



Diagnostic and prognostic role of BTA, NMP22, survivin and cytology in urothelial carcinoma

Yu-Wen Gong^{1#^}, Yi-Ran Wang^{1#}, Guang-Rui Fan¹, Qian Niu¹, You-Li Zhao¹, Hanzhang Wang², Robert Svatek², Ronald Rodriguez², Zhi-Ping Wang^{1^}

¹Institute of Gansu Nephro-Urological Clinical Center, Department of Urology, Institute of Urology, Key Laboratory of Urological Disease of Gansu Province, Lanzhou University Second Hospital, Lanzhou, China; ²Department of Urology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA

Contributions: (I) Conception and design: YW Gong; (II) Administrative support: ZP Wang; (III) Provision of study materials or patients: YR Wang; (IV) Collection and assembly of data: YR Wang, GR Fan, YL Zhao; (V) Data analysis and interpretation: YW Gong, Q Niu, H Wang, R Svatek, R Rodriguez; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Zhi-Ping Wang. 82 Cuiyingmen, Lanzhou 730000, China. Email: wangzplzu@163.com.

Background: Cytology is a recommended noninvasive urine test for the detection and surveillance of bladder cancer and upper-tract urothelial carcinoma. It is however characterized by poor sensitivity in low-grade tumors. This study aims to determine the diagnostic and prognostic role of BTA, BTA-stat, NMP22, and Survivin.

Methods: Urine samples were collected from a total of 105 patients (bladder cancer (n=61), upper-tract urothelial carcinoma (n=44), and controls (n=52)). The samples were directly assessed using cytology, BTA-stat (Qualitative test), BTA (chemiluminescence test), NMP22 (Qualitative test), and Survivin (enzyme-linked immunosorbent assay). Cancer progression and recurrence were assessed after a median follow-up of 32 months (4–47 months). Univariate and multivariate analyses were performed using Kaplan-Meier survival analysis and Cox proportional hazards regression.

Results: The triple combination of Survivin + BTA + Cytology was the most promising model for discriminating bladder cancer or upper-tract urothelial carcinoma from controls (UTUC group: the area under the curve value 0.97, sensitivity 86%, specificity 96%; BC group: the area under the curve value 0.86 sensitivity 67%, specificity 96%). Univariate survival analysis, showed Cytology (P=0.02; HR=5.35) and Survivin (HR=3.24; P=0.03) to have a significant association with the progression-free survival, while Survivin (HR=4.15; P=0.04) was statistically associated with cancer-specific survival in the bladder cancer group. The multivariable analysis did not show any of these markers as independent prognostic factors.

Conclusions: These biomarkers showed a higher sensitivity than cytology, but a poorer specificity. All biomarkers exhibited good diagnostic performance in both bladder cancer and upper-tract urothelial carcinoma. Combining Survivin + BTA + Cytology was superior to the use of a single marker or combining other biomarkers.

Keywords: Biomarkers; urothelial carcinoma (UC); combined detection; diagnosis; prognosis

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[^] ORCID: Yu-Wen Gong, 0000-0003-3134-5696; Zhi-Ping Wang, 0000-0002-8140-591X.

Introduction

Urothelial carcinomas (UCs), are the fourth most common tumors and mainly include bladder cancers (BC) and upper tract urothelial carcinomas (UTUC) (1). BC is the most common urinary tract malignancy accounting for 90–95% of UCs while UTUC is a rare lethal disease accounting for approximately 5% of UCs (2).

Approximately 75% of patients with BC, are initially diagnosed with non-muscle-invasive bladder cancer (NMIBC) (3). Although these lesions are not immediately life-threatening, they have a high probability of recurrence (up to 50%) and progression (up to 20%) (4). Despite UTUC and BC sharing many characteristics, they are two distinct diseases (5,6). 60% of patients with UTUC have an invasive disease while 7% have metastasis on the first diagnosis. Cancer recurrence in the bladder occurs in 22–47% of UTUC patients compared with 2–6% recurrence in the contralateral upper tract following surgery (2,7). Therefore, early diagnosis and stringent follow-up are crucial.

The methods currently used in the detection and surveillance of BC and UTUC include endoscopic examination (cystoscopy or ureteroscopy), imaging and urine cytology. Cytology is recommended as a noninvasive urine test due to high specificity. Urine cytology is unreliable as it has poor sensitivity in low-grade BC (16%) (8), cytological interpretation is highly observer-dependent and evaluation is hampered by low cellular yield, urinary tract infections, stones, or intravesical installations (3). The U.S. Food and Drug Administration (FDA) approved FISH, Immunocyt, BTA, and NMP22 as auxiliary testing methods for BC. However, these markers showed low specificity and only a few studies have investigated these biomarkers in the Chinese population. These biomarkers have also not been recommended by guidelines for UTUC diagnosis. However, some biomarkers, such as E-cadherin, HER2, and Ki-67 have been mentioned in guidelines for UTUC prognosis (9,10). Studies done to evaluate the prognostic and diagnostic role of the biomarkers have limitations of retrospective design and small sample size. Therefore, numerous urinary tests have been developed for the diagnosis and surveillance of BC. In this study, cytology was combined with BTA-stat, quantitative BTA, NMP22, and Survivin in a prospective analysis of prognostic and diagnostic tests in BC and UTUC.

We present the following article in accordance with the REMARK reporting checklist (available at <https://dx.doi.org/10.21037/tcr-21-386>).

Methods

Patient cohort and sample

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of Lanzhou University Second Hospital (approval number: 2016A-058). Written informed consent was obtained from all participants before the samples were collected. A total of 157 cases of the UC group (BC group, UTUC group) and control group (healthy individuals and patients with other urinary diseases) were included in this study carried out between October 2015 and May 2017.

