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Evaluation of the Application Value of Combined Detection of BTA, BTA stat, NMP22, and Survivin in the Diagnosis of Urothelial Carcinoma

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Abstract: *Objective:* To explore the diagnostic value of combined detection of bladder tumor antigen (BTA), bladder tumor antigen-associated test (BTA stat), nuclear matrix protein 22 (NMP22), and survivin in urothelial carcinoma. *Methods:* Sixty patients with urothelial carcinoma admitted from January 2024 to January 2025 were selected as the observation group for this study, and 60 healthy individuals were selected as the control group. BTA, BTA stat, NMP22, and survivin tests were performed on both groups, respectively. The test results were analyzed to evaluate the diagnostic value of combined detection. *Results:* The levels of BTA, BTA stat, NMP22, and survivin in the observation group were higher than those in the control group ($P < 0.05$). The specificity, sensitivity, and accuracy of combined detection of BTA, BTA stat, NMP22, and survivin were higher than those of single detection methods ($P < 0.05$). There were significant differences in the positive rates of BTA, BTA stat, NMP22, and survivin among patients with different tumor diameters, tumor numbers, pathological grades, clinical stages, and lymph node metastasis status ($P < 0.05$). *Conclusion:* In the diagnosis of urothelial carcinoma, the combined detection of BTA, BTA stat, NMP22, and survivin has high diagnostic value and can be promoted and applied in clinical diagnosis.

Keywords: BTA; BTA stat; NMP22; Survivin; Urothelial carcinoma

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1. Introduction

Bladder urothelial carcinoma, as a malignant tumor, is relatively common in the urinary system. Early symptomatic treatment can timely inhibit tumor progression, reduce patient mortality, and effectively extend the patient's life cycle. Currently, there is no clear conclusion on the specific pathogenesis of urothelial carcinoma, and it is generally believed to be closely related to the genomic stability of the bladder mucosal epithelium. Cystoscopy is the gold standard for diagnosing urothelial carcinoma, which can provide patients with accurate diagnosis

results. However, this method is an invasive examination that may induce urinary tract infections during testing, and some patients have relatively low acceptance, which limits its clinical application. Although urine exfoliative cytology does not cause trauma to patients during the examination, its sensitivity is related to tumor grading. If the patient has a poorly differentiated tumor, the diagnostic sensitivity is relatively poor, and it is easily affected by other lesions, resulting in certain interference with the final diagnosis. Therefore, a more scientific diagnostic method is needed to provide timely and effective treatment for patients. Bladder tumor antigen (BTA), bladder tumor antigen-associated test (BTA stat), nuclear matrix protein 22 (NMP22), and survivin are all tumor markers for diagnosing urothelial carcinoma, and there are relatively few clinical reports on the effect of their combined detection. Based on this, this article mainly explores the application value of combined detection of BTA, BTA stat, NMP22, and survivin in the diagnosis of urothelial carcinoma.

2. Materials and methods

2.1. General information

60 cases of urothelial carcinoma treated from January 2024 to January 2025 were selected for the study, and 60 healthy individuals who underwent physical examination during the same period were selected as controls. The patients and healthy individuals were assigned to the observation group and the control group, respectively. The control group had a male-to-female ratio of 34:26, with ages ranging from 38 to 76 years old, and an average age of 57.28 ± 7.31 years old. The observation group had a male-to-female ratio of 36:24, with ages ranging from 39 to 75 years old, and an average age of 57.26 ± 7.29 years old. There was no significant difference in general information between the two groups ($P > 0.05$). Inclusion criteria: (1) The observation group was diagnosed with urothelial carcinoma by pathological examination; (2) None of them had immune system diseases or blood system diseases; (3) The clinical data of the two groups were recorded in detail and preserved intact; (4) The key points of this experiment were fully informed to the enrolled personnel, and informed consent forms were signed. Exclusion criteria: (1) History of other tumors; (2) Acute cardio-cerebrovascular diseases; (3) No urine samples were collected for examination; (4) Those who withdrew due to personal factors and had missing data.

