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Original Article

Prognostic value of the tumor-stroma ratio in patients with T1 high-grade bladder cancer undergoing transurethral resection of bladder tumor

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Abstract: Tumor-stroma ratio (TSR) has recently been identified as an independent prognostic parameter for several solid tumors. This study aims to evaluate the prognostic value of TSR in T1 high-grade bladder cancer (BC). A cohort of 179 BC patients was included. TSR was estimated on hematoxylin-and-eosin-stained tissue sections from the most invasive part of the primary tumor. With a cutoff value of 50% stroma, patients were classified as TSR-low or TSR-high groups. Correlations between TSR and clinicopathological factors were evaluated. Prognostic values of TSR in T1 high-grade BC were estimated using Cox's regression. The difference in tumor category, along with tumor size and p53 and Ki-67 expressions, between TSR-high and TSR-low groups was statistically significant (all $P<0.05$). The bivariate correlation analysis revealed a positive correlation between Ki-67 expression (r=0.190, $P=0.011$), p53 expression (r=0.326, $P<0.001$), tumor size (r=0.393, $P<0.001$), tumor invasiveness (r=0.327, $P<0.001$), and TSR. Furthermore, the partial correlation analysis showed a positive correlation between tumor invasiveness and TSR (r=0.254, $P=0.001$). For 81 patients with T1 high-grade BC, multivariate analysis revealed that elevated TSR was independently associated with worse recurrence-free survival (RFS) [hazard ratio (HR): 2.140; 95% confidence interval (CI): 1.045-4.380, $P=0.037$] and progression-free survival (PFS) (HR: 3.168; 95% CI: 1.281-7.836, $P=0.013$). Conclusion: TSR is an independent prognostic predictor of clinical outcomes in T1 high-grade BC. This parameter can serve as a new predictive histological characteristic in high-risk BC to improve assessment of prognosis and guide therapy.

Keywords: Tumor-stroma ratio, bladder cancer, prognosis, recurrence, progression

Introduction

Bladder cancer (BC) is the ninth most common cancer worldwide, with more than 380,000 new cases and more than 150,000 deaths annually [1]. Specifically, nearly 75% of patients present with non-muscle-invasive bladder cancer (NMIBC) [2]. The traditional strategy used in treating and predicting the disease outcome for staging BC is based on the TNM classification of the International Union against Cancer (UICC). The standard treatment for NMIBC is transurethral resection of the bladder tumor (TURBT) with intravesical chemotherapy or intravesical bacillus Calmette-Guérin (BCG) immunotherapy [3]. However, the 5-year recurrence and progression rates of NMIBC still range from 31% to 78% and from 1% to 45% [4]. When NMIBC progresses to muscle-invasive bladder cancer (MIBC), the cancer-specific survival is significantly worse compared with patients with MIBC without a history of NMIBC [5]. As proposed in the NMIBC guidelines of the European Association of Urology (EAU), immediate radical cystectomy is recommended to patients with non-muscle-invasive tumor who are at high risk of progression, particularly for T1 high-grade BC [5, 6]. However, other studies confirm that the 10-year RFS rate is similar to that with TURB and BCG maintenance therapy in T1 high-grade BC [7, 8]. Currently, controversies in treatment decisions for T1 high-grade

BC still exist. Thus, additional pretreatment predictors of outcome are being evaluated to further improve the ability to select the appropriate treatment for each individual patient.

Moreover, emerging evidence has indicated that the molecular classification of BC may facilitate individualized treatment and targeted therapy [9, 10]. However, until recently, several molecular stratification systems for BC based on expression patterns of tumor messenger RNA have not provided adequate prognostic or predictive information for clinical application [10].

Currently, no single biomarker can be recommended in daily clinical practice because of the complexity and multistep development of a human tumor [11]. Although multigene prediction models based on tumor cell were proposed to estimate prognosis, these models need to be verified in large clinical samples before they can be widely used in clinical practice [12]. Therefore, intratumor stroma, as an important part of the tumor, should also be included in the evaluation system. The tumor micro-environment plays a crucial role in the initiation, progression, and metastasis of cancers [13]. Recently, the percentage of intratumor stroma has attracted a growing interest in the prediction of tumor prognosis [14]. Tumor-stroma ratio (TSR), as the evaluation index of tumor stroma, has a prognostic value for several solid tumors, including esophageal [15-17], breast [18, 19], and colon cancers [20] and early cervical carcinoma [21, 22] and hepatocellular carcinoma [23]. To our knowledge, the predictive value of TSR has never been explored for BC.