About 10 mL of the first-morning urine was collected from each individual for the detection of BTA, BTA-STAT, NMP22, and Survivin. The entire urine volume of the second voided morning urine from both study groups was then collected for cytological examinations. Collected data variables included: patient demographics, date of the urine collection, surgical pathological examination, recurrence, and follow-up. Inclusion criteria: The patients in the UC group were diagnosed based on histological examination while the healthy individuals (normal medical reports) underwent a physical examination in the Physical Examination Center affiliated to Lanzhou University second hospital and patients with other urinary diseases were recruited from the Department of Urology, Lanzhou University second hospital. Exclusion criteria: patients who had undergone invasive examination, patients who had suffered from both UTUC and BC, patients with incomplete clinical information, and patients who declined to sign the informed consent.

Urine detection

Cytology

Urine cytology was examined in the Department of Pathology in Lanzhou University second hospital with Thinprep cytologic test (TCT) and interpreted by two experienced pathologists. The cytopathological findings were classified as negative or positive, in correspondence to the Papanicolaou classes I-III and IV-V, respectively.

NMP22 and BTA-stat

Both the NMP22 (Alere Inc. USA) and BTA-stat assays (Polymedco CDP, LLC. USA) were point-of-care devices. The assay was carried out by dripping the urine into the device and reading the results after 30 minutes in the NMP22 test and after 5 minutes in the BTA-stat test. Both results were interpreted by a single observer who was

blinded to the result of various clinical examination.

Quantitative BTA and surviving

Urine samples were centrifuged as soon as possible at 3,000 rpm, 4 °C, for 10 minutes. The supernatant was collected and stored at -80 °C. All samples were brought to room temperature before carrying out chemiluminescence for quantitative BTA (Pergrande, Beijing, China), and enzyme-linked immunosorbent assay (ELISA) for Survivin (R&D systems, MN, USA).

Follow-up

To analyze the prognostic performance, tumor recurrence-free survival (RFS), progression-free survival (PFS), and cancer-specific survival (CSS) were determined. Recurrence was defined to have occurred if patients presented with the same grade and stage as the previous tumor, or lower stage or grade than the previous tumor, or presented with a non-muscle invasive tumor in the bladder in UTUC patients. Progression was said to have occurred if patients presented with a higher grade or stage than the recurrent tumor, metastasis, or with a muscle-invasive tumor in the bladder in UTUC patients.

Statistical calculations

Statistical analysis was performed using SPSS version 19.0 and GraphPad prism7. The diagnostic performance of these markers was determined using receiver operating characteristic (ROC) curves and the area under the curve (AUC) values. The optimal cutoff values for the single markers were determined by calculation of the Youden index. In combination tests, both parallel and serial tests were analyzed. The Chi-square test was used to analyze differences between two classified variable groups while the Mann-Whitney U test was used for independent samples with a continuous variable. Recurrence, progression, and death risk were evaluated by the Kaplan-Meier method, with the log-rank test, and multivariable analysis with Cox proportional hazards models. All tests were two-sided, and P values <0.05 were considered statistically significant.

Results

Patient characteristics

The median age of patients with UCs was 70 (60–76) years old, with males accounting for about 69% and females 31%. A

total of 105 participants were included in the study. The stage and grade of 61 patients with BC were as follows: 30 NMIBC low grade (LG), 15 NMIBC high grade (HG), and 16 MIBC HG. UTUC included 22 patients with renal pelvic cancer, 14 patients with ureter cancer, and 8 patients with both renal pelvic cancer and ureter cancer. The stage and grade of 44 patients with UTUC were as follows: 7 non-invasive UTUC LG, 7 non-invasive UTUC HG, 1 flat lesion, 8 invasive carcinoma LG, and 21 invasive carcinoma HG. The control group (n=52) consisted of 32 patients with other urinary diseases including benign prostate hyperplasia, urinary tract infection, hematuria, lithiasis, and 20 healthy volunteers. Their clinicopathological data are summarized in *Table 1*.

The median follow-up period for BC patients and UTUC patients was 32 months (4–47 months). During the follow-up, 4 patients in the BC group and 3 patients in the UTUC group were lost to follow-up. Four patients experienced local tumor recurrence, 15 patients experienced progression and 10 patients died due to cancer-related events in the BC group. Two patients experienced low-grade BC, 13 patients had cancer progression, and 7 patients died due to cancer-related events in the UTUC group.

Single test performance

The AUCs of BTA, BTA-stat, NMP22, Survivin and cytology for differentiating BC from the control group were 0.84, 0.67, 0.69, 0.84 and 0.59, respectively. Sensitivity values of each marker for BC were as follows: C, 18% (3% for LG and 37% for HG, 11% for NMIBC and 37% for MIBC); Bs, 69% (50% for LG and 93% for HG; 55% for NMIBC and 95% for MIBC); N, 59% (35% for LG and 89% for HG; 53% for NMIBC and 74% for MIBC); and B, 74% (56% for LG and 96% for HG; 66% for NMIBC and 95% for MIBC); S, 77% (59% for LG and 96% for HG; 66% for NMIBC and 95% for MIBC).

The AUCs of BTA, BTA-stat, NMP22, Survivin and cytology for differentiating UTUC from control group were 0.93, 0.81, 0.76, 0.88 and 0.65, respectively. Sensitivity values of each marker for UTUC were as follows: C, 30% (13% for LG and 38% for HG; 31% for TaT1 tumor and 28% for T2-T4 tumor); Bs, 98% (93% for LG and 100% for HG; 100% for TaT1 tumor and 97% for T2-T4 tumor); N, 73% (60% for LG and 79% for HG; 54% for TaT1 tumor and 79% for T2-T4 tumor); and B, 95% (87% for LG and 97% for HG; 92% for TaT1 tumor and 94% for T2-T4 tumor); S, 89% (87% for LG and 90% for HG; 85% for TaT1 tumor and 90% for T2-T4 tumor).