2.2. Methods

After enrollment, 100 ml of the first clean urine in the morning was collected from the subjects. The urine was centrifuged at 1000 r/min for 15 minutes, and the supernatant was taken for testing. BTA detection: The level of BTA in urine was detected by enzyme-linked immunosorbent assay. The detection instrument was an automatic multi-functional microplate reader [1-3], and the detection operation needed to be carried out step by step according to the requirements of the kit instructions. BTA stat detection: The immune chromatographic assay kit was used for detection. Five drops of fresh urine were dropped into the BTA stat test paper sample addition hole using a sterile dropper. When the urine flowed through the detection area, if there was a bladder tumor-related antigen in the urine sample, it would promote the formation of antigen conjugates. After 5 minutes, a red band appeared in the test area, which was positive. If no red band appeared, it was negative. If this red band was not observed in the control area, it meant that the rapid kit was faulty. NMP22 detection: Enzyme-linked immunosorbent assay was used for detection, using anti-NMP22-digoxin conjugate and digoxin-horseradish peroxidase display system, enzyme-linked 490 nm wavelength measurement and calculation, and drawing a straight-line regression graph

to calculate the antigen concentration of the urine sample. Survivin detection: Detected by streptomycin avidin-peroxidase method, subject to the requirements of the instruction manual. Survivin was selected as a ready-to-use rabbit anti-human polyclonal antibody. The sections were dewaxed, hydrated, and soaked in 3% hydrogen peroxide-microwave-methanol at room temperature for 10 minutes to inactivate hydrogen peroxide enzyme activity. Microwave antigen was continuously repaired for 20 minutes, with a pH of 7.6, and EDTA antigen repair solution was used as the repair solution. After being cooled to room temperature, the primary antibody was added and incubated in a wet box at 4°C overnight. On the second day, the samples were washed three times with TBS, followed by the addition of a biotin-labeled secondary antibody and incubation at room temperature for 10 minutes. Horseradish peroxidase-labeled streptavidin was then added and incubated at room temperature for another 10 minutes. DAB color development was performed for 2 minutes, followed by hematoxylin counterstaining for 2 minutes. The samples were then dehydrated, rendered transparent, sealed, and observed under a microscope. To avoid affecting the staining quality, each section should use positive tissue as a positive control, and use phosphate buffered saline instead of the primary antibody as a negative control. The test results were reviewed by two pathologists with rich experience in reading films using a double-blind method. If there was a disagreement, the results would be discussed and negotiated. The final result was that the cell nucleus or cytoplasm appeared brown, which could be judged as positive.

2.3. Observation indicators

The levels of BTA, BTA stat, NMP22, and survivin were compared between the two groups to analyze the diagnostic value of single and combined detection of BTA, BTA stat, NMP22, and survivin for urothelial carcinoma. The positive rates of BTA, BTA stat, NMP22, and survivin in patients with different clinical features were also compared.

2.4. Statistical methods

SPSS 26.0 was used to process the research data, and *t*-test and chi-square test were used to measure the data (mean ± standard deviation [SD]) and count data (%). When the research result was $P < 0.05$, it indicated that the research was statistically significant.

3. Results

3.1. Comparison of BTA, BTA stat, NMP22, and survivin levels between the two groups

There were significant differences in the levels of BTA, BTA stat, NMP22, and survivin between the two groups ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of BTA, BTA stat, NMP22, and survivin between the two groups (mean ± SD)

Group	BTA (ng·mL^{-1})	BTA stat (ng·mL^{-1})	NMP22 (U·mL^{-1})	Survivin (%)
Observation group (n = 60)	23.16 ± 8.81	26.37 ± 5.53	5.02 ± 1.78	22.32 ± 2.39
Control group (n = 60)	9.82 ± 5.26	18.21 ± 4.46	2.16 ± 0.91	13.58 ± 1.25
t value	10.070	8.897	11.082	25.101
P value	< 0.001	< 0.001	< 0.001	< 0.001

3.2. Comparison of diagnostic effectiveness of BTA, BTA stat, NMP22, and survivin for urothelial carcinoma

Significant differences ($P < 0.05$) were observed in sensitivity, specificity, and accuracy between combined detection and single detection. See **Table 2.**

Table 2. Diagnostic effectiveness of BTA, BTA stat, NMP22, survivin, and combined detection for urothelial carcinoma [*n* (%)]

Group	Sensitivity	Specificity	Accuracy
BTA	83.33% (50/60)	78.33% (47/60)	81.67% (49/60)
BTA stat	85.00% (51/60)	76.67% (46/60)	80.00% (48/60)
NMP22	86.67% (52/60)	80.00% (48/60)	83.33% (50/60)
Survivin	86.67% (52/60)	81.67% (49/60)	85.00% (51/60)
Combined detection	98.33% (59/60)	95.00% (57/60)	98.33%(59/60)
χ^2 value $_1$	0.063/0.261/0.261/8.107	0.048/0.051/0.208/7.212	0.054/0.058/0.240/9.259
P value $_1$	0.803/0.609/0.609/0.004	0.827/0.822/0.648/0.007	0.817/0.810/0.624/0.002
χ^2 value $_2$	0.069/0.069/6.982	0.196/0.455/8.292	0.223/0.520/10.439
P value $_2$	0.794/0.794/0.008	0.658/0.500/0.004	0.637/0.471/0.001
χ^2 value $_3$	0.000/5.886	0.054/6.171	0.063/8.107
P value $_3$	1.000/0.015	0.817/0.013	0.803/0.004
χ^2 value $_4$	5.886	5.175	6.982
P value $_4$	0.015	0.023	0.008