The present study aims to evaluate the prognostic value of TSR in T1 high-grade BC and the relationship of TSR with other prognostic factors.

Patients and methods

Patients and tissue samples

A total of 179 patients with T1-T4a BC who underwent TURBT or radical cystectomy between January 2011 and December 2014 were selected from the database of the Department of Urology of the Shanghai Tenth People's Hospital of Tongji University. All surgical specimens have a confirmed diagnosis of urothelial carcinoma of the bladder. Exclusion criteria included patients with double tumors, other malignancies in the past, death or recurrence of tumor (distant or locoregional) within the 1-month postoperative period, and mixed urothelial carcinoma and other histological subtypes and patients who received neoadjuvant chemotherapy and preoperative radiotherapy. Clinical and pathologic parameters were retrieved from the Medical Records Room. The hematoxylin-and-eosin (H&E)-stained tissue sections of all patients were retrieved from the Department of Pathology. In this study, all patients signed the informed consent, and the protocol was approved by the Ethics Committee of Shanghai Tenth People's Hospital of Tongji University.

Histopathological protocol

On microscopic examination, the 5 μm H&E-stained sections from the primary tumor were routinely analyzed. Using a ×4 objective, the most invasive part of the tumor was selected in all slides. Subsequently, using a ×10 objective, the TSR was visually evaluated at least one microscopic field, where both stroma and tumor were present, and tumor cells were seen on all sides of the image field. In case of tumor heterogeneity, the highest of the stromal percentages was deemed decisive, and the estimate was then recorded as the TSR. A 50% cutoff value was used as determined earlier by Mesker et al., who confirmed that the optimal threshold level of TSR is 50%. Using this protocol, all tissue samples were independently estimated by two pathologists. When the two observers disagreed,the decision of a third pathologist was decisive. The stroma-rich category (the proportion of stroma ≥50%) was defined as TSR high (i.e., ≥50%), whereas stroma poor (the proportion of stroma <50%) was categorized as TSR low (i.e., <50%). Then, TNM staging was done according to UICC guidelines. Tumor grade was classified as moderately differentiated based on the 1973 World Health Organization (WHO) grading system [24]. These specimens were then re-evaluated by a genitourinary pathologist in accordance to the 2004 WHO grading system. The risk stratificationfor T1 high tumors were categorized as high and highest risk for progression, according to the European Association of Urology guidelines [3],

Table 1. Clinicopathologic characteristics of the study cohort

Variables	Total	TSR<50%	TSR≥50%	*P*
		N (%)	N (%)	
Age (years), median (IQR)	68 (59-79)	69 (60-79)	67 (59-80)	0.786*
Sex				0.111
Female	32 (17.9%)	18 (17.3%)	14 (21.1%)	
Male	147 (82.1%)	104 (85.2%)	43 (75.4%)	
Tumor category				<0.001
T1	129 (72.1%)	98 (80.3%)	31 (54.4%)	
T2-4a	50 (27.9%)	24 (19.7%)	26 (45.6%)	
Tumor grade				1.000
Low	13 (7.3%)	9 (7.4%)	4 (7.0%)	
High	166 (92.7%)	113 (92.6%)	53 (93.0%)	
Tumor size				<0.001
<3 cm	76 (42.5%)	68 (55.7%)	8 (14.0%)	
≥3 cm	103 (57.5%)	54 (44.3%)	49 (86.0%)	
Tumor size, median (IQR)	3.0 (2.0-4.0)	2.5 (2.0-3.5)	3.5 (3.0-5.0)	<0.001*
Number of tumors				0.173
Single	103 (57.5%)	66 (54.1%)	37 (64.9%)	
Multiple	76 (42.5%)	56 (45.9%)	20 (35.1%)	
p53 expression				<0.001
Negative	59 (33.0%)	53 (43.4%)	6 (10.5%)	
Positive	120 (67.0%)	69 (56.6%)	51 (89.5%)	
Ki-67 expression				0.011
Negative	47 (26.3%)	39 (32.0%)	8 (14.0%)	
Positive	132 (73.7%)	83 (68.0%)	49 (86.0%)	

TSR: tumor-stroma ratio; *Mann-Whitney U.

based on the number of tumors, tumor size, prior recurrence rate, T category, concurrent carcinoma in situ, and tumor grade.

