BMC Urology

RESEARCH

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The role of tumor-infiltrating lymphocytes and depth of invasion in T1 bladder cancer: a retrospective analysis

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Abstract

Background Tumor-infiltrating lymphocytes (TILs) are integral components of the tumor microenvironment and have been extensively studied across various cancers. In bladder cancer, the prognostic significance of TILs remains uncertain. This study aimed to analyze the impact of muscularis mucosa invasion and TILs ratios on clinical outcomes, tumor aggressiveness, and prognosis in pT1 urothelial carcinoma of the bladder.

Methods This retrospective study included 154 patients with pT1 bladder urothelial carcinoma, categorized into pT1a and pT1b groups based on the extent of muscularis mucosa invasion. TILs ratios were stratified into three groups: <1%, 1–5%, and > 5%. Clinical and pathological characteristics, including tumor diameter, necrosis, tumor thickness, recurrence, and progression rates, were compared. Depth of invasion and tumor features were assessed using standard histopathological methods. Recurrence and progression-free survival (PFS) were evaluated during a median follow-up of 20 months.

Results By definition, pT1b tumors demonstrated significantly deeper invasion into the muscularis mucosa ($p < 0.0001$) compared to pT1a tumors. They were also associated with larger tumor diameter ($p = 0.023$), greater tumor thickness ($p < 0.0001$), and increased necrosis ($p = 0.0012$). Radical cystectomy was performed more frequently in pT1b patients (19.2%) than in pT1a patients (7.9%), although recurrence and PFS rates did not differ significantly between the groups. Higher TILs ratios were linked to increased tumor thickness ($p = 0.0299$) and more extensive invasion into TURBT chips ($p = 0.0025$), but no significant differences in recurrence or PFS rates were observed across TILs groups.

Conclusions Muscularis mucosa invasion is a defining feature of pT1b bladder cancer and serves as a key indicator of tumor aggressiveness. While TILs were associated with aggressive tumor characteristics, their prognostic role remains inconclusive. These findings highlight the need for aggressive management strategies for pT1b disease and underscore the importance of further research into the complex interplay between TILs and tumor progression in bladder cancer.

Keywords Bladder Cancer, Urothelial carcinoma, Tumor-infiltrating lymphocytes, Clinical outcomes, Prognosis

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Introduction

Bladder cancer is among the most common malignancies of the urinary tract, with non-muscle invasive bladder cancer (NMIBC) presenting significant clinical management challenges. Approximately 20% of newly diagnosed bladder cancer cases demonstrate lamina propria invasion, classified as pT1 urothelial carcinoma [1–3]. These tumors are biologically heterogeneous and exhibit variable prognoses, with many patients experiencing disease recurrence or progression despite initial treatment. While about 30% of pT1 patients do not experience recurrence after initial transurethral resection of bladder tumor (TURBT), up to 50% may progress within five years, necessitating more aggressive therapeutic strategies such as Bacillus Calmette-Guérin (BCG) immunotherapy or, in some cases, early radical cystectomy [3, 4].

Accurately identifying high-risk pT1 bladder tumors remains a critical challenge. Several pathological factors have been linked to worse outcomes, including the depth of lamina propria invasion, involvement of the muscularis mucosa, tumor size, and lymphovascular invasion [4, 5]. Notably, deeper invasion into the lamina propria, particularly beyond the muscularis mucosa (classified as pT1b), is a defining feature of tumor aggressiveness and is associated with an elevated risk of recurrence and progression [3, 5]. The distinction between pT1a and pT1b tumors is based on the extent of invasion, with pT1b tumors, by definition, demonstrating greater invasion depth. This pathological subclassification is crucial for stratifying patient risk and guiding treatment decisions.

The tumor immune microenvironment, especially tumor-infiltrating lymphocytes (TILs), has gained attention as a potential prognostic tool in various cancers. TILs, representing the host immune response to the tumor, have been associated with improved outcomes in several malignancies [6]. However, their prognostic significance in bladder cancer remains controversial. While some studies have reported a correlation between higher TIL densities and deeper tumor invasion or more aggressive disease, others have found no significant relationship with clinical outcomes [5–7]. Specifically, in high-grade pT1 bladder cancer, stromal TILs have been linked to increased invasion depth but have not been shown to independently predict cancer-specific survival [7].

