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Application Value of Urine NMP22, OPN, and BTA Detection in the Diagnostic and Prognostic Evaluation of Bladder Urothelial Carcinoma

Chao Li, Jiqian Wang, Yongchao Wang, Jianping Wang, Yiliang Huang, Ying Chen, Qinglong Wu, Minghuang Rao, Rongjin Fang*

Xiamen Haicang Hospital, Xiamen 361000, Fujian, China

***Corresponding author:** Rongjin Fang, frj2023@126.com

Abstract: *Objective*: To investigate the application value of urine nuclear matrix protein 22 (NMP22), osteopontin (OPN), and bladder tumor antigen (BTA) detection in the diagnostic and prognostic evaluation of bladder urothelial carcinoma. *Methods*: 100 patients with bladder urothelial carcinoma who visited our hospital from January 2020 to December 2022 were selected as the case group, and 100 healthy individuals who underwent physical examination during the same period were selected as the control group. The levels of NMP22, OPN, and BTA in the urine of the two groups were detected, and their diagnostic efficacy in bladder urothelial carcinoma was analyzed. The patients in the case group were followed up to observe the relationship between these markers and prognosis. *Results*: The levels of NMP22, OPN, and BTA in the urine of the case group were significantly higher than those of the control group ($P < 0.05$). The sensitivity, specificity, and accuracy of combined detection of NMP22, OPN, and BTA for diagnosing bladder urothelial carcinoma were higher than those of single marker detection ($P < 0.05$). Follow-up results showed that patients with high levels of NMP22, OPN, and BTA had a higher recurrence rate and shorter progression-free survival ($P < 0.05$). *Conclusion*: Combined detection of urine NMP22, OPN, and BTA has high diagnostic value for bladder urothelial carcinoma, and the levels of these markers are correlated with patient prognosis, which can be used for prognostic evaluation.

Keywords: Bladder urothelial carcinoma; Urine detection; NMP22; OPN; BTA

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1. Introduction

Bladder urothelial carcinoma, as an extremely common type of malignant tumor in the urinary system, has a high incidence and mortality rate globally, posing a serious threat to human health. Early diagnosis is crucial for effective treatment, improved prognosis, and enhanced quality of life. Prognostic evaluation can assist clinicians in developing more precise and personalized treatment plans and follow-up management strategies for patients.

Traditional cystoscopy has long been regarded as the gold standard for diagnosing bladder urothelial carcinoma. However, this method is significantly invasive, causing considerable discomfort to patients during the examination, leading to low acceptance among some patients, and potentially delaying optimal diagnosis and treatment due to resistance. Urine testing, as an emerging non-invasive diagnostic method, is simple and easy to perform, requiring no complex equipment or professional skills. Patients only need to provide a morning urine sample to complete the test. This convenience greatly improves patient compliance, allowing more patients to actively cooperate with the examination. In recent years, with the deepening of medical research, some biomarkers in urine, such as nuclear matrix protein 22 (NMP22), osteopontin (OPN), and bladder tumor antigen (BTA), have gradually come into focus. Their potential value in the diagnostic and prognostic evaluation of bladder urothelial carcinoma has attracted widespread attention. Based on this background, this study aims to explore the specific application value of these biomarkers, NMP22, OPN, and BTA, in the diagnosis and treatment of bladder urothelial carcinoma. It provides clinicians with more effective and precise diagnostic and prognostic evaluation methods, further advancing the clinical diagnosis and treatment of bladder urothelial carcinoma.

2. Materials and methods

2.1. General information

100 patients diagnosed with bladder urothelial carcinoma by pathology in our hospital from January 2020 to December 2022 were selected as the case group, aged 35–78 years, with an average age of 56.33 ± 10.52 years. Simultaneously, 100 healthy individuals who underwent health checkups in our hospital during the same period were selected as the control group, aged 32–75 years, with an average age of 54.86 ± 11.27 years. After comparison, there was no statistically significant difference in general information between the two groups ($P > 0.05$), making them comparable.