Immunohistochemical staining of tissue sections was then performed to evaluate the Ki-67 and p53 expression for all tumors. While Ki-67 staining was performed by sections using antibody GM001 (GT2094, Gene Technology, China), p53 staining was done using BP-53-12 antibody (GT2095, Gene Technology, China). Consequently, Ki-67 immunoreactivity was considered positive when samples showed >10% nuclear reactivity, whereas nuclear p53 immunoreactivity was considered positive when samples demonstrated at least 10% nuclear reactivity.

Follow-up and evaluation

All patients with T1 tumors were initially treated with TURBT, and a second TURBT was routinely performed, with the aim of a complete resection of the bladder tumor. A total of 81 patients with T1 high-grade NMIBC were followed up after TURBT every 3 months for 2 years, and every 6 months for another 3 years, followed by annual follow-ups. Postoperative investigations primarily included cystoscopy and urinary cytology, and imaging was performed only if clinically indicated. Missing survival data were obtained by telephone contact. The primary endpoint was RFS and PFS. RFS was defined as the time from the initial TURBT to the date of first recurrence in the bladder, regardless of the tumor stage. PFS was defined as the time from initial TURBT to the date of first increase in T category from T1 to T2 or higher, lymph node (N+) disease, or distant metastasis (M1) based on the criteria for progression in NMIBC recommended by the International Bladder Cancer Group [25].

Statistics

Statistical calculations were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL). Differences in clinical and pathological characteristics of patients were analyzed using the Mann-Whitney *U* test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Intra-observer variability was assessed using the Cohen's kappa coefficient. Univariate and multivariate correlation analyses were performed to evaluate the relationship between TSR and clinicopathological variables. The *P*-values <0.05 (two sided) were considered statistically significant. Survival curves were calculated through the Kaplan-Meier method and compared through the log-rank test. Cox regression models were used to perform univariate and multivariate analyses to