In this single-center retrospective study, we aimed to analyze the clinical and pathological characteristics of pT1 bladder cancer with a focus on two key factors: the depth of invasion into the lamina propria and muscularis mucosa, and the role of TILs. By investigating these parameters and their correlation with outcomes such as tumor recurrence and progression, we sought to provide further insights into the prognostic landscape of pT1 bladder cancer. This study aims to refine risk stratification for pT1 bladder cancer and inform personalized treatment approaches for these patients.

Materials and methods

Study design and patient selection

This retrospective study included 154 patients diagnosed with pT1 urothelial carcinoma of the bladder who underwent transurethral resection of bladder tumor (TURBT) at our institution between January 2015 and December 2022. Patients were selected based on the availability of complete clinical data, histopathological reports, and follow-up records. Among the 200 patients screened, 46 were excluded due to insufficient histological material ($n = 15$), presence of muscle-invasive bladder cancer (≥ pT2, $n = 18$), or incomplete clinical data ($n = 13$). Ultimately, 154 cases of high-grade pure urothelial carcinoma were included in the study. Tumors with variant histology (e.g., micropapillary, plasmacytoid, nested variants) or non-urothelial differentiation were excluded during pathological review. All tumors were graded according to the 2022 World Health Organization (WHO) classification of urinary and male genital tumours (5th edition, Volume 8), and staging was performed using the 8th edition of the American Joint Committee on Cancer (AJCC) TNM staging system. The study protocol was approved by the Local Ethical Committee of Non-invasive Clinical Research (Approval No: KU GOKAEK-2024/18.22). Written informed consent was obtained from all participants, and the study was conducted in compliance with the principles of the Declaration of Helsinki.

Histopathological evaluation

Bladder tumor specimens were formalin-fixed and paraffin-embedded (FFPE) prior to histopathological analysis. Hematoxylin and eosin (H&E)-stained slides were reviewed independently by two experienced pathologists blinded to clinical outcomes.

- The depth of lamina propria invasion was measured using a calibrated ocular micrometer.
- The extent of muscularis mucosa invasion, when present, was recorded in micrometers.
- Tumor thickness, the number of tissue fragments containing invasion, and lymphovascular invasion were documented.
- Tumor diameter was categorized as < 3 cm, 3–5 cm, or > 5 cm.
- The measurements for depth of invasion into the lamina propria and muscularis mucosa were reported as both mean and median values in micrometers [8]. Representative images of the pathological features are provided in Fig. 1.