Table 1 Demographic and clinicopathological characteristics of study subjects

Group	N (%)
UCs	105 (67)
Sex	
Male	73 (70)
Female	32 (30)
BC	
History of UC	
Primary	49 (80)
Recurrence	12 (20)
Grade	
PUNLMP	3 (5)
LG	31 (51)
HG	27 (44)
Stage	
Ta-T1	47 (77)
T2-T4	14 (23)
UTUC	
History of BC	
No	43 (98)
Yes	1 (2)
Location	
Renal pelvis	22 (50)
Ureter	14 (32)
Pelvis and ureter	8 (18)
Grade	
LG	15 (34)
HG	29 (66)
Stage	
Tis only	1 (2)
Ta-T1	14 (32)
T2-T4	29 (66)
Control	52 (33)
Sex	
Male	35 (67)
Female	17 (33)

Table 1 (continued)

Table 1 (continued)

Group	N (%)
Other disease	32 (62)
Healthy	20 (38)

UCs, urothelial carcinomas; BC, bladder cancer; UTUC, upper-tract urothelial carcinoma; PUNLMP, papillary urothelial neoplasm of low malignant potential; LG, low grade; HG, high grade; Tis, tumor in situ. Grade according to 2004 WHO classification; Stage according to 2009 TNM classification;

Specificity results of each marker were as follows: C, 100%; Bs, 63%; N, 79%; B, 81%; and S, 83% (Figure 1, Table 2). The cut-off of BTA and Survivin as quantitative tests were 88 U/mL and 21.8 pg/mL, respectively (Figure 1, Table 2).

Combination test performance

A combination of urinary markers with cytology, a total of 15 combined models can be generated (B+C, Bs+C, S+C, N+C, B+S+C, BS+S+C, S+N+C, B+N+C, B+BS+C, BS+N+C, B+BS+N+C, BS+S+N+C, B+S+N+C, BS+B+S+C, BS+B+S+N+C). Among the combination of a single tumor marker with C, the B+C yielded the best diagnostic performance in UTUC group (AUC 0.91, sensitivity 96%, specificity 81%, PPV 81%, NPV 95%); while S+C had the best performance in BC group (AUC 0.81, sensitivity 77%, specificity 83%, PPV 84%, NPV 75%). After comprehensive consideration, the triple combination of S+B+C was the most promising models in all models, with cost-savings, and valuable in both UTUC group and BC group (UTUC group: AUC 0.97, sensitivity 86%, specificity 96%, PPV 95%, NPV 89%; BC group: AUC 0.86, sensitivity 67%, specificity 96%, PPV 95%, NPV 78%) (Figure 1, Table 3).

Associations of the urinary markers and cytology with clinical parameters

In the BC group, cytology (P=0.02), BTA-stat (P=0.017), and BTA (P=0.001) were statistically associated with tumor stage and all these markers included cytology were statistically associated with tumor grade (Table 4). Tumor recurrence and size ≥ 3 cm had a significant correlation to a positive test of NMP22 and BTA-stat, respectively. In the UTUC group, only the expression of Survivin had a

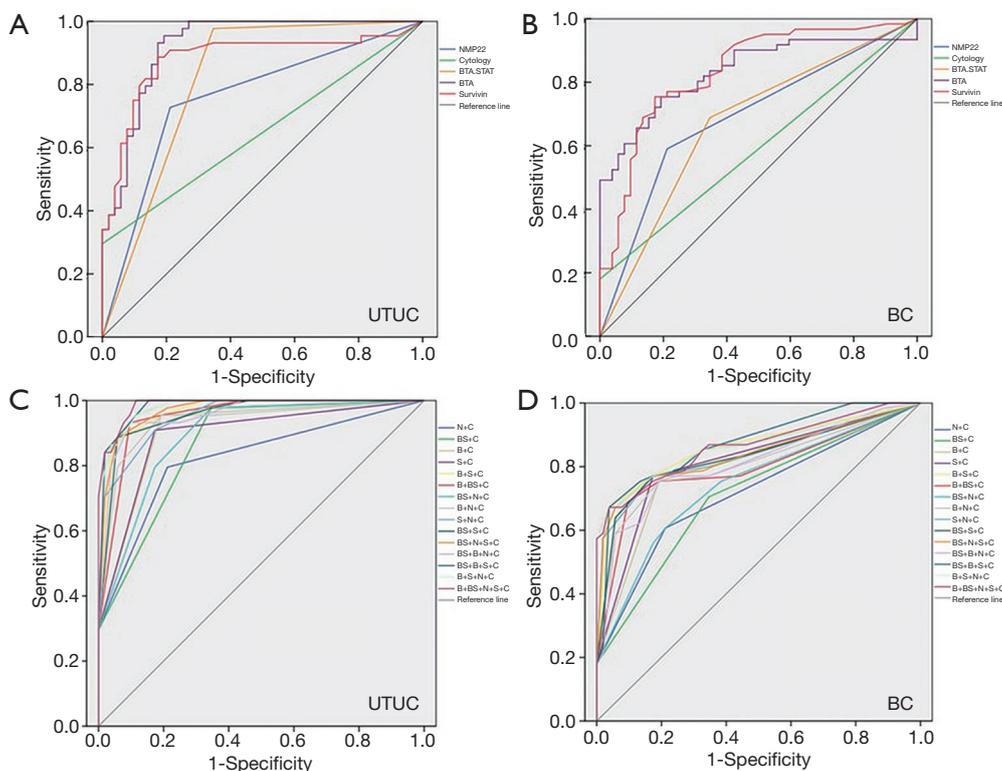


Figure 1 Diagnostic performance of single urine biomarker and combinations. (A) ROC for single urine tests in UTUC. (B) ROC for single urine tests in BC. (C) ROC for different combinations of cytology and other biomarkers in UTUC. (D) ROC for different combinations of cytology and other biomarkers in BC. Youden Index cutoffs that maximized sum of sensitivity and specificity were determined for each test. ROC, area under the curve; UTUC, upper-tract urothelial carcinoma; BC, bladder cancer.

significant correlation to a higher tumor stage (Table 4).

