



En bloc resection improves the identification of muscularis mucosae in non-muscle invasive bladder cancer

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Abstract

Purpose The T1 substage, according to the relationship between muscularis mucosae (MM) and tumors, is a promising prognostic factor for T1 bladder cancer. However, the identification rate of MM is low in specimens, and it is, therefore, not widely used in clinical practice. In this study, we investigated whether en bloc resection of non-muscle invasive bladder cancer (NMIBC) could improve the identification of muscularis mucosae (MM), which may further accurate identification of the T1 substage.

Patients and methods Specimens from 158 patients with primary NMIBC were retrospectively reviewed by two independent pathologists to assess the presence of MM and stratify the T1 substage. Of 158 specimens, 70 specimens were obtained via TURBt with a plasma kinetic loop and 88 were obtained via front-firing potassium-titanyl-phosphate (KTP) green-light laser en bloc resection. Univariable and multivariable logistic regression models were used to analyze the relationship between the clinical characteristics and the presence of MM.

Results The mean age was 58.22 years (range 18–85 years). Multivariable logistic regression analysis showed that the KTP laser resection method was associated with the presence of MM in specimens ($P=0.008$). In addition, tumors with smaller sizes, which could also be en bloc resected with TURBt (e.g., ≤ 1 cm), had a higher presence of MM ($P=0.047$).

Conclusions En bloc resection improves the identification rate of MM, which may enhance the accurate identification of the T1 substage.

Keywords T1 substage · En bloc resection · Muscularis mucosae · Non-muscle invasive bladder cancer

Introduction

Bladder cancer is the 10th most common form of cancer worldwide [1], and up to 80% of the cases are non-muscle invasive bladder cancers (NMIBCs). The standard treatment is transurethral resection of a bladder tumor (TURBt)

followed by intravesical therapy. However, there is a relatively high recurrence and progression rate, which emphasizes the importance of predictive parameters before surgery. Indeed, conventional parameters listed in the EORTC table [2], such as tumor grade, stage, numbers, diameter, concurrent CIS, and prior recurrence rate, help predict the prognosis of patients with bladder cancer, but even these parameters cannot accurately predict patient outcome, especially for patients with T1 tumors [3].

To predict patients' outcomes accurately, the T1 substage was studied, and it was determined to be another promising prognostic factor for T1 bladder cancer. Two common systems of determining T1 substage, termed T1a/b/c and T1m/e, respectively, were applied in clinical studies. In detail, the T1a/b/c system was used according to the position of muscularis mucosae (MM), which consists of discontinuous smooth muscle bundles in the submucosa of the bladder wall and invasion in front of the tumor. T1a is defined as the invasion front of the tumor above the MM; T1b is defined as

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tumor invasion to the MM; and T1c is defined as tumor invasion across the MM. The T1m/e system was used according to the number or diameter of microinvasive tumors in the submucosa. However, neither of these two kinds of systems is better than the other, because both of them have drawbacks. The main drawback of the T1a–c system is the low identification rate of MM in specimens, which hinders its clinical application and weakens its advantage over the other system, even though it has been studied for nearly 30 years [4–6]. Can we overcome this problem?

The main contributors to the low identification rate of MM in specimens are the limited experience of pathologists, the fragmentation and/or the coagulation of tumor tissues from TURBt, and the discontinuous nature of MM [7]. In our previous study, we developed a novel technique to en bloc enucleate NMIBC with a front-firing green-light laser, which could maintain the integrity and architecture of tumor specimens [8]. Whether en bloc resection could improve the identification rate of MM is still unknown. Therefore, we performed a retrospective study to detect its potential role in stratification of the T1 substage for NMIBC.

Patients and methods

Patients

From January 2017 to December 2017, 203 specimens from patients with primary NMIBC were collected in the First Affiliated Hospital of Xi'an Jiaotong University. Cases were confirmed as NMIBC after review of the entire specimen, and 45 specimens without muscularis propria were excluded. All specimens were reviewed by two independent pathologists who were blind to the clinical information of the patients. All patients underwent either TURBt or green laser en bloc resection (see “Surgical methods”), and pirarubicin (30 mg/50 ml) was chosen for intravesical instillation.

Surgical methods

In this study, front-firing potassium-titanyl-phosphate (KTP) green-light laser en bloc resection was chosen to complete the laser en bloc resection, which was reported in our previous study [8]. TURBt was performed by stratified resection with an Olympus plasma kinetic loop. However, en bloc resection could also be completed by TURBt in smaller tumors.