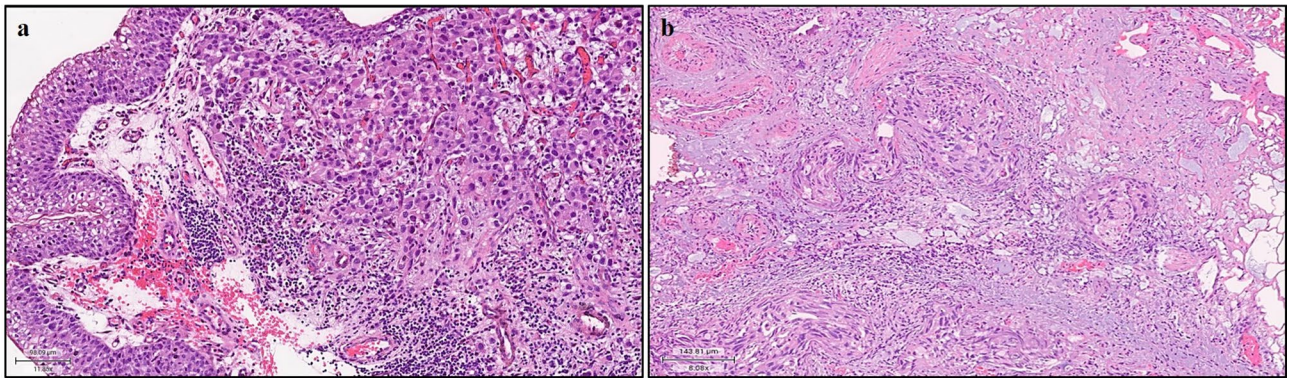


Fig. 1 **a**. Hematoxylin and eosin (H&E)-stained section showing neoplastic cells arranged in irregular nests and single cells invading the lamina propria. **b.** H&E-stained section illustrating deeper tumor invasion extending into the muscularis mucosa

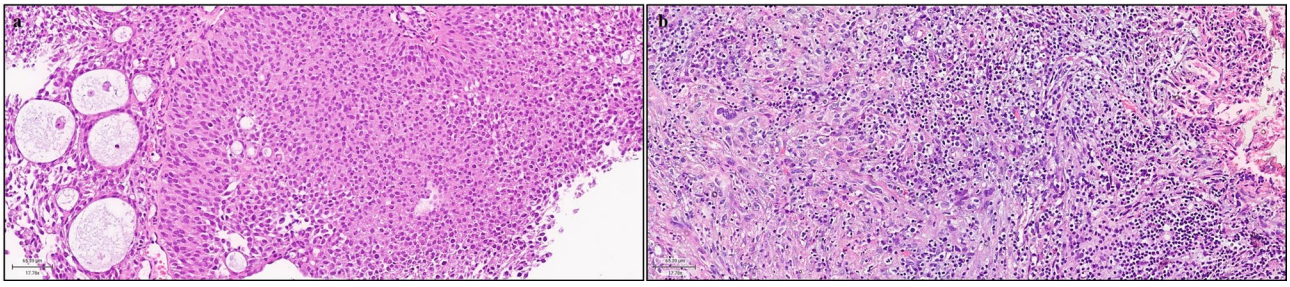


Fig. 2 **a**. H&E-stained section demonstrating low density of tumor-infiltrating lymphocytes (TILs) within the tumor stroma. **b.** H&E-stained section showing high density of TILs infiltrating the tumor microenvironment

Tumor-infiltrating lymphocytes (TILs) assessment

Tumor-infiltrating lymphocytes (TILs) were evaluated on H&E-stained sections in the stromal areas adjacent to invasive tumor foci. Areas with necrosis or significant artifacts were excluded from analysis. The percentage of the stromal area occupied by TILs was semi-quantitatively estimated by two independent pathologists blinded to patient outcomes, and any discrepancies in categorization were resolved through joint review and consensus. TILs were categorized into three groups: <1%, 1–5%, and >5% infiltration. These cut-off values were selected based on previous literature suggesting their prognostic relevance in urothelial and other epithelial malignancies [6, 7], aiming to balance practicality in routine pathology with clinical applicability. Representative images for TIL categorization are shown in Fig. 2.

Clinical data collection

Patient demographic and clinical data, including age, gender, smoking status, and body mass index (BMI), were obtained from electronic medical records. Clinical outcomes, including recurrence, progression, and survival, were also documented. Recurrence was defined as histologically confirmed bladder cancer detected after the initial TURBT. Progression was defined as the development of muscle-invasive bladder cancer. Patients were followed for a median of 20 months (range: 12–84 months), with routine cystoscopic evaluations performed every three months during the first two years and every six months thereafter.

Statistical analysis

All statistical analyses were performed using GraphPad Prism. Descriptive statistics were presented as mean ± standard deviation, median, and range. Normality of data distribution was tested using the Kolmogorov-Smirnov test. For normally distributed data, comparisons between two groups were conducted using the Student's t-test, and comparisons between three or more groups were analyzed using one-way ANOVA with Tukey-Kramer post-hoc tests. For non-normally distributed data, Mann-Whitney U and Kruskal-Wallis tests were used, with Dunn's test for post-hoc comparisons. Multivariable analysis was performed using logistic regression to assess the independent prognostic significance of histopathological parameters and TILs. Statistical significance was set at $p < 0.05$, with a 95% confidence interval (CI).