Survival prediction

We developed the Kaplan-Meier survival analysis of each urinary biomarker and cytology (Figures 2-4). Cytology ($P=0.02$), and Survivin ($P=0.03$) had a significant association with PFS, and Cytology ($P=0.03$), Survivin ($P=0.04$) was also statistically associated with CSS in the BC group. None of the markers showed a significant association to the PFS or CSS in the UTUC group. (There were too few recurrence cases to analyze the relationship between the urine markers and recurrence-free survival).

We also analyzed the predicting performance of clinicopathological parameters with PSF and CSS. The univariate analysis predicted poor prognosis with a higher stage ($P=5.75E-05$), and HG ($P=2.08E-04$) in the BC group. In the UTUC group, tumor number ≥ 2 had a significantly increased risk of tumor progression ($P=0.02$),

while the tumor grade showed a significant association with CSS ($P=0.03$). Multivariate analysis revealed that the tumor stage was an independent prognostic factor for both PFS and CSS ($P=0.03$; $P=0.004$), while tumor number ≥ 2 was an independent prognostic factor for CSS ($P=0.01$) in the BC group. And the tumor grade was an independent prognostic factor for CSS ($P=0.04$). However, none of these biomarkers was an independent prognostic factor in BC or UTUC (Tables 5,6).

Discussion

In this study, both UTUC and BC samples were included for the detection of BTA, BTA-stat, NMP22, Survivin, and cytology. The results revealed that all tumor markers and urine cytology were effective in the diagnosis of both BC and UTUC, with superior effectiveness in the UTUC group. Urine cytology is recommended as an auxiliary examination. However, it is plagued by low sensitivity

Table 2 Test results of single urinary tests in UTUC and BC group

Test	Sensitivity (%) (UTUC/BC)	Specificity (%)	PPV (%) (UTUC/BC)	NPV (%) (UTUC/BC)	AUC (UTUC/BC)
Cytology	30/18	100	100/100	51/39	0.65/0.59
LG	13/3				
HG	38/37				
TaT1	31/11				
T2-T4	28/37				
BTA-STAT	98/69	63	70/70	97/64	0.81/0.67
LG	93/50				
HG	100/93				
TaT1	100/55				
T2-T4	97/95				
NMP22	73/59	79	74/77	72/62	0.76/0.69
LG	60/35				
HG	79/89				
TaT1	54/53				
T2-T4	79/74				
BTA	93/74	81	81/82	95/72	0.93/0.84
LG	87/56				
HG	97/96				
TaT1	92/66				
T2-T4	94/95				
Survivin	89/75	83	81/84	90/75	0.88/0.84
LG	87/59				
HG	90/96				
TaT1	85/66				
T2-T4	90/95				

LG, low grade; HG, high grade; BC, bladder cancer; UTUC, upper-tract urothelial carcinoma; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value. Grade according to 2004 WHO classification; Stage according to 2009 TNM classification.

in detecting low-grade noninvasive lesions, and lack of standardized diagnostic criteria. In 2016, a standardized reporting system redefining urinary cytology diagnostic categories was published by the Paris Working Group (11). Since patients were enrolled in this study before 2015 before the publication of the Paris Working Group, the cytology results were classified according to the Papanicolaou classes. This study revealed that cytology has a high specificity, but low sensitivity 18%, especially for low-grade bladder tumors. These results were consistent with similar

reported findings (12). Surprisingly, the sensitivity of the cytology test was higher in UTUC than BC group and was correlated with the tumor grade, which was consistent with earlier reports (13,14).

BTA is a “bladder tumor antigen” human complement factor H-related protein (15). In this study we performed both qualitative and quantitative tests. The AUC of the BTA qualitative test for BC was 0.67, and the diagnostic sensitivity and specificity were 69% and 63%, respectively. These results revealed a higher sensitivity and a lower

Table 3 Test results of combinations of cytology and other urinary tests in UTUC and BC group

The combination tests	Sensitivity (%) (UTUC/BC)	Specificity (%)	PPV (%) (UTUC/BC)	NPV (%) (UTUC/BC)	AUC (UTUC/BC)
N+C	80/61	79	76/77	82/63	0.82/0.72
BS+C	98/71	65	71/71	97/65	0.87/0.71
B+C	96/75	81	81/82	95/74	0.91/0.80
S+C	91/77	83	82/84	91/75	0.90/0.81
B+S+C	86/67	96	95/95	89/78	0.97/0.86
B+BS+C	93/69	90	89/89	94/71	0.95/0.80
BS+N+C	80/56	83	80/79	83/61	0.89/0.74
B+N+C	80/75	81	81/82	95/74	0.94/0.81
S+N+C	91/77	83	82/84	91/75	0.95/0.83
BS+S+C	89/64	94	93/93	91/69	0.96/0.83
BS+N+S+C	70/53	98	97/97	80/64	0.97/0.84
BS+B+N+C	80/56	96	95/94	85/65	0.97/0.81
BS+B+S+C	84/61	98	97/97	88/68	0.98/0.87
B+S+N+C	70/57	100	100/100	80/67	0.98/0.86
B+BS+N+S+C	93/67	92	91/91	94/71	0.98/0.87

N, NMP22; BS, BTA–BTAT; S, Survivin; B, BTA; C, cytology; AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

Table 4 Association of clinical parameters with urinary markers in UTUC and BC group