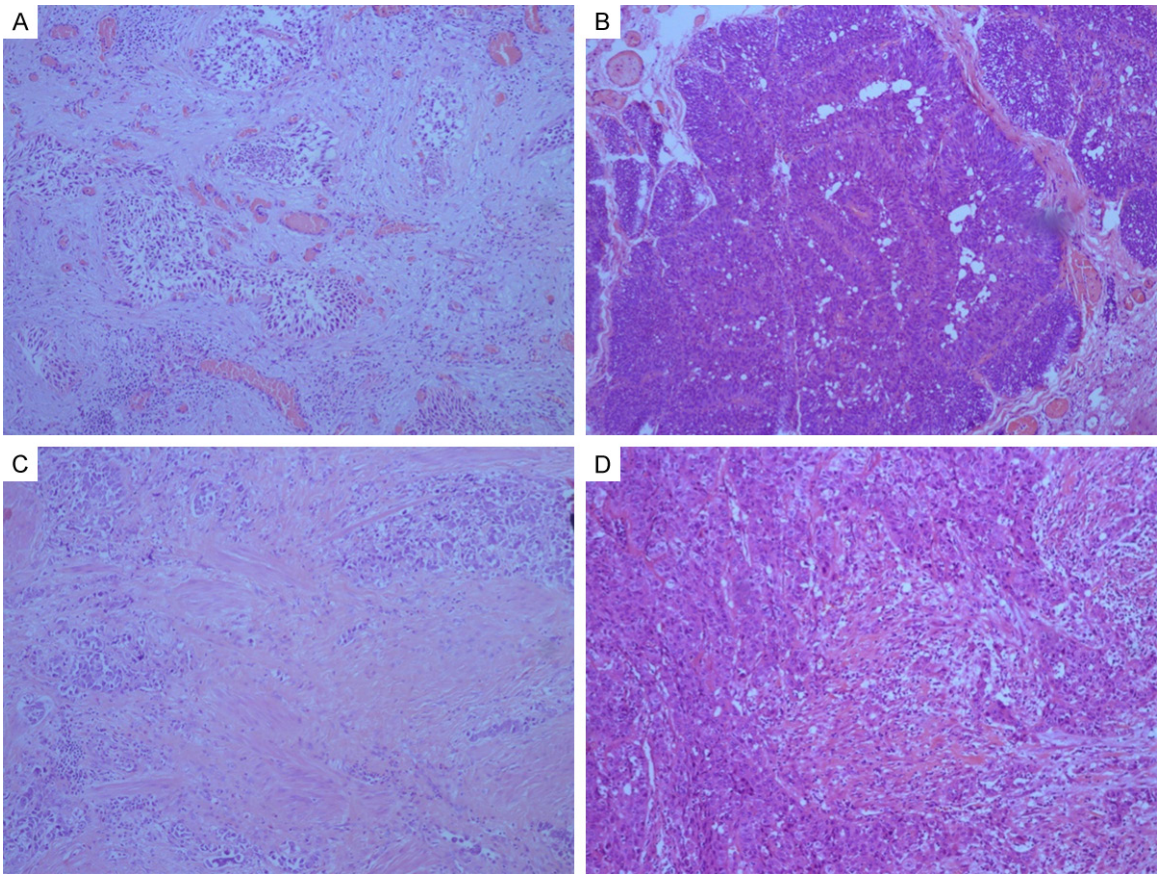

Figure 1. Hematoxylin-and-eosin stained 5 μm sections of bladder cancer (BC) (original magnification ×100). T1 BC: A. TSR high (the proportion of stroma ≥50%), B. TSR low (the proportion of stroma <50%). T2-T4a BC: C. TSR high (the proportion of stroma ≥50%), D. TSR low (the proportion of stroma <50%).

Table 2. Univariate and partial correlation coefficients of TSR with clinicopathologic characteristics

Variables	TSR (Univariate)		TSR (partial)	
	r	*P*	r	*P*
Age	-0.020	0.787		
Sex	-0.119	0.112		
Tumor invasiveness	0.327	<0.001	0.254	0.001
Tumor grade	0.006	0.932		
Tumor size	0.393	<0.001	0.256	0.001
Number of tumors	-0.102	0.175		
p53 expression	0.326	<0.001	0.166	0.028
Ki-67 expression	0.190	0.011	0.006	0.427

TSR: tumor-stroma ratio.

determine the association between explanatory variables, RFS, and PFS. Variables that were introduced in multivariate analysis were thosethat were associated ($P<0.05$) with RFS or PFS in univariate analyses.

Result

Patient characteristics

A total of 179 patients, consisting of 147 males and 32 females, were included in this study. A total of 129 patients (72.1%) had T1 tumor category, and 166 patients (92.7%) had high-grade tumor. In total, 95 patients with stage T1 tumors underwent TURBT, and 34 patients with stage T1 tumors and 50 patients with MIBC underwent radical cystectomy. Clinicopathological characteristics stratified with a cutoff value of 50% TSR are shown in **Table 1**. Out of 179 patients, 98 patients with stage T1 and 24 patients with stage T2-T4a were in the low TSR group, and 31 patients with stage T1 tumors and 26 patients with stage T2-T4a tumors were in the high TSR group (**Figure 1**). Cohen's kappa coefficient showed an almost perfect agreement between the 2 observers who assessed the TSR from tissue sections (kappa =0.818).