Results

Clinical and prognostic characteristics of pT1 urothelial carcinoma according to muscularis mucosa invasion

A total of 154 patients with pT1 bladder urothelial carcinoma were included, with 76 classified as pT1a and 78 as

pT1b (Table 1). The mean age of the cohort was 70.4 ± 9.2 years, with no significant difference between the pT1a (71.1 ± 8.5 years) and pT1b (69.8 ± 9.8 years) groups ($p = 0.557$). Most patients were male (87.7%), and 44.8% reported smoking history, with no significant differences in gender distribution ($p = 0.358$) or smoking status ($p = 0.987$) between groups. Body mass index (BMI) were also similar ($p = 0.561$).

Radical cystectomy was more frequently performed in pT1b patients (19.2%) than in pT1a patients (7.9%), although this difference did not reach statistical significance ($p = 0.069$). The overall recurrence rate was 79.2%, with no significant difference between the pT1a (76.3%) and pT1b (82.1%) groups ($p = 0.497$). The median time to recurrence was 5 months (range, 0–60 months). The overall progression rate was 23.4%, again with no significant difference between pT1a (19.7%) and pT1b (26.9%) patients ($p = 0.388$). The median progression-free survival (PFS) was 0 months (range, 0–70 months), and the median follow-up duration for the entire cohort was 20 months (range, 12–84 months), with no significant difference between the pT1a (23 months) and pT1b (17.5 months) groups ($p = 0.126$) (Table 1).

Pathologic characteristics of pT1 urothelial carcinoma according to muscularis mucosa invasion

The median tumor diameter was significantly larger in the pT1b group (3.0 cm) compared to the pT1a group (2.0 cm) ($p = 0.023$). The proportion of tumors > 5 cm was significantly higher in the pT1b group (15.4%) than in the pT1a group (5.2%) ($p = 0.029$). The frequency of multiple tumors was comparable between groups ($p = 0.597$). By definition, the depth of invasion was significantly greater in pT1b tumors (median: 150 µm vs. 70 µm; $p < 0.0001$), confirming their greater invasion into the muscularis mucosa. Tumor thickness was also significantly higher in pT1b tumors (median: 2.85 mm vs. 1.0 mm; $p < 0.0001$). The presence of necrosis was significantly more common in the pT1b group (60.3%) compared to pT1a (32.9%) ($p = 0.0012$). Invasion into TURBT chips was significantly higher in pT1b tumors (median: 90% vs. 50%; $p < 0.0001$). However, lymphovascular invasion ($p = 0.130$) and carcinoma in situ (CIS) presence ($p = 0.144$) did not significantly differ between groups (Table 2).

Clinical and prognostic characteristics according to tumor infiltrating lymphocytes (TILs) ratio

Patients were categorized into three groups based on TILs ratio: **<1% ($N = 55$), 1–5% ($N = 59$), and >5% ($N = 40$)** <1% ($N = 55$), 1–5% ($N = 59$), and >5% ($N = 40$) (Table 3). The age, gender, smoking status, and BMI were not significantly different among the groups ($p > 0.05$). The recurrence rate was high across all groups, with no statistically significant differences ($p = 0.578$), and the median recurrence duration was also similar ($p = 0.629$). Similarly, progression rates and median PFS duration did not significantly differ among the TILs groups ($p = 0.403$

Table 1 Comparison of the clinical and prognostic characteristics of pT1 bladder urothelial carcinoma according to the muscularis mucosa invasion

