensitivity was defined as the number of true positive UUT cancer cases classified as positive by a test. Specificity was defined as the number of true negative cases classified as negative by a test. The positive predictive value of a test was defined as the probability that the patient had UUT cancer, given the test was positive. The negative predictive value of a test was defined as the probability that the patient was free of UUT cancer, given the test was negative. The kappa test was used for agreement.

Results

Patient Characteristics

The average age of 34 patients with UUT carcinoma (22 patients with pyelon and/or calix tumor and 12 patients with tumors of the ureter) was 63.4 ± 6.2 years, while the average age of the control group of 25 patients (17 with pyelon calculus and 8 with calix calculus) was 45.3 ± 7.8 years. Out of 34 patients with tumors of the UUT, 12 patients (36%) were females. The control group consisted of 14 (63%) women and 8 (27%) men.

Table 1. The NMP22 test in relation to the diagnosis of UUT carcinoma

Diagnostic test		UUT tumor	
		no	yes
NMP22 in separated urine	–	22	9
	+	3	25
NMP22 in voided urine	–	23	10
	+	2	24
Cytology, separated urine	–	24	12
	+	1	22
Cytology, voided urine	–	24	14
	+	1	20

Table 2. Overall sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV)

Diagnostic test	Sn, %	Sp, %	PPV, %	NPV, %
NMP22, separated urine	73.5	88	89.2	70.9
NMP22, voided urine	70.5	92	92.3	69.6
Cytology, separated urine	64.7	96	95.6	66.6
Cytology, voided urine	58.8	96	95.2	63.1
Combination separated urine ¹	79.4	88	90	75.8
Combination voided urine ¹	79.4	88	90	75.8

¹ NMP22 or cytology positive.

The NMP22 Test and Urine Cytology in Relation to the Diagnosis of UUT Tumor

Distribution of patients with UUT carcinoma and control subjects in relation to the positive or negative NMP22 test results and cytology in separated and voided urine is presented in table 1.

Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of the Urine NMP22 Test and Cytology in Separated and Voided Urine in Detecting UUT Carcinoma

Analyzing the sensitivity of the studied methods, it was found that the highest sensitivity of NMP22 is in the separated urine, followed by voided urine. The sensitivity of urine cytological analyses was lower. However, the specificity of cytology was higher compared to NMP22. The combination of NMP22 and cytology (one or both positive tests) has the highest sensitivity but lower specificity than cytology alone. The results are shown in table 2.

Table 3. Stage and grade of UUT carcinoma in relation to the NMP22 test and urine cytology

UUT tumor	NMP22 separated urine		NMP22 voided urine		Cytology separated urine		Cytology voided urine	
	–	+	–	+	–	+	–	+
Stage								
Ta (n = 8)	5	3	5	3	6	2	7	1
T1 (n = 8)	2	6	2	6	3	5	4	4
T2 (n = 4)	1	3	1	3	2	2	2	2
T3 (n = 13)	1	12	2	11	1	12	1	12
T4 (n = 1)	0	1	0	1	0	1	0	1
Grade								
GI (n = 7)	5	2	5	2	6	1	6	1
GII (n = 15)	3	12	4	11	4	11	6	9
GIII (n = 12)	1	11	1	11	2	10	2	10

Table 4. UUT tumor risk in relation to NMP22 and cytology

Risk	NMP22 separated urine		NMP22 voided urine		Cytology separated urine		Cytology voided urine	
	–	+	–	+	–	+	–	+
Low (n = 8)	5	3	5	3	6	2	7	1
Medium (n = 8)	2	6	2	6	3	5	4	4
High (n = 18)	2	16	3	15	3	15	3	15

Comparison of Patients with UUT Tumors in Relation to Tumor Stage, Grade and Risk

Only the patients who had UUT tumors were taken into further consideration and analyses. They were divided according to the disease stage and grade.

Table 3 shows the distribution of patients in relation to the disease stage according to the result of the NMP22 test and cytology in separated and voided urine.

The ability of the NMP22 test to detect tumors in the lower stages of disease (Ta and T1) was greater; NMP22 was better at detecting T2 stage tumors, while for T3 and T4 stages the results were almost identical. The same analysis was performed for the grade of the disease. NMP22 was better at detecting grade I tumors. It detected grade II tumors with the best accuracy in separated urine, and it was more accurate than cytology at detecting grade III tumors. The patients were divided into three groups according to the degree of risk: those with low risk (Ta GI/GII), medium risk (TaGIII, T1GI/GII) and high

risk (T2, T3, T4). The distribution of patients according to the risk in relation to the results of NMP22 and cytology in the separated and voided urine is shown in table 4.