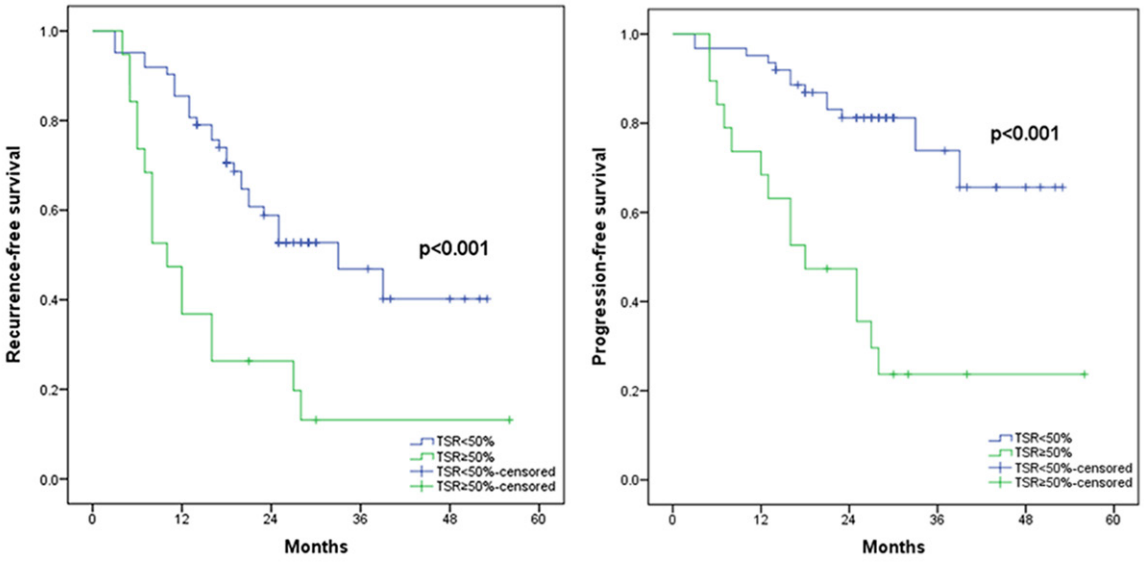

Figure 2. Kaplan-Meier curves for recurrence-free survival and progression-free survival of patients with T1 high-grade bladder cancer undergoing transurethral resection of bladder tumor, stratified through tumor-stroma ratio (TSR).

Associations between TSR and clinicopathological features

Tumor invasiveness (P<0.001), tumor size (P<0.001), and TSR had statistically significant relation. The median tumor size of the TSR-low group was 2.5 cm (range: 0.5-7 cm) and that of the TSR-high group was 3.5 cm (range: 0.8-10 cm). When the tumor size was analyzed as a continuous variable, a positive relation was still found between TSR and tumor size. In terms of p53 and Ki-67, a statistically significant difference between the TSR-low (P<0.001) and TSR-high groups (P=0.011) was noted. The correlation tests revealed a positive correlation between p53 status (r=0.326, P<0.001), Ki-67 status (r=0.190, P=0.011), tumor size (r=0.393, P<0.001), tumor invasiveness (r=0.327, P<0.001), and TSR. Furthermore, the partial correlation test remained a positive correlation between tumor invasiveness and TSR (r=0.254, P=0.001) (**Table 2**).

Survival and multivariate analysis for TSR in T1 high-grade NMIBC

Out of the 95 T1-stage patients with TURBT, the tumor grade of 6 patients was low, and 4 patients were lost in the follow-up. A total of 16 patients with G2 tumors were reassessed by the pathologist according to the 2004 WHO grading system; among them, 12 patients were confirmed to be in high grade, whereas the remainder were in low grade. Thus, the T1 high-grade NMIBC cohort comprised 81 patients for survival analysis. All patients received intravesical chemotherapy. Overall, 45 (55.6%) patients had disease relapse, and 27 (33.3%) patients had disease progression. In the TSR-high group, the 2-year RFS rate was 26.3% versus 58.8% from the TSR-low group. For the PFS, the 2-year survival rates for TSR-high and TSR-low groups were 47.4% and 81.2%. Kaplan-Meier survival analysis showed significantly worse RFS (log-rank test P<0.001) and PFS (log-rank test P<0.001) for the TSR-high group (**Figure 2**). These findings were consistent with the univariate Cox regression analyses. High TSR (HR: 3.090; 95% CI: 1.666-5.730, P<0.001), tumor size (HR: 3.170; 95% CI: 1.744-5.760, P<0.001), prior recurrence history (HR: 2.409; 95% CI: 1.011-5.742, P=0.047), concurrent CIS (HR: 9.822; 95% CI: 2.180-44.252, P=0.003), p53 expression (HR: 2.238; 95% CI: 1.062-4.715, P=0.034), and Ki-67 expression (HR: 4.092; 95% CI: 1.597-10.486, P=0.003) were associated with worse RFS (**Table 3**). Meanwhile, age (HR: 1.042; 95% CI: 1.005-1.081, P=0.026), high TSR (HR: 4.767; 95% CI: 2.235-10.169, P<0.001), tumor size (HR: 4.725; 95% CI: 2.048-10.902, P<0.001), prior recurrence history (HR: 3.610; 95% CI: 1.335-9.762, P=0.011), concurrent CIS (HR: 4.385; 95% CI:

Table 3. Univariate and multivariate Cox regression analyses for recurrence-free survival in T1 high-grade bladder cancer