Parameters	Total patients (*N* = 154)	pT1a (*N* = 76)	pT1b (*N* = 78)	*P* value
Age, Mean ± SD	70.4 ± 9.2	71.1 ± 8.5	69.8 ± 9.8	0.557
<65-year, N (%)	36 (23.4)	13 (17.1)	23 (29.5)	0.104
≥65-year, N (%)	118 (76.6)	63 (82.9)	55 (70.5)	
Gender, N (%)				
Male	135 (87.7)	69 (90.8)	66 (84.6)	0.358
Female	19 (12.3)	7 (9.2)	12 (15.4)	
Smoking, N (%)	69 (44.8)	34 (44.7)	35 (44.9)	0.987
BMI (kg/m2), Mean ± SD	24.4 ± 4.3	24.3 ± 3.8	24.5 ± 4.9	0.561
Intravesical BCG therapy, N (%)				
6	20 (13.0)	11 (14.5)	9 (11.5)	0.523
7–12	104 (67.5)	47 (61.8)	57 (73.1)	
>12	10 (6.5)	6 (7.9)	4 (5.1)	
Radical cystectomy, N (%)	21 (13.6)	6 (7.9)	15 (19.2)	0.069
Recurrence				
N (%)	122 (79.2)	58 (76.3)	64 (82.1)	0.497
Duration (month) (Median [Min-Max])	5 [0–60]	5 [0–28]	5 [0–60]	0.361
Progression				
N (%)	36 (23.4)	15 (19.7)	21 (26.9)	0.388
PFS Duration (month) (Median [Min-Max])	0 [0–70]	0 [0–36]	0 [0–72]	0.239
Follow-up, month				
Median [Min-Max]	20 [12–84]	23 [12–79]	17.5 [12–84]	0.126

BMI: Body-mass index, PFS: Progression free survival

Table 2 Comparison of the pathologic characteristics of pT1 bladder urothelial carcinoma according to the muscularis mucosa invasion

Parameters	Total patients (*N* = 154)	pT1a (*N* = 76)	pT1b (*N* = 78)	*P* value
Tumor Diameter, Median [Min-Max]	3.0 [1.0–10.0]	2.0 [1.0–10.0]	3.0 [1.0–8.0]	**0.023**
<3 cm, N (%)	71 (46.1)	42 (55.3)	29 (37.2)	**0.029**
3–5 cm, N (%)	67 (43.5)	30 (39.5)	37 (47.4)	
>5 cm, N (%)	16 (10.4)	4 (5.2)	12 (15.4)	
Tumor number, N (%)				
Single	61 (39.6)	28 (36.8)	33 (42.3)	0.597
Multiple	93 (60.4)	48 (63.2)	45 (57.7)	
Invasion into Chips from TURBT (%)				
Median [Min-Max]	80 [5-100]	50 [5-100]	90 [10–100]	**< 0.0001**
Lymphovascular invasion, N (%)	7 (4.5)	1 (1.3)	6 (7.7)	0.130
CIS, N (%)	73 (47.4)	31 (40.8)	42 (53.8)	0.144
Necrosis, N (%)	72 (46.7)	25 (32.9)	47 (60.3)	**0.0012**
TILs ratio group				
<%1	55 (35.7)	33 (43.4)	22 (28.2)	0.123
%1-%5	59 (38.3)	27 (35.5)	32 (41.0)	
> %5	40 (26.0)	16 (21.1)	24 (30.8)	
Depth of invasion (µm)				
Median [Min-Max]	110 [5-220]	70 [5-140]	150 [80–220]	**< 0.0001**
Tumor thickness (mm)				
Median [Min-Max]	1.5 [0.3-6.0]	1.0 [0.3-3.0]	2.85 [0.8-6.0]	**< 0.0001**

TURBT = transurethral resection of bladder tumor, TILs: Tumor infiltrating lymphocytes, CIS: Carcinoma in situ

Table 3 Comparison of the clinical and prognostic characteristics of pT1 bladder urothelial carcinoma according to the TILs ratio groups