Statistical analyses

Fisher's exact test and the Chi-squared test were performed to assess the difference between TURBt and KTP lasers. Univariable and multivariable logistic regression models

were used to evaluate the association between clinicopathological features or surgical methods and the identification rate of MM. All tests were two-sided, and statistical significance was defined as $P < 0.05$. SPSS v18.0 was used for analysis (IBM Corp, Armonk, NY, USA).

Results

In Fig. 1, representative pictures of H&E staining are shown, including an integrated specimen obtained from the KTP laser (A) and a fragmented specimen from TURBt (B). MM could then be easily identified, which facilitated the stratification of the T1 substage for NMIBC.

Of the 158 patients, the mean patient age was 58.22 years (range 18–85 years). The detailed information is shown in Table 1. Most of the clinical characteristics showed no difference between the KTP laser and TURBt methods, except for sex. Univariable and multivariable logistic regression models for analyzing the association between clinic characteristics and the presence of MM in tumor specimens are shown in Table 2. In univariable analysis, the KTP laser group, a higher tumor grade, and stage were associated with an increased identification rate of MM ($P=0.005$, $P=0.024$, and $P=0.028$, respectively). After adjusting the effects of tumor stage in the multivariable analysis, a higher tumor grade and KTP laser were associated with an increased identification rate of MM ($P=0.043$ and $P=0.008$, respectively).

In addition, the relationship between tumor size and MM in bladder specimens is shown in Table 3. Despite TURBt, tumors with smaller sizes, such as ≤ 1 cm, had a higher presence rate of MM in the specimens ($P=0.047$), which may be due to small tumors also being en bloc resected. However, tumor size has no association with the presence of MM in specimens from the KTP laser, regardless of the cutoff for tumor size, since all tumors were en bloc resected with the KTP laser.

Discussion

Numerous studies have previously shown that substage T1, depending on the location of MM in the submucosa, has an association with the prognostics of bladder cancer [9]. However, mainly due to the low identification rate of MM, the substage of T1 was not included in the TNM staging system for bladder cancer and is not widely used in clinical practice. Although some studies recommended the vascular plexus instead of MM when MM was not present in the surgical specimens, the value of the vascular plexus was suspected due to the discrepancy of their exact location in the submucosa and their anatomical relationship to MM in the literature [10, 11].