Tumor variable	Tumor type	Statistic Index	Cytology	BTA–stat	NMP22	Statistic Index	BTA	Survivin
Tumor history	BC	χ^2	0.028	0.972	6.126	Z	–0.354	–0.272
		P	0.868	0.324	0.013	P	0.723	0.786
Tumor stage	BC	χ^2	5.428	5.748	2.534	Z	–3.309	–1.395
		P	0.02	0.017	0.111	P	0.001	0.163
	UTUC	χ^2	0.296	N/A	1.12	Z	–0.648	–2.133
		P	0.587	0.674	0.29	P	0.517	0.033
Tumor grade	BC	χ^2	11.071	8.163	15.017	Z	–4.525	–2.092
		P	0.001	0.004	<0.001	P	<0.001	0.036
	UTUC	χ^2	2.01	N/A	2.516	Z	–0.612	–0.192
		P	0.156	0.349	0.113	P	0.541	0.848
Number of tumors	BC	χ^2	0.818	0.566	0.514	Z	–1.732	–1.524
		P	0.366	0.452	0.474	P	0.083	0.128
	UTUC	χ^2	0 [†]	N/A	0 [†]	Z	–1.723	–1.682
		P	1 [†]	0.14	1 [†]	P	0.09	0.97
Size of tumor	BC	χ^2	1.498	3.881	2.527	Z	–2.045	–0.642
		P	0.221	0.05	0.112	P	0.41	0.521
	UTUC	χ^2	0.402	N/A	0.078	Z	–0.593	–1.083
		P	0.526	0.419	0.779	P	0.553	0.279

BC, bladder cancer; UTUC, upper-tract urothelial carcinoma. [†]means this value is infinitely close to 1 or 0. N/A means that the difference between the two groups of samples is too large to be suitable for analysis

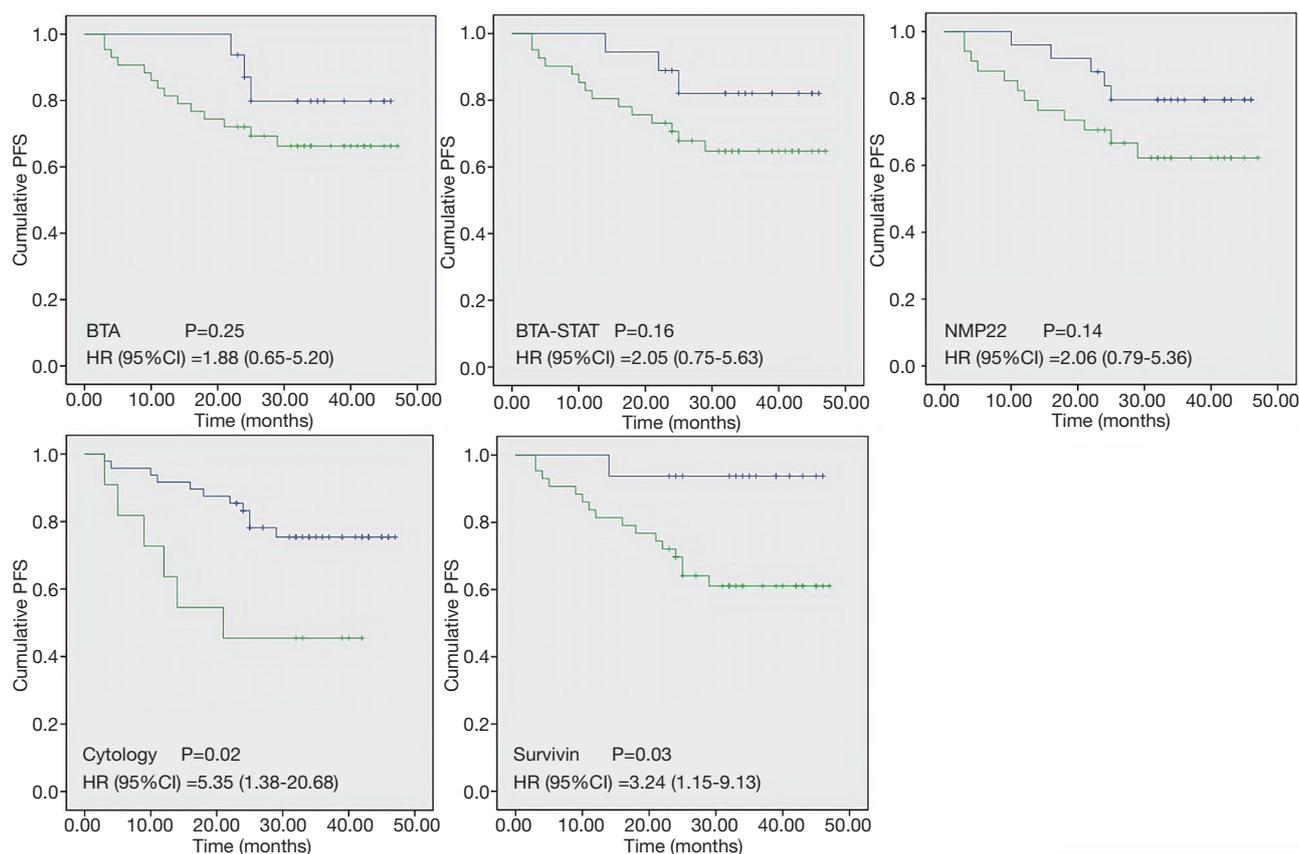


Figure 2 Kaplan-Meier curves for PFS as a function of BTA, BTA-STAT, NMP22, cytology and Survivin in BC. Differences between both groups were calculated by the log-rank test. The green line and blue line represent positive and negative, respectively. Censored cases (+) mark patients that developed no progression during the follow-up or withdraw from the study. PFS, progression-free survival; BC, bladder cancer.

specificity compared to the results obtained by Yafi (8). The BTA quantitative assay had an AUC of 0.84 in the diagnosis of BC, with a sensitivity and specificity of 74% and 81%, respectively, which were higher than previously reported results (72%, 53%) (16). In this study, the BTA quantitative detection showed a good diagnostic performance which was consistent with Irani J's findings (17), but contrary to Babjuk's findings that reported that the BTA qualitative detection was more sensitive, while BTA quantitative detection was more specific (18). In the UTUC group, the AUC of the qualitative and quantitative detection of BTA was 0.81 and 0.93, respectively, which were superior to that seen in bladder cancer.