Parameters	<%1 (*N* = 55)	%1–5 (*N* = 59)	>%5 (*N* = 40)	*P* value
Age, Mean ± SD	70.2 ± 9.9	70.9 ± 8.2	70.0 ± 9.5	0.615
<65-year, N (%)	15 (27.3)	10 (16.9)	11 (27.5)	0.332
≥65-year, N (%)	40 (72.7)	49 (83.1)	29 (72.5)	
Gender, N (%)				
Male	49 (89.1)	52 (88.1)	34 (85.0)	0.828
Female	6 (10.9)	7 (11.9)	6 (15.0)	
Smoking, N (%)	23 (41.8)	26 (44.1)	20 (50.0)	0.723
BMI (kg/m2), Mean ± SD	23.6 ± 3.8	24.7 ± 4.9	25.1 ± 4.1	0.079
Intravesical BCG therapy, N (%)				
6	6 (10.9)	9 (15.3)	5 (12.5)	0.381
7–12	37 (67.3)	40 (67.8)	27 (67.5)	
>12	6 (10.9)	1 (1.7)	3 (7.6)	
Radical cystectomy, N (%)	8 (14.5)	6 (10.2)	7 (17.5)	0.563
Recurrence				
N (%)	43 (78.2)	50 (8.5)	31 (77.5)	0.578
Duration (month), Median [Min-Max]	5 [0–39]	6 [0–41]	4.5 [0–60]	0.629
Progression				
N (%)	12 (21.8)	17 (28.8)	7 (17.5)	0.403
PFS Duration (month), Median [Min-Max]	0 [0–36]	0 [0–36]	0 [0–72]	0.297
Follow-up, month				
Median [Min-Max]	19 [12–80]	19 [12–84]	24 [12–70]	0.863

BMI: Body-mass index, PFS: Progression free survival

Table 4 Comparison of the pathologic characteristics of pT1 bladder urothelial carcinoma according to the TILs ratio groups

Parameters	<%1 (*N* = 55)	%1–5 (*N* = 59)	>%5 (*N* = 40)	*P* value
Tumor Diameter, Median [Min-Max]	2.0 [1.0–8.0]	2.0 [1.0–6.0]	3.0 [1.0–10.0]	0.489
<3 cm, N (%)	26 (47.3)	30 (50.8)	15 (37.5)	0.759
3–5 cm, N (%)	23 (41.8)	24 (40.7)	20 (50.0)	
>5 cm, N (%)	6 (10.9)	5 (8.5)	5 (12.5)	
Tumor number, N (%)				
Single	26 (47.3)	20 (33.9)	15 (37.5)	0.328
Multiple	29 (52.7)	39 (66.1)	25 (62.5)	
Invasion into Chips from TURBT (%)				
Median [Min-Max]	60 [5-100]	80 [1-100]	80 [2-100]	**0.0025**
Lymphovascular invasion, N (%)	3 (5.5)	3 (5.1)	1 (2.5)	0.767
CIS, N (%)	24 (43.6)	27 (45.8)	22 (55.0)	0.521
Necrosis, N (%)	21 (38.2)	32 (54.2)	19 (47.5)	0.228
Invasion into Muscularis Mucosa, N(%)	22 (40.0)	32 (54.2)	24 (60.0)	0.123
Depth of invasion (mm)				
Median [Min-Max]	90 [5-220]	120 [10–200]	120 [20–210]	0.310
Tumor thickness (mm)				
Median [Min-Max]	1.3 [0.4-6.0]	2.0 [0.6-5.0]	2.1 [0.3-5.0]	**0.0299**

TURBT = transurethral resection of bladder tumor, TILs: Tumor infiltrating lymphocytes, CIS: Carcinoma in situ

and $p = 0.297$, respectively). The median follow-up duration was also comparable across groups ($p = 0.863$).

Pathological characteristics according to tumor infiltrating lymphocytes (TILs) ratio

Pathological findings according to TILs groups revealed that tumor diameter was not significantly different ($p = 0.489$). However, invasion into TURBT chips was significantly higher in patients with increased TILs ratios ($p = 0.0025$), suggesting a correlation between immune response and tumor invasion. Tumor thickness was significantly greater in patients with higher TILs ratios ($p = 0.0299$). Other pathological characteristics, including depth of invasion, lymphovascular invasion, CIS, and necrosis, did not differ significantly between TILs groups ($p > 0.05$) (Table 4).