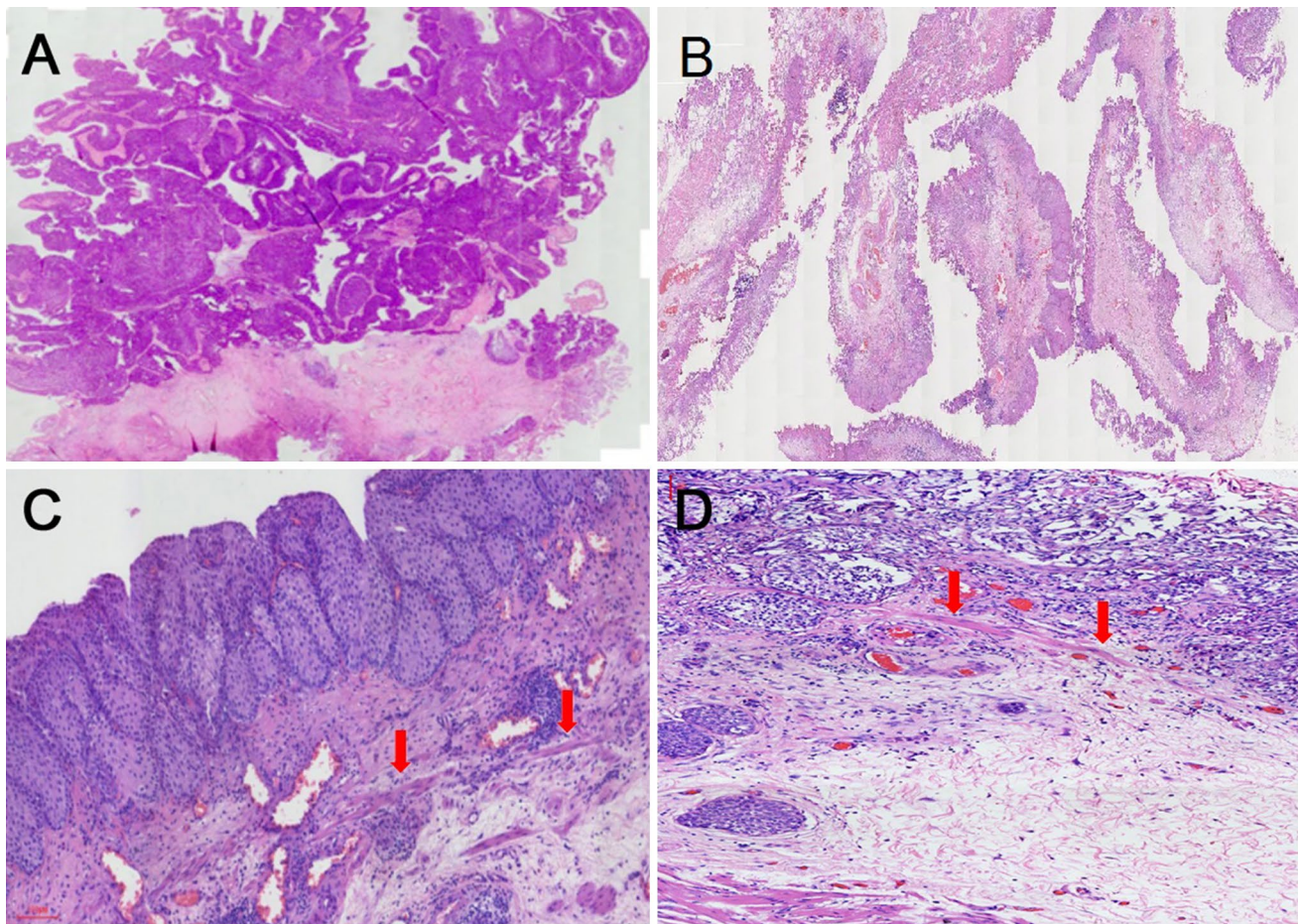


Fig. 1 **a, b**, H&E photograph of specimen from KTP laser en bloc resection (**a**) and TURBt (**b**), magnification 20 \times . **c, d** H&E photograph of specimen from KTP laser en bloc resection, magnification 100 \times . **c** was classified as T1a, and **d** was T1c

To solve the problem with MM, two other substage systems were developed, which depended on the depth or the diameter (T1m/T1e) of tumor invasion in the lamina propria [12, 13]. However, both systems lacking objective criteria, such as MM, were not standardized and were highly dependent on the subjective view of the pathologist [14]. Therefore, the substage system based on the MM was more promising, and now, the major problem to be solved is how to improve the identification rate of MM in tumor specimens.

To our knowledge, this is the first study to investigate a strategy to increase the identification rate of MM. The low identification rate of MM could be explained by the experience of the pathologist, the TURBt surgery process which could have fragmented and/or coagulated tumor specimens, and the discontinuous nature of MM. The advantages of laser en bloc resection for bladder cancer have been confirmed by many studies, such as maintaining the tumor integrity to prevent the fragmentation of the tumor tissue and a inducing high amount of floating cancer cells; improving the presentation rate of the detrusor muscle in the specimen;

and enhancing the accuracy of pathological staging [15]. Since *en bloc* resection could maintain the tumor tissue integrity, we suspected that it could overcome the discontinuous nature of MM, which might be further fragmented by the TURBt procedure, and, ultimately, improve the identification rate of MM.

The safety and effectiveness of the KTP laser have been confirmed in previous works [8, 16]. Furthermore, the same conclusion was reached from two trials comparing the KTP laser with TURBt in the treatment of NMIBC [17, 18]. In this retrospective study, the KTP laser was chosen to complete en bloc resection of bladder cancer regardless of the tumor size, and then, we compared the outcome with TURBt. We found that the KTP laser was associated with a higher identification rate of MM in both univariable and multivariable logistic regression analyses. In addition, despite the coagulation and/or fragmentation of specimens from TURBt, en bloc resection was also completed by TURBt when the tumor size was ≤ 1 cm, which further confirmed the superiority of en bloc resection in terms of