Concerning patients with low and medium risk, tumor detection was better with NMP22 (in separated and voided urine), while with the high risk patients, tumor detection was almost identical in all four methods.

Analysis of the NMP22 Test and Cytology Agreement in Separated and Voided Urine

The kappa test for agreement was used to compare these four methods. The agreement between NMP22 results for voided and separated urine was kappa = 0.795 ($p < 0.01$). The agreement between cytology results for voided and separated urine was kappa = 0.710 ($p < 0.01$), and the agreement between cytology results for separated urine and NMP22 results for voided urine was kappa = 0.756 ($p < 0.01$). The agreement between NMP22 results for voided urine and cytology results for separated urine was kappa = 0.544 ($p < 0.01$).

These results indicate that the agreement is higher with the NMP22 test for voided and separated urine as well as cytology for separated and voided urine, while the agreement between NMP22 and cytology is high when it comes to NMP22 and voided urine and cytology and separated urine. The agreement between NMP22 results for voided and cytology results for separated urine was the poorest.

Discussion

The general characteristics of the test group fully corresponded to test group characteristics described in other reports [5, 6]. The resulting sensitivity and specificity of the NMP22 test in separated and voided urine is slightly lower compared to the results published so far in the literature, which are about 88 and 96% [7–9]. The sensitivity of cytological methods was considerably lower than that of NMP22, but the specificity of the cytological methods, whether in voided or separated urine, was higher than with NMP22, which corresponds to the literature findings – sensitivity of NMP22 being 65–78%, cytology 30–54%, and specificity of NMP22 being 40–70% and cytology 78–99% [10, 11].

Direct correlation between T stage tumors and the results of the NMP22 test and urine cytology has been proven in many studies [7, 12, 13]. In our study, NMP22 has proven to be a better marker than cytology when it comes to lower tumor stages. Regarding the higher stages (T3 and T4), cytology and NMP22 show almost identical results.

Depending on the pathohistological grade of the tumor, the NMP22 test has proven to be superior to cytology not only for the separated but also for the voided urine. In all grades, NMP22 has shown greater number of positive results than cytology [14, 15]. Looking at the results obtained from the three groups of patients divided according to the level of risk, it is obvious that the most frequent positive results of both the NMP22 test and cytology occur in patients from the high risk study group. NMP22 has proved to be more accurate than cytology in detecting low and medium risk tumors. The connection between tumor stage and grade and the level of risk the patient has and the results of the NMP22 test and cytology is direct and clearly detectable. The connection can be explained by the fact that the higher the tumor grade and stage, the more atypical the tumor cells, and thus the cells are more easily detected by cytology. In other words, the level of proteins in urine detectable by the NMP22 test is higher, so the values of sensitivity range from 15% with low grade to almost 100% with high grade, i.e. from 17 to 97% depending on the T stage of the disease [1, 12]. Analysis of agreement showed that the result of NMP22 in separated urine corresponds well with that in voided urine. The same goes for cytological analyses. Given that the examination of any of the two urine samples has almost identical diagnostic accuracy, it allows exemption of patients from invasive sam-

pling of urine by catheterization through the bladder and marks the NMP22 test as a quick and simple test for detecting upper urothelium tumors, as well as for monitoring patients treated with conservative surgical methods. The results show that the combination of the NMP22 test and cytology provides higher sensitivity and specificity than when only one of the two tests is done, which is especially important in low grade surface tumors, and also reduces the frequency of endoscopic examinations [16, 17].

In conclusion, the NMP22 test has greater sensitivity but lower specificity than urine cytology. In the lower tumor stages, NMP22 is a better detector of the presence of tumors, while in the higher stages it is almost identical to cytology. The combination of NMP22 and cytology has the best ratio of sensitivity and specificity, indicating that this combination is useful when it comes to screening and upper urothelial tumor follow-up. Good result agreement was achieved by the NMP22 test in separated and voided urine, indicating that a noninvasive urine sampling technique can provide us with almost identical results in the upper urothelium tumor diagnosis. Results obtained in this study point out that ureterorenoscopy can be replaced with the NMP22 test during patient follow-up or in the primary diagnostics, especially the screening of regions with endemic nephropathy.

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