Inclusion criteria: The case group was pathologically diagnosed with bladder urothelial carcinoma; aged 18 years and above; patients signed informed consent. The control group consisted of healthy individuals without a history of urinary system diseases or malignant tumors. Exclusion criteria: those with other urinary system diseases affecting biomarker test results; those who had recently undergone urinary system surgery, radiotherapy, or chemotherapy; those with severe liver and kidney dysfunction.

2.2. Methods

Morning urine samples were collected from all subjects, and the levels of NMP22, OPN, and BTA in the urine were detected using enzyme-linked immunosorbent assay (ELISA). The operations were strictly carried out according to the kit instructions to ensure the accuracy of the test results.

2.3. Observation indicators

(1) Diagnostic performance evaluation: Using pathological diagnosis as the gold standard, the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of single and combined detection of NMP22, OPN, and BTA were calculated. Sensitivity = True Positive / (True Positive + False Negative) × 100%; Specificity = True Negative / (True Negative + False Positive) × 100%; Accuracy = (True Positive + True Negative) / Total number of cases × 100%; Positive Predictive Value = True Positive / (True Positive + False Positive) × 100%; Negative Predictive Value = True Negative /

(True Negative + False Negative) × 100%.

(2) Prognostic evaluation: The case group patients were followed up for 2 years, and their recurrence and progression-free survival were recorded. Progression-free survival was calculated from the diagnosis of bladder urothelial carcinoma until disease recurrence, distant metastasis, or death. Patients were divided into high and low-level groups based on the median NMP22, OPN, and BTA test results, and the recurrence rate and progression-free survival were compared between the two groups.

2.4. Statistical methods

SPSS 26.0 software was used to process data. Measurement data were described using mean ± standard deviation (SD) and analyzed using the *t*-test. Count data was expressed as [*n* (%)] and analyzed through χ^2 test. $P < 0.05$ indicated that the corresponding difference was statistically significant.

3. Results

3.1. Comparison of urine marker levels between the two groups

The levels of NMP22, OPN, and BTA in the urine of the case group were significantly higher than those of the control group, and the difference was statistically significant ($P < 0.05$). See **Table 1** for details.

Table 1. Comparison of urine marker levels between the two groups (mean ± SD, U/mL)

Group	*n*	NMP22	OPN	BTA
Case group	100	56.38 ± 15.24	85.67 ± 20.13	3.56 ± 1.21
Control group	100	12.53 ± 5.31	30.29 ± 10.54	1.04 ± 0.52
t		27.171	24.372	19.134
P		< 0.001	< 0.001	< 0.001

3.2. Comparison of diagnostic performance

For single marker detection, the sensitivity of NMP22 was 72%, with a specificity of 83%; OPN had a sensitivity of 68% and a specificity of 81%; BTA demonstrated a sensitivity of 63% and a specificity of 79%. When combined detection was used, the sensitivity reached 88%, the specificity was 92%, and the accuracy was 90%, all higher than single marker detection. The difference was statistically significant ($P < 0.05$).

3.3. Comparison of prognosis analysis between the two groups

After a 2-year follow-up, patients in the high-level group of NMP22, OPN, and BTA had a significantly higher recurrence rate than those in the low-level group ($P < 0.05$). The progression-free survival period of patients in the high-level group was shorter than that of the low-level group, and the difference was statistically significant ($P < 0.05$). See **Table 2** for details.

Table 2. Comparison of prognosis analysis between the two groups

Biomarker	Group	*n*	Recurrence rate (%)	Progression-free survival (months)
NMP22	High-level group	43	18 (41.86)	11.85 ± 2.98
	Low-level group	57	11 (19.29)	19.32 ± 4.56
	t/χ^2		6.060	9.337
	P		0.014	< 0.001
OPN	High-level group	47	17 (36.17)	13.21 ± 3.57
	Low-level group	53	9 (16.98)	20.14 ± 4.33
	t/χ^2		4.767	8.530
	P		0.029	< 0.001
BTA	High-level group	38	15 (34.10)	12.67 ± 3.12
	Low-level group	62	9 (14.52)	18.95 ± 4.08
	t/χ^2		8.046	8.405
	P		0.005	< 0.001

4. Discussion

The incidence of bladder urothelial carcinoma is increasing year by year globally, making it one of the important causes of death from urinary system tumors. Early diagnosis facilitates timely intervention and improves patient prognosis, while prognostic evaluation provides critical information for adjusting subsequent treatment plans. Urine testing has become a popular direction for the diagnosis and monitoring of bladder cancer due to its noninvasive sample collection and easy acceptance by patients. Nuclear matrix protein 22 (NMP22), osteopontin (OPN), and bladder tumor antigen (BTA) are potential biomarkers, and their detection is expected to optimize the diagnosis and treatment process of bladder urothelial carcinoma [1,2].