NMP22 is a protein specific to mitosis and is involved in the distribution of chromatids to daughter cells. The concentration of NMP22 is up to 25 times greater in bladder cancer cell lines than in normal urothelium (8).

This study showed an improved sensitivity with 59%, and a decreased specificity of 79% compared to Yifa's findings (19). The diagnostic efficiency in UTUC was better than that in BC with an AUC of 0.76 and a sensitivity of 73%.

Survivin is a member of the inhibitor of apoptosis protein (IAP) family and has a unique role in apoptosis and control of cell division (20). A meta-analysis reported a pooled sensitivity of 0.79, with a specificity of 0.87 of the Survivin protein test for bladder cancer (21). In this study, the AUC of Survivin for BC was 0.84, with a sensitivity and specificity of 75% and 83%, respectively. The results were better than Akhtar's findings (22), however the sensitivity of 75% was slightly lower than the sensitivity of 81% in this study (23). In the UTUC, Survivin had a better diagnostic performance.

Although the single marker assays are more sensitive than cytology, the latter has a higher specificity. The clinical

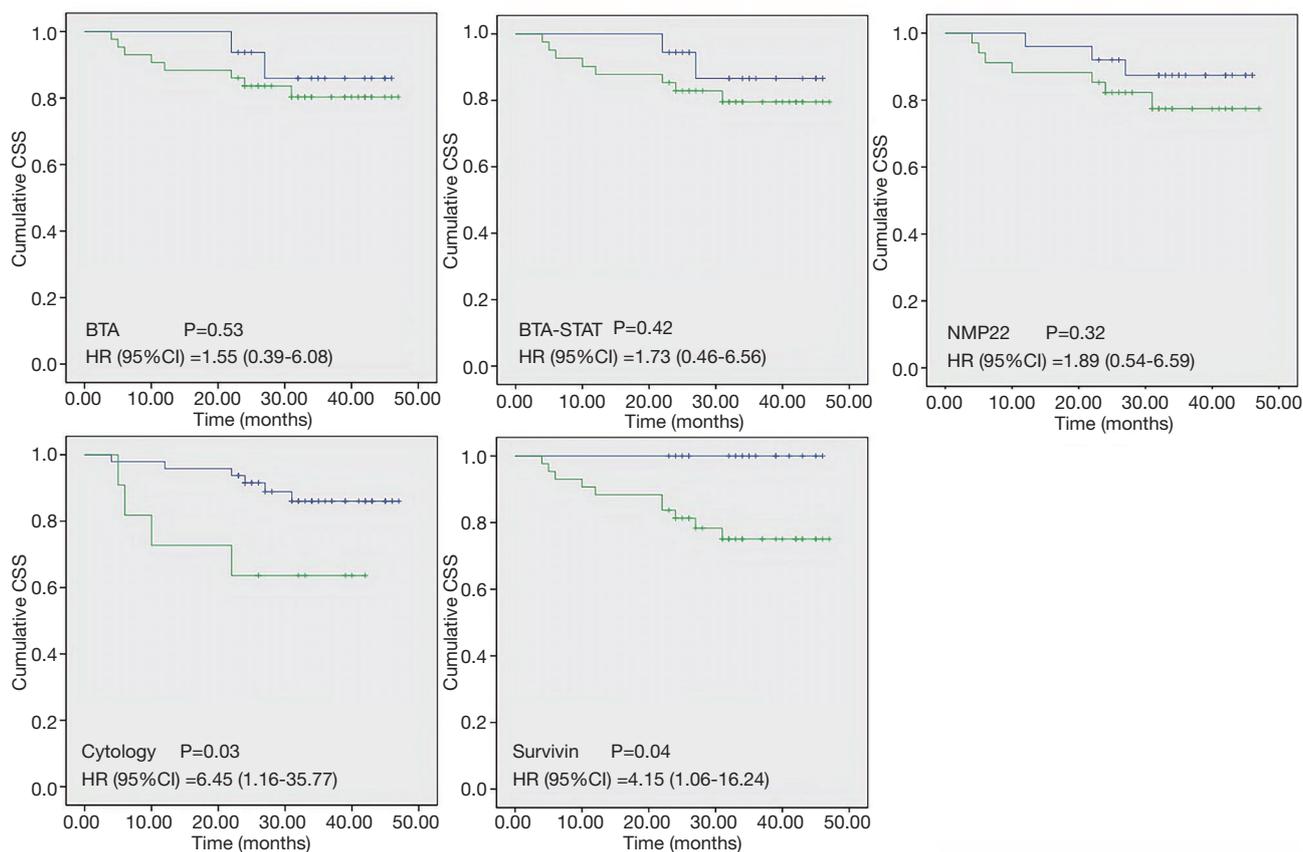


Figure 3 Kaplan-Meier curves for CSS as a function of BTA, BTA-STAT, NMP22, cytology and Survivin in BC. Differences between both groups were calculated by the log-rank test. The green line and blue line represent positive and negative, respectively. Censored cases (+) mark patients that developed no progression during the follow-up or withdraw from the study. CSS, cancer-specific survival; BC, bladder cancer.

application of these tumor markers is limited by false positives. In this study, BTA-stat, BTA, and NMP22 were detected as false positives in urinary tract inflammation and benign prostatic hyperplasia. Tumor markers were negative in 6 cases of patients with haematuria however, hematuria in patients without a malignant disease can result in false-positive BTA assays (16). Therefore, in-depth research requires a large sample size.