Table 1 Comparison of Characteristics between TURBt and KTP laser

Characteristic	Cohort (N=158)	TURBt (N = 70)	KTP laser (N = 88)	P
Age, years, n (%)				0.101
< 75	142 (89.9)	66 (94.3)	76 (86.4)	
≥ 75	16 (10.1)	4 (5.7)	12 (13.6)	
Sex, n (%)				0.011
Male	129 (81.6)	51 (72.9)	78 (88.6)	
Female	29 (18.4)	19 (27.1)	10 (11.4)	
Grade, n (%)				0.182
High	68 (43.0)	26 (37.1)	42 (47.7)	
Low	90 (57.0)	44 (62.9)	46 (52.3)	
Stage, n (%)				0.07
T1	96 (60.8)	37 (52.9)	59 (67.0)	0.07
Ta	62 (39.2)	33 (47.1)	29 (33.0)	
Tumor numbers, n (%)				0.560
Single	116 (73.4)	53 (75.7)	63 (71.6)	
Multiple	42 (26.6)	17 (24.3)	25 (28.4)	
Size, cm, n (%)				0.483
< 2 cm	115 (72.8)	49 (70.0)	66 (75.0)	
≥ 2 cm	43 (27.2)	21 (30.0)	22 (25.0)	
Concomitant CIS, n (%)				0.324
Yes	37 (23.4)	19 (27.1)	18 (20.5)	
No	121 (76.6)	51 (72.9)	70 (79.5)	

Table 2 Univariable and multivariable analyses assessing the relationship between the clinic characteristics and MM present in NMIBC

Characteristic	Univariable			Multivariable		
	OR	95% CI	P	OR	95% CI	P
Age (≥ 75 vs. < 75)	2.338	0.785–6.962	0.127			
Sex (Female vs. Male)	0.688	0.241–1.958	0.483			
Grade (High vs. Low)	2.427	1.125–5.235	0.024	2.252	1.025–4.950	0.043
Stage (T1 vs. Ta)	2.639	1.111–6.289	0.028	1.727	0.618–4.831	0.297
Numbers (Multiple vs. Single)	1.617	0.719–3.637	0.245			
Size (≥ 2 cm vs. < 2 cm)	0.604	0.242–1.508	0.280			
Surgical						
KTP laser vs. TURBt	3.430	1.445–8.143	0.005	3.241	1.352–7.770	0.008
Concomitant CIS						
Yes vs. no	1.422	0.609–3.323	0.416			

Table 3 The relationship between tumor size and the presence of MM in bladder specimens

Tumor size (cm)	The presence of MM in TURBt, n(%)		P ^a	The presence of MM in KTP laser, n(%)		P
	Yes	No		Yes	No	
Size (n, %)			0.095			0.894
> 2	0(0)	21(100)		7(31.8)	15(68.2)	
≤ 2	8(16.3)	41(83.7)		20(30.3)	46(69.7)	
Size (n, %)			0.047			0.148
> 1	3(6.1)	46(93.9)		24(34.3)	46(65.7)	
≤ 1	5(23.8)	16(76.2)		3(16.7)	15(83.3)	

^aFisher's exact test was used

the identification of MM. However, the role of KTP laser in transurethral resection of bladder cancer is still controversial due to the limited cases reported. Therefore, more cases are needed to further confirm our conclusion whether KTP laser can improve the identification of muscularis mucosae in en bloc bladder cancer specimens.

Although the identification rate of MM in KTP laser specimens was improved, this did not confirm that the accuracy in identifying substage T1 also improved, since some specimens had no tumor cells around the MM or elsewhere. To answer this question, all pT1 specimens in which the MM was present were reviewed by two independent pathologists to identify substage T1. However, this work was not finished due to the samples were too small to finish the kappa test between these two independent pathologists in terms of staging for substage T1. Despite the limitations of this study, we still hypothesized that en bloc resection of the tumor may improve the accuracy of identifying substage T1, because the presence rate of MM could be improved by en bloc resection, and MM is the cornerstone of the estimation of the T1 substage. This hypothesis, however, still needs further confirmation in a large study in the future.

In addition to the small samples of this study, other limitations exist because of the retrospective analysis methods. For example, patients with recurrence tumors were excluded, which resulted in the absence of analysis regarding whether a scar of the bladder wall can influence the identification of MM. In addition, we did not review the bladder cystectomy specimens to further confirm the value of en bloc resection; however, Ro and his colleagues showed that the identification rate of MM in cystectomy specimens was as high as 94% [5].

Conclusions

The T1 substage, one of the most promising parameters in predicting the prognosis of NMIBC, was not widely used in clinical practice. This study suggests that en bloc resection, which maintains tumor integrity, could improve the identification rate of MM, and may facilitate the stratification of the T1 substage in NMIBC specimens.

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Author contributions JHF: perform operation, project development, and manuscript editing. HL: data collection, manuscript writing, view pathological specimens. TY: data collection and manuscript writing.

KJW: project development and manuscript writing. DLH: manuscript editing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval For this type of study, formal consent is not required.

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