Note: χ^2_1 tests the comparison between BTA and BTA stat, NMP22, survivin, and combined detection; χ^2_2 tests the comparison between BTA stat and NMP22, survivin, and combined detection; χ^2_3 tests the comparison between NMP22 and survivin, and combined detection; χ^2_4 tests the comparison between survivin and combined detection.

3.3. Comparing the positive rates of BTA, BTA stat, NMP22, and survivin among patients with different clinical characteristics

There were significant differences in the positive rates of BTA, BTA stat, NMP22, and survivin among patients with different tumor diameters, tumor numbers, pathological grades, clinical stages, and the presence or absence of lymph node metastasis ($P < 0.05$). See **Table 3** for details.

Table 3. Comparison of positive rates of BTA, BTA stat, NMP22, and survivin among patients [*n* (%), *n* = 60]

Group	Number of cases	BTA			BTA stat			NMP22			Survivin		
		Positive rate	χ^2	*P*	Positive rate	χ^2	*P*	Positive rate	χ^2	*P*	Positive rate	χ^2	*P*
Age (years)			0.024	0.876		0.184	0.668		0.156	0.693		0.203	0.887
< 60	29	72.41% (21)			75.86% (22)			72.41% (21)			75.86% (22)		
≥ 60	31	74.19% (23)			70.97% (22)			67.74% (21)			77.42% (24)		
Gender (cases)			0.074	0.785		0.016	0.898		0.278	0.598		0.196	0.658
Male	36	80.56% (29)			77.78% (28)			77.78% (28)			83.33% (30)		
Female	24	83.33% (20)			79.17% (19)			83.33% (20)			87.50% (21)		
Tumor diameter (cm)			5.432	0.020		4.705	0.030		4.271	0.039		4.812	0.028
< 3	29	65.52% (19)			58.62% (17)			68.97% (20)			72.41% (21)		
≥ 3	31	90.32% (28)			83.87% (26)			90.32% (28)			93.55% (29)		
Tumor number			4.973	0.026		4.877	0.027		4.184	0.041		5.939	0.015
Single	27	59.26% (16)			55.56% (15)			70.37% (19)			70.37 % (19)		
Multiple	33	84.85% (28)			81.82% (27)			90.91% (30)			93.9% (31)		
Pathological grade			5.001	0.025		4.242	0.039		5.001	0.025		4.434	0.035
Low	26	57.69% (15)			53.85% (14)			57.69% (15)			61.53% (16)		
High	34	82.35% (28)			76.47% (26)			82.35% (28)			85.29% (29)		
Clinical stage			5.284	0.022		5.180	0.023		5.143	0.023		6.655	0.010
T^1~T^2	25	52.00% (13)			56.00% (14)			60.00% (15)			60.00% (15)		
T^3~T^4	35	80.00% (28)			82.86% (29)			85.71% (30)			88.57% (31)		
Lymph node metastasis			4.106	0.043		5.000	0.025		4.261	0.039		8.298	0.004
Yes	20	75.00% (15)			80.00% (16)			90.00% (18)			100.00% (20)		
No	40	47.50% (19)			50.00% (20)			65.00% (26)			67.50% (27)		

4. Discussion

Bladder urothelial carcinoma is a malignant tumor with a high incidence in the clinical urinary system. With the advent of an aging society, the incidence and death toll of urothelial carcinoma continues to increase [4]. Therefore, timely and accurate screening and treatment are needed. Currently, clinical practices mainly adopt methods such as urinary exfoliative cytology, cystoscopy, and ultrasonography for diagnosis. However, due to various factors, the implementation effects of the above diagnostic methods are relatively mediocre. Moreover, some patients cannot accept certain examinations due to tolerability and examination costs, necessitating the search for more ideal diagnostic markers [5]. In recent years, various tumor markers have been widely used in the diagnosis of urothelial carcinoma. However, the sensitivity and accuracy of single detection still cannot achieve the desired goals, making combined detection and diagnosis a hot spot of clinical attention [6].