To improve the diagnostic efficacy of tumor markers, combined detection of several tumor markers should be analyzed, which was a useful diagnostic strategy to complement each other and improve the detection value. Several previous studies reported superior efficacy in detection of combining tumor markers compared to the use of single tumor markers (8,24,25). However, a contrary report was given (19). In this study, a triple combination of S+B+C yielded the most valuable combination model with a

specificity rate of 97% and a sensitivity of 86% and 67% in UTUC and BC, respectively. In general, the performance of combined detection of tumor markers was evaluated by statistical analysis, however it was hard to using statistical software to determine patient's results of combined tests in clinical work. From our perspective, it was necessary to propose a appropriate recommendation to guide doctors in clinical practice. We suggest that at least the two of Survivin, BTA, and cytology were positive, then the result of the combined detection was considered positive. This recommendation was consistent with our statistical analysis and compensated for the lack of sensitivity of urinary cytology while maintaining high specificity. However, the hypothesis needs more further verification with large sample size.

In this study, there were 49 and 12 cases of primary and recurrent bladder cancer, respectively, and one case of

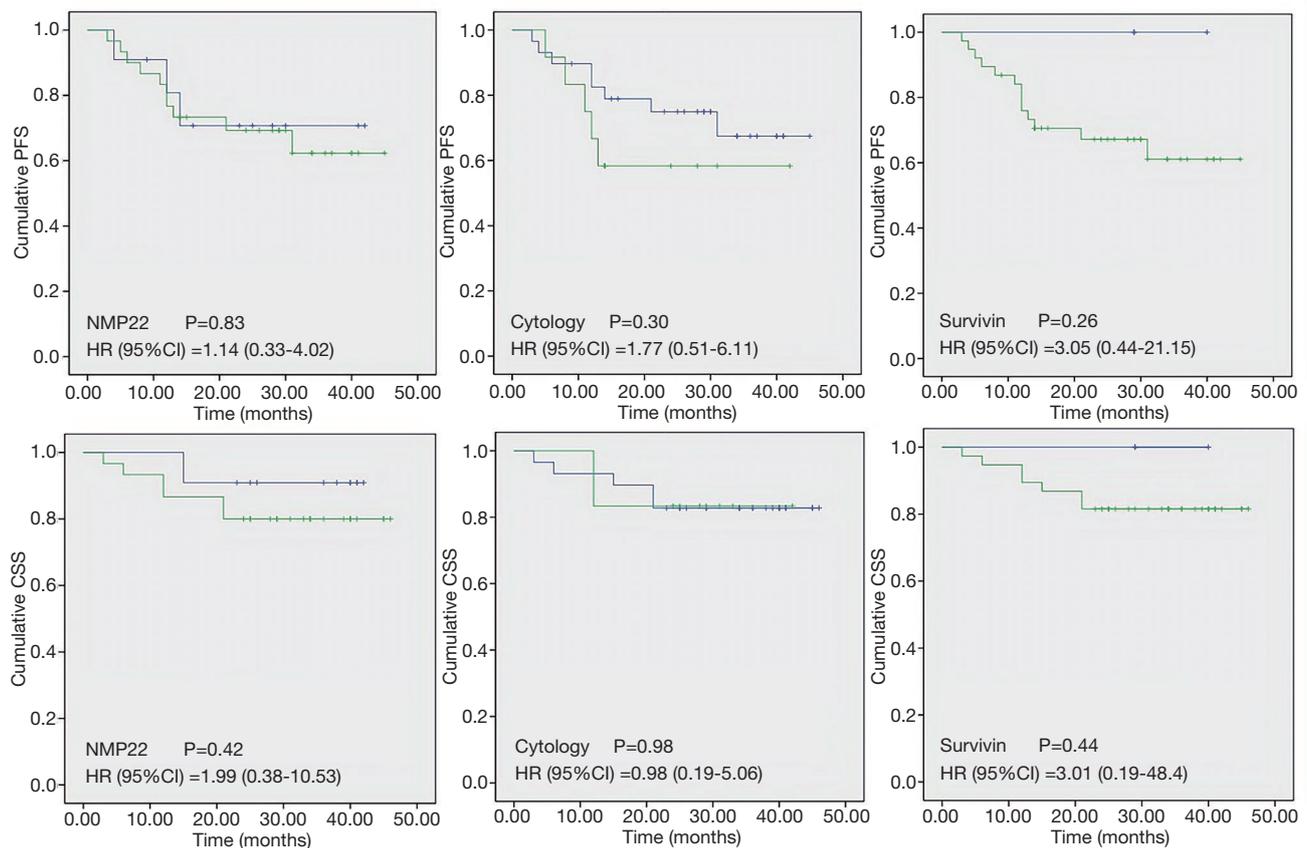


Figure 4 Kaplan-Meier curves for PFS and CSS as a function of the cytology, Survivin and NMP22 in UTUC (the difference of samples between positive group and negative group in both BTA-STAT and BTA are too large to be suitable for analysis). Differences between both groups were calculated by the log-rank test. The green line and blue line represent positive and negative, respectively. Censored cases (+) mark patients that developed no progression during the follow-up or withdraw from the study. PFS, progression-free survival; CSS, cancer-specific survival; UTUC, upper-tract urothelial carcinoma.

recurrence in UTUC. We analyzed the relationship between the tumor markers and the primary and recurrent status of BC. All markers and cytology were no significant correlations with the tumor status except for NMP22 ($P=0.013$). NMP22 was also associated with bladder tumor grade ($P<0.001$). According to Yafi's report, NMP22 was associated with stage and grade of BC, but not with other parameters (8), while Jeong's study, NMP22 was only related to the tumor grade (19). This difference might arise as a result of different populations, or the grades and stages of the tumors. Quantitative BTA, and cytology were significantly associated with the stage and grade of BC, while Qualitative BTA-stat was associated with tumor grade and cytology was related to tumor stage (8). The association of the Survivin expression with the histopathological parameters had contradictory viewpoints. In this study, Survivin was not associated with any

parameters in BC. In the UTUC group, we observed that Survivin was related to the tumor stage ($P=0.033$). None of the urinary markers were associated with the tumor number or size in both BC and UTUC groups.