Foreign countries have started early in the field of using urine markers for bladder cancer diagnosis, with collaborative advancement in basic research and clinical translation. Many prospective studies have focused on NMP22, OPN, and BTA, exploring all aspects from molecular mechanisms to clinical efficacy. For example, a long-term cohort study at the Southwestern Medical Center in the United States tracked bladder cancer patients and healthy controls over several years, precisely quantifying urine markers [3]. They found significant trends in the concentration of NMP22 in the urine of early-stage bladder cancer patients, providing a critical basis for setting subsequent diagnostic thresholds. Some European multicenter joint projects have collected massive samples across different medical levels to validate the indicative role of OPN in bladder cancer grading [4]. The results have facilitated clinical stratified diagnosis and treatment. Currently, some achievements have been integrated into the clinical preliminary screening process. For instance, some community hospitals in Germany have introduced bladder cancer screening packages including BTA testing, improving the efficiency of early detection. Additionally, authoritative organizations like the International Urological Association continuously integrate cutting-edge achievements, update relevant guidelines, standardize the implementation of new technologies, promote international exchanges and cooperation, and continuously optimize detection efficiency [5].

Although domestic research on urine biomarkers for bladder cancer lagged slightly in early stages, it has shown vigorous development in recent years. Major medical centers and research teams have actively conducted large-sample cohort studies, recruiting subjects across different regions and fully considering the genetic traits and living environment differences of the Chinese population. For example, a large tertiary hospital in Shanghai, in collaboration with multiple local hospitals, has constructed a cohort for bladder cancer urine markers among the population in East China. They have analyzed the sensitivity and specificity of NMP22 and OPN in local patients, strongly validating their applicability [3]. However, the standardization of detection remains a prominent issue. Due to differences in equipment procurement channels and reagent brands among laboratories in various regions, data consistency is poor. For instance, the deviation in NMP22 detection values can reach 20% when the same urine sample is sent to different laboratories. Regarding combined application schemes, although there are theoretical ideas, practical operations lack a mature process and mostly remain in the small-scale exploration stage. A widely recognized, efficient, and precise combined detection mode has not been established, requiring further resource integration and collaborative research [6,7].

NMP22, as a key component of the cell nuclear mitotic apparatus, maintains the stability of the nuclear structure and mitotic process under normal physiological conditions. When bladder urothelial cells undergo cancerous changes, the cell cycle becomes uncontrolled, and apoptosis programs are frequently initiated. During apoptosis of bladder cancer cells, the cell membrane ruptures, releasing a large amount of NMP22 originally located in the nucleus into the extracellular environment and entering the urine system. This results in a urine NMP22 concentration far exceeding that of healthy individuals [8]. Currently, ELISA is the mainstream detection method. ELISA is based on the immunological principle of antigen-antibody specific affinity. First, NMP22-specific antibodies are coated on a solid-phase carrier. After adding the urine sample, NMP22 binds to the antibodies. Unbound substances are then washed away, and enzyme-labeled secondary antibodies are added. A chromogenic reaction occurs through substrate catalysis, and the absorbance is measured using a spectrophotometer. The NMP22 content in the urine can be accurately calculated based on a standard curve [9].