BTA, a hydrolytic fragment formed during the development of urothelial carcinoma, is mainly related to the degradation of the basement membrane of urothelial cells. It can affect the complement activation pathway, protect the tumor from immune attack, and promote tumor growth [7]. An increase in BTA levels in urine indicates the possibility of urothelial carcinoma [8]. BTA can diagnose superficial and small bladder tumors. However, if patients have received treatment for bladder cancer or have urinary system infections, it may cause false-positive results in clinical testing, which restricts its application [9]. BTA stat, as the second-generation bladder tumor antigen reagent, can be detected using monoclonal antibodies. It can bind to complement C3b and inhibit the formation of the membrane attack complex, laying the foundation for tumor growth [10]. NMP22, a cellular structural protein, plays a role in DNA replication and transcription, improving cell proliferation activity. In healthy individuals, its expression level is relatively low. The malignant transformation of urothelial mucosa, accelerates the proliferation and apoptosis of urothelial cells, promoting the massive release of NMP22 into the urine [11]. Thus, significantly elevated NMP22 levels can be detected in the urine of patients with urothelial carcinoma [12]. Survivin, a member of the apoptosis-inhibiting protein family, can regulate the cell cycle and inhibit apoptosis. Survivin is highly expressed in multiple tumor tissues, appearing only in the testes, thymus, and secretory uterus in normal tissues [13,14]. Analyzing the detection results of the two groups, the levels of BTA, BTA stat, NMP22, and survivin in the observation group were higher than those in the healthy controls, suggesting an association between the expression levels of these markers and the occurrence of urothelial carcinoma. They can be used for the diagnosis of urothelial carcinoma. Meanwhile, comparing single and combined detections of BTA, BTA stat, NMP22, and survivin in patients with urothelial carcinoma revealed that the diagnostic efficacy of combined detection was superior to single detection. This indicates the relatively high value of combined detection of BTA, BTA stat, NMP22, and survivin in the diagnosis of urothelial carcinoma. Different tumor markers have varying diagnostic advantages. Single tumor marker detection can lead to missed diagnosis or misdiagnosis, affecting the accuracy of the final diagnosis. Combined detection facilitates complementary advantages and provides more assistance for patient diagnosis and treatment [15].

After analyzing the positive rates of BTA, BTA stat, NMP22, and survivin in patients with urothelial carcinoma with different clinical features, it was found that tumor diameter, number of tumors, pathological grade, clinical stage, and the presence of lymph node metastasis all significantly affect the positive rates of these markers. However, age and gender do not have a significant impact on the positive rates. These results suggest that BTA, BTA stat, NMP22, and survivin all play a role in the occurrence and development of urothelial carcinoma. Changes in their levels can be used to assess the severity of the patient's condition and understand disease progression and treatment directions. Therefore, these markers can serve as important indicators for diagnosing and treating

patients [16]. Patients with a tumor diameter of ≥ 3cm, multiple tumors, high pathological grade, clinical stage T3–T4, and lymph node metastasis have higher levels of these tumor markers. This is mainly because the positive rates of BTA and BTA stat increase with the pathological grade of urothelial carcinoma. By understanding the positive rates of BTA and BTA stat, the pathological grade of the patient can be judged [17]. NMP22 remains highly sensitive in tumors with low pathological grades and clinical stages. As the tumor size increases, the number of tumors increases, and the staging rises, the positive rate of NMP22 also increases. Therefore, NMP22 can not only be used for early diagnosis of urothelial carcinoma but also to analyze the prognosis of patients based on changes in its expression level [18]. The positive expression rate of survivin is closely related to the pathological grade, staging, and lymph node metastasis of urothelial carcinoma. The positive expression of survivin can be detected early in cellular malignancy, and its overexpression can disrupt the balance between cell proliferation and apoptosis, playing an important role in the early stages of tumor development. Additionally, survivin can protect growth factors, induce the formation of new blood vessels in tumor tissue, and provide a favorable environment for tumor growth and invasion, thereby promoting tumor development [19]. Overall, the high positive expression rates of BTA, BTA stat, NMP22, and survivin promote the malignant transformation of urothelial carcinoma, and their abnormal expression is closely related to the clinicopathological features of urothelial carcinoma patients. This allows for better patient identification, and combined detection can improve diagnostic sensitivity and ensure diagnostic efficacy [20].

5. Conclusion

In summary, the combined detection of BTA, BTA stat, NMP22, and survivin has definite application value in the diagnosis of urothelial carcinoma patients. It can provide an important reference for patient condition evaluation and is recommended for active use in the diagnosis and treatment of urothelial carcinoma.

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Disclosure statement

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