In the BC group, histopathological parameters such as a higher tumor grade and an invasive tumor were predictive of progression and survival on univariate analysis. Multivariable analysis revealed that only the tumor grade could predict a shorter PFS and CSS, as earlier reported (26). In the UTUC, tumor grade was shown to have a significant association with the tumor progression by multivariate analysis, which was consistent with other studies (27,28). Previous studies reported that tumor size >3.0 cm was a significant predictor of CSS and RFS (29,30), which was not seen in this study.

Considering the role of tumor markers and cytology in tumor progression and survival, C ($P=0.01$) and S ($P=0.03$)

Table 5 Univariate and multivariate of survival analysis in bladder cancer

Patient variable	PFS (univariate log rank)		PFS (multivariate Cox)		CSS (univariate log rank)		CSS (multivariate Cox)	
	Mean survival time (95% CI)	P	HR (95% CI)	P	Mean survival time (95% CI)	P	HR (95% CI)	P
Gender								
Female	37.77 (31.87–43.66)	0.94			37.77 (31.87–43.66)	0.94		
Male	37.08 (32.39–41.78)				37.08 (32.39–41.78)			
Age								
≤60	42.76 (38.52–46.99)	0.08			NA	0.04		
>60	35.01 (29.96–40.06)				NA			
Tumor history								
Initial	37.94 (33.61–42.27)	0.51			41.76 (38.31–45.21)	0.87		
Recurrent	33.18 (23.06–43.30)				39.27 (30.81–47.74)			
Tumor stage								
NMIBC	42.47 (39.03–45.92)	5.75E–05	3.73 (1.17–11.93)	0.03	45.28 (43.40–47.15)	0.001	27.25 (2.88–257.42)	0.004
MIBC	24.58 (17.33–31.83)				30.78 (23.41–38.15)			
Tumor grade								
Low grade	43.21 (40.65–45.77)	2.08E–04			44.00 (41.85–46.16)	0.04		
High grade	28.44 (21.24–35.64)				37.16 (30.78–43.54)			
Number								
1	38.66 (34.48–42.84)	0.37			42.92 (39.78–46.06)	0.13	10.90 (1.70–70.03)	0.01
≥2	32.15 (22.94–41.37)				35.58 (27.46–43.70)			
Size								
<3 cm	37.63 (32.92–42.34)	0.84			41.78 (37.87–45.68)	0.93		
≥3 cm	34.13 (27.88–40.38)				37.91 (33.11–42.71)			

HR, hazard ratio; CI, confidence interval; UTUC, upper-tract urothelial carcinoma; NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer.

showed significant association with progression, but only the C ($P=0.04$) was statistically associated with CSS in the BC group by using the Kaplan-Meier survival analysis. These results were consistent with these reports (31–33). In the UTUC group, none of the makers had a significant association with the tumor progression and CSS. Among these makers, Survivin was the only maker analyzed in UTUC before, which did not find prognostic value in these studies (34,35), on the contrary, Survivin was considered as an independent prognostic factor (36). A likelihood of false-negative results may be seen with the small sample size.

Thus a multicenter clinical trial considering more factors in the prognostic analysis in the UC group should be carried out to verify the predictive effect of the biomarkers.

Conclusions

All biomarkers exhibited diagnostic utility in both BC and UTUC, with a better performance in UTUC. The triple combination S+B+C seems to be the most promising model to serve as a valuable noninvasive method in identifying patients with BC and UTUC.

Table 6 Univariate and multivariate of survival analysis in UTUC

Patient variable	PFS (univariate log rank)		PFS (multivariate Cox)		CSS (univariate log rank)	
	Mean survival time (95% CI)	P	HR (95% CI)	P	Mean survival time (95% CI)	P
Gender						
Female	34.39 (28.67–40.11)	0.23			41.57 (37.21–45.93)	0.81
Male	30.94 (23.73–38.14)				41.16 (37.35–44.96)	
Age						
≤60	40.17 (36.89–43.45)	0.07			NA	0.11
>60	30.99 (24.77–37.21)				NA	
Stage						
NMIBC	31.62 (23.90–39.33)	0.88			40.15 (33.97–46.34)	0.84
MIBC	33.33 (27.07–39.58)				40.04 (35.16–44.92)	
Grade						
Low grade	39.90 (33.30–46.49)	0.06	5.86 (1.07–32.24)	0.04	NA	0.03
High grade	27.90 (21.66–34.14)				NA	
Number						
1	35.43 (30.36–40.50)	0.02			40.67 (36.69–44.65)	0.79
≥2	13.60 (5.95–21.25)				33.20 (21.28–45.12)	
Size						
<3 cm	35.23 (28.06–42.39)	0.57			39.11 (32.95–45.28)	0.48
≥3 cm	30.48 (23.98–36.98)				37.83 (33.40–42.25)	

HR, hazard ratio; CI, confidence interval; UTUC, upper-tract urothelial carcinoma; NMIBC, non-muscle-invasive bladder cancer; MIBC, muscle-invasive bladder cancer.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Lanzhou University Second Hospital (approval number: 2016A-058). Written informed consent was obtained from all participants before the samples were collected.

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