Discussion

Our study provides a detailed analysis of pT1 urothelial carcinoma of the bladder, focusing on the prognostic significance of muscularis mucosa invasion and TILs. The findings reveal key insights into the aggressive nature of pT1b tumors and the nuanced role of TILs in bladder cancer prognosis. These results contribute to the ongoing efforts to refine risk stratification and management strategies for patients with this heterogeneous disease.

By definition, pT1b bladder cancers demonstrate deeper invasion into the muscularis mucosa compared to pT1a tumors, as confirmed in our study (median depth: 150 µm vs. 70 µm; $p < 0.0001$). This deeper invasion is intrinsically linked to the classification of pT1b tumors and underscores their more aggressive pathological and clinical behavior. Our findings align with prior studies that have reported increased tumor thickness and higher rates of necrosis as additional markers of poor prognosis in pT1b bladder cancer [9–11].

Interestingly, despite these aggressive features, recurrence and progression-free survival (PFS) rates did not significantly differ between pT1a and pT1b groups in our cohort. However, the higher frequency of radical cystectomy in pT1b patients suggests that clinicians recognize the elevated risk of progression associated with these tumors and are more likely to pursue aggressive treatment strategies. Although we did not include treatment modality as a variable in our multivariable analyses due to the limited number of patients undergoing radical cystectomy ($n = 21$), we acknowledge that this may represent a potential confounding factor influencing recurrence and progression outcomes. This limitation has now been highlighted for future consideration. The absence of a significant survival difference may reflect the relatively short follow-up period in our study, as long-term studies have demonstrated worse outcomes for pT1b tumors over extended durations [12–17]. These findings reinforce the necessity for early and aggressive management, including radical cystectomy, in high-risk pT1b cases.

TILs are increasingly recognized as critical components of the tumor immune microenvironment, with potential prognostic implications across various malignancies. In bladder cancer, however, their role remains less clear [6, 7]. In our study, higher TIL densities were associated with increased tumor thickness and more extensive invasion into TURBT chips, consistent with the notion that TILs reflect a heightened immune response to more aggressive tumors. Nevertheless, no significant differences in recurrence or progression rates were observed across TILs groups, indicating that their prognostic value may be limited in this context.

It is important to clarify that the reported "median PFS duration of 0 months" does not imply immediate progression in all patients. Rather, this finding reflects the distribution of progression events: a subset of patients progressed early, resulting in a median of 0, while the majority did not progress during follow-up. This statistical outcome underscores the limitation of using median values when event rates are low.

Similarly, regarding recurrence duration, we have corrected the misstatement in the >5% TIL group. As reported in Table 3, the actual minimum time to recurrence in this group was 4.5 months—not 0 months. The manuscript text has been updated to reflect this accurately and prevent misinterpretation.

This apparent paradox may be explained by the functional heterogeneity of tumor-infiltrating lymphocytes. While some subsets, such as CD8 + cytotoxic T cells, mediate anti-tumor effects, others—including FOXP3 + regulatory T cells—may contribute to immune suppression and tumor progression. Without specific immunophenotypic characterization, the net biological role of TILs remains uncertain. The absence of a clear survival benefit in patients with higher TILs ratios may be attributed to the dual role of TILs in tumor biology. While they represent an anti-tumor immune response, aggressive tumors often provoke a stronger immune reaction. Furthermore, certain tumors may evade immune detection through mechanisms such as immune checkpoint activation, potentially neutralizing the protective effects of TILs. These complexities highlight the need for further research to elucidate the interplay between TILs and immune escape mechanisms, particularly in the context of emerging immunotherapies [8, 17–21].

The significant association between pT1b tumors and markers of aggressiveness, such as tumor thickness and necrosis, underscores the importance of tailored treatment strategies for this subgroup. Early consideration of radical cystectomy may be warranted to mitigate the risk of progression to muscle-invasive disease in high-risk pT1b tumors. At the same time, the role of TILs in bladder cancer prognosis remains uncertain, requiring additional investigation to clarify their clinical utility.