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.018 (0.992-1.045)	0.185		
Sex	0.884 (0.437-1.790)	0.732		
Tumor size (≥3 cm vs. <3 cm)	3.170 (1.744-5.760)	<0.001	2.259 (1.159-4.404)	0.017
Number of tumors (multiple vs. single)	0.889 (0.491-1.609)	0.697		
Prior recurrence history	2.409 (1.011-5.742)	0.047	1.661 (0.578-4.778)	0.346
Concurrent CIS	9.822 (2.180-44.252)	0.003	2.210 (0.354-13.815)	0.396
TSR (≥50% vs. <50%)	3.090 (1.666-5.730)	<0.001	2.140 (1.045-4.380)	0.037
p53 expression (positive vs. negative)	2.238 (1.062-4.715)	0.034	1.557 (0.716-3.383)	0.264
Ki-67 expression (positive vs. negative)	4.092 (1.597-10.486)	0.003	3.157 (1.185-8.408)	0.021

CIS: carcinoma in situ, *TSR:* tumor-stroma ratio.

Table 4. Univariate and multivariate Cox regression analyses for progression-free survival in T1 high-grade bladder cancer

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.042 (1.005-1.081)	0.026	1.014 (0.975-1.054)	0.496
Sex	0.584 (0.246-1.389)	0.224		
Tumor size (≥3 cm vs. <3 cm)	4.725 (2.048-10.902)	<0.001	3.616 (1.420-9.207)	0.007
Number of tumors (multiple vs. single)	1.786 (0.829-3.845)	0.138		
Prior recurrence history	3.610 (1.335-9.762)	0.011	5.607 (1.493-21.053)	0.011
Concurrent CIS	4.385 (1.025-18.762)	0.046	0.203 (0.028-1.495)	0.118
TSR (≥50% vs. <50%)	4.767 (2.235-10.169)	<0.001	3.168 (1.281-7.836)	0.013
p53 expression (positive vs. negative)	4.370 (1.308-14.604)	0.017	3.798 (1.066-13.530)	0.039
Ki-67 expression (positive vs. negative)	3.326 (0.996-11.112)	0.051		

CIS: carcinoma in situ, *TSR:* tumor-stroma ratio.

1.025-18.762, P=0.046), and p53 expression (HR: 4.370; 95% CI: 1.308-14.604, P=0.017) were associated with worse PFS (**Table 4**). In multivariate analyses, after adjusting for prior recurrence history, concurrent CIS, and p53 expression, TSR (HR: 2.140; 95% CI: 1.045-4.380, P=0.037) was an independent prognostic factor for RFS, along with tumor size (HR: 2.259; 95% CI: 1.159-4.404, P=0.017) and Ki-67 expression (HR: 3.157; 95% CI: 1.185-8.408, *P*=0.021) (**Table 3**). After adjusting for age and concurrent CIS, TSR (HR: 3.168; 95% CI: 1.281-7.836, P=0.013), prior recurrence history (HR: 5.607; 95% CI: 1.493-21.053, P=0.011), tumor size (HR: 3.616; 95% CI: 1.420-9.207, P=0.007), and p53 expression (HR: 3.798; 95% CI: 1.066-13.530, *P*=0.039) remained to be independent prognostic factors for PFS (**Table 4**).

Prediction accuracy of risk stratification based on TSR combined with the risk stratification of the European Association of Urology guidelines

To identify the highest-risk patients with T1 high-grade tumors who might benefit from radical cystectomy, the EAU guidelines proposed several high-risk criteria for clinical implementation. These criteria include the number of tumors, tumor diameter, prior recurrence rate, tumor category, concurrent CIS, and tumor grade. We compared the efficiency of the risk stratification system in the identification of highest-risk patients with TSR parameter. Based on the risk stratification tables, 67% of 81 patients were classified as highest risk. With the addition of TSR parameter to the risk tables, 73% of these patients were classified

as highest risk. The addition of TSR parameter improved the false-negative rate of the risk stratification system, (i.e., number of patients who were not classified as highest risk by the risk stratification system, but indeed developed disease recurrence or progression in the follow-up period. In conclusion, the 1-year recurrence and 1-year progression rates were correctly predicted to increase, as evidenced by 9.5% and 11.1% increase, when using the risk category combined with TSR parameter.