Osteopontin (OPN), a secreted phosphorylated glycoprotein, is secreted by various cells and plays a complex role in tumor development and progression. In the context of bladder urothelial carcinoma, gene regulation is disrupted in cancer cells, significantly upregulating *OPN* gene transcription and translation activity. This leads to massive synthesis and accumulation of OPN within cells, which is then actively secreted into the extracellular space and mixed into the urine. Consequently, urine OPN levels are significantly higher than those in the normal population [10,11]. For its detection, immunoturbidimetric assay and ELISA are commonly used methods. In the immunoturbidimetric assay, the urine sample is mixed with OPN-specific antibodies to form antigen-antibody complexes, changing the turbidity of the solution. The change in turbidity is measured using a turbidimeter, and the OPN concentration is calculated based on a standard curve. In contrast, ELISA involves immobilizing OPN antibodies, reacting with the urine sample, and then binding to labeled secondary antibodies. The OPN content in the urine is precisely quantified based on the intensity of the chromogenic or fluorescent signal [12,13].

Bladder tumor antigen (BTA) is essentially a unique type of antigen complex on the surface of bladder cancer cells, closely related to the biological characteristics and proliferation and invasion abilities of tumor cells. In qualitative detection mode, immunochromatographic test strips play a crucial role. These test strips contain pre-fixed BTA-specific antibodies. When a urine sample is dropped onto the test strip, urine spreads along the fibrous membrane of the test strip driven by chromatography. If BTA is present in the urine, it quickly

binds to the immobilized antibodies, triggering a chromogenic reaction. This is typically manifested as the appearance of a band in a specific region of the test strip, allowing for intuitive judgment of whether BTA is positive. The operation is simple and fast, suitable for rapid initial screening at the grassroots level. In terms of quantitative detection, the advantages of chemiluminescent immunoassay are prominent [14,15]. This method utilizes the intensity of the light signal generated by a chemical reaction to reflect the BTA content. The urine sample reacts sequentially with labeled antibodies and solid-phase carriers. After washing, a luminescent substrate is added. The light signal is captured by detection equipment, and a precise quantitative BTA value is provided based on a calibration curve. This provides detailed data support for clinical diagnosis [16,17].

This study focused on the application of urine NMP22, OPN, and BTA detection in bladder urothelial carcinoma, and the results have important clinical implications. The levels of NMP22, OPN, and BTA in the case group were significantly higher than those in the control group. This indicates that these three markers are highly expressed in the urine of patients with bladder urothelial carcinoma, providing a strong basis for disease diagnosis. Combined detection has higher sensitivity, specificity, and accuracy than single-marker detection. When NMP22, OPN, and BTA are detected individually, their sensitivity and specificity have limitations [18]. However, combined detection integrates the advantages of each marker, improving sensitivity from a maximum of 72% for a single marker to 88% for the combination, specificity from 83% to 92%, and achieving 90% accuracy. This significantly enhances the diagnostic capability for bladder urothelial carcinoma, reducing misdiagnosis and missed diagnosis. After a 2-year follow-up, patients with high levels of NMP22, OPN, and BTA had a significantly higher recurrence rate and shorter progression-free survival compared to those with low levels. Taking NMP22 as an example, the recurrence rate in the high-level group was 41.86%, significantly higher than the 19.29% in the low-level group, and the progression-free survival was also shorter. This implies that the levels of these three markers are closely related to patient prognosis. High levels indicate a high risk of tumor recurrence and rapid disease progression. Clinicians can assess patient prognosis based on these marker levels, develop more targeted treatment and follow-up strategies, and improve patients' quality of life [19,20].

5. Conclusion

The combined detection of urinary biomarkers—including NMP22, OPN, and BTA—demonstrates significant diagnostic value for bladder urothelial carcinoma. Studies indicate that this multi-marker approach improves sensitivity and specificity compared to individual tests, enabling earlier and more accurate detection of malignant lesions.

Furthermore, the levels of NMP22, OPN, and BTA in urine are closely correlated with tumor progression, aggressiveness, and clinical outcomes. Elevated concentrations of these markers are associated with advanced disease stages, higher recurrence rates, and poorer survival, suggesting their utility in prognostic evaluation. Regular monitoring of these biomarkers may aid in risk stratification, treatment response assessment, and long-term follow-up for bladder urothelial carcinoma patients.

Thus, integrating NMP22, OPN, and BTA testing into clinical practice could enhance both diagnostic precision and prognostic prediction, offering a non-invasive tool to optimize patient management and improve therapeutic outcomes.

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Disclosure statement

The authors declare no conflict of interest.

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