The retrospective design and relatively short follow-up period of the present study may limit the ability to assess long-term outcomes; however, the findings contribute to the growing body of evidence on tumor aggressiveness and immune response in non-muscle-invasive bladder cancer. The semi-quantitative assessment of TILs, although inherently subjective, highlights their potential role in tumor progression. We did not use digital pathology tools or image analysis algorithms, which may have introduced some observer variability. Yet, the absence of data on specific TIL subtypes (e.g., CD3+, CD8+, FOXP3 + lymphocytes) prevents a more detailed understanding of their functional impact on prognosis. Future studies should aim to incorporate immunohistochemical or molecular characterization of TILs, along with digital pathology approaches, to improve the objectivity and reproducibility of immune profiling. A more refined classification of TIL subsets through immunohistochemistry or molecular profiling could offer deeper insights into immune-tumor interactions. Furthermore, the observed association between TILs and tumor aggressiveness in our study supports the emerging concept that the tumor immune microenvironment may significantly influence therapeutic response. This is particularly relevant in light of recent advances in immunotherapy, such as immune checkpoint inhibitors like pembrolizumab, which have shown clinical benefit in patients with BCG-unresponsive NMIBC [19]. Although the single-center nature of our study may limit generalizability, it ensured consistency in histopathological evaluation and patient management. Future multicenter studies with extended follow-up and comprehensive immunological analyses are warranted to refine risk stratification and assess the clinical utility of TILs in guiding personalized treatment decisions. Another important limitation is the lack of molecular or immunohistochemical characterization of the TIL subsets. Future studies incorporating markers such as CD3, CD8, or FOXP3 may provide a more nuanced understanding of the immune contexture in pT1 tumors.

Conclusion

This study highlights the significance of muscularis mucosa invasion as a marker of tumor aggressiveness in pT1 bladder cancer. The findings confirm that pT1b tumors exhibit greater invasion depth, increased tumor thickness, and a higher prevalence of necrosis compared to pT1a tumors. While these characteristics suggest a more aggressive disease course, recurrence and progression-free survival rates were not significantly different between pT1a and pT1b groups, potentially reflecting the impact of early radical cystectomy in high-risk cases. The role of TILs in bladder cancer prognosis remains unclear, as higher TILs ratios were associated with more extensive tumor invasion and greater tumor thickness, but no significant differences in recurrence or progression rates were observed. This may be due to the functional heterogeneity of TILs, including the presence of immunosuppressive subtypes, and highlights the limitations of morphology-based evaluation alone. The median PFS value of 0 months reported in TIL groups reflects early progression in a small subset of patients and does not indicate universal or immediate progression. Likewise, recurrence duration in the >5% TIL group began at 4.5 months, and the manuscript now reflects this correction to avoid ambiguity. These findings imply a potential

association between TILs and tumor-immune activity; however, the prognostic significance of TILs in pT1 bladder cancer remains inconclusive and warrants further exploration in larger, prospective cohorts. Future studies should integrate molecular and immunohistochemical analyses, along with digital quantification methods, to better define the immune landscape and its clinical relevance in non-muscle-invasive bladder cancer.

Acknowledgements
The authors thank Elif Yaprak, PhD (ORCID:0000-0001-6691-1235) for her assistance in editing the article and professional English translation.

Author contributions
BYB, HND, KT, HY collected the data and BYB drafted the manuscript. BYB, HND edited the manuscript, participated in the study design and coordination. All authors (BYB, HND, KT, HY, OD) read and approved the final manuscript.

Funding
No funding was received for conducting this study.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval
The study protocol was approved by the Ethical Committee of Non-invasive Clinical Research of Kocaeli University (Decision No: KU GOKAEK-2024/18.22, Project No: 2024/456). This study was conducted according to the Helsinki principles, patients signed informed consent for participation, and nothing invasive of patients' privacy was done. Written informed consent was obtained from patients for publication of this original article and all accompanying images.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 21 January 2025 / Accepted: 12 May 2025
Published online: 24 May 2025

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