Discussion

In this study, we found that TSR was associated with advanced pathologic category in BC. The study also showed that TSR was a significant predictor of RFS and PFS in the T1 high-grade BC cohort. These findings remained significant after adjusting for clinicopathological factors, suggesting an independent association of elevated TSR with worse oncologic outcomes in patients with T1 high-grade BC treated with TURBT. Furthermore, this parameter can be used in combination with the tumor size and Ki-67 expression to identify patients with a relatively worse prognosis. With the addition of TSR parameter to the risk stratification, the risk stratification system improved the prediction accuracy for recurrence and progression rates in T1 high-grade BC. Thus, considering immediate radical cystectomy to these high-risk patients is reasonable.

In addition to tumor cell characteristics, tumor stroma, as the main part of the tumor microenvironment, might frequently be associated with different stages of tumor development, including tumorigenesis, progression, invasion, and metastasis [13]. Bioactive molecules secreted by stroma cells play important roles on the whole tumor development by regulating the growth, progression, and differentiation of all cell types, including cancer cells, fibroblasts, endothelial cells, immune inflammatory cells, and pericytes, in the tumor microenvironment [11].

TSR was initially defined by Meske et al. [14]. A 50% cutoff level was determined to be the optimal threshold value of TSR on the basis of a maximum discriminating power for overall survival and disease-free survival. Subsequently, TSR, an inexpensive, reproducible, and readily available tumor tissue indicating intratumor stroma percentage, has a prognostic value in a variety of cancers according to diverse studies. A high intratumor stroma percentage is associated with poor prognosis in colorectal [20], esophageal [15-17], and breast cancer [18, 19] and early cervical carcinoma [21, 22] and hepatocellular carcinoma [23]. In addition, cancer-associated fibroblasts and leukocyte infiltration into tumors, including neutrophils, macrophages, mast cells, B and T cells, have also been shown to play a key role in tumor cell invasion and may serve as potential prognostic factors for BC [26-28]. However, the value of TSR in BC remained poor, despite the potential clinical utility of TSR in risk stratification and treatment decision. This study demonstrates that TSR is an independent prognostic predictor of clinical outcomes in patients with BC following TURBT. Our results show that Cohen's kappa coefficient was 0.818, showing that the observations of two pathologists were in perfect agreement.

The relationship between tumor stroma, recurrence, and progression is complex and yet remains to be elucidated. The tumor-associated stroma includes the extracellular matrix, immune cells, endothelial cells, and cancer-associated fibroblasts [11]. These different cell types in the stromal environment can be recruited by malignant cells to support tumor growth and facilitate metastatic dissemination [11]. Studies have suggested that tumor invasion occurs in the microenvironment, where tumor cells and stroma exchange cytokines and enzymes. For instance, paracrine cytokines play an important role in promoting invasive and migratory capacities, including various growth factors, cytokines, and angiogenic molecules in the tumor microenvironment [29]. Furthermore, the principal enzymes, including the matrix metalloproteinases, tissue serine proteases, and adamalysin-related membrane proteinases, secreted by host stroma cells are essential for tumor invasion [30]. In addition, cancer-induced immune suppression and tumor-associated inflammation, resulting from the interactions between cancer cells and stromal compartment, facilitate the activation of epithelial-mesenchymal transition (EMT), angiogenesis, invasion, and metastasis [31, 32]. Thus, the stroma around tumor cells does not only act as a support for the tumor cells, but also actively stimulates tumor cell growth, differentiation, and movement.

The study limitations include its retrospective and nonrandomized nature, which may have led to a recall bias. The sample size of this study is also relatively small, and the follow-up time is short. Furthermore, this is a single-institution study and, as such, requires external validation. Finally, despite the use of standard treatment protocols, information regarding the drug of intravesical instillations was lacking. However, to our knowledge, this is the first study to assess the prognostic values of TSR in patients with NMIBC undergoing TURBT. We confirmed that TSR is a potential prognostic marker for the prediction of disease progression and recurrence of T1 high-grade BC. This parameter warrants further validation as a potential selection criterion for risk factor-stratified patient management in NMIBC.

In conclusion, TSR seems to be an independent predictor of disease progression and recurrence in high-risk NMIBC patients. TSR shows promising usefulness in the management of patients with NMIBC, both for improved assessment of prognosis and guidance of therapy. Large-scale prospective and multi-institution studies are warranted to validate our findings.

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Informed consent was obtained from all individual participants included in the study.

Disclosure of conflictof interest

None.

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