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REVIEW AND PERSPECTIVES

pT1 high-grade bladder cancer: histologic criteria, pitfalls in the assessment of invasion, and substaging

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Abstract

Most patients with bladder carcinoma are diagnosed with non-muscle-invasive disease, stage Ta, and pT1. Stage remains as the single most important prognostic indicator in urothelial carcinoma. Among the pT1 bladder cancer patients, recurrence and progression of disease occur in 50% and 10%, respectively. The identification of high-risk patients within the pT1 subgroup remains an important clinical goal and an active field of research. Substaging of pT1 disease has been claimed as important histologic discriminator by the 2016 World Health Organization (WHO) classification of the genitourinary tract tumors and by the 8th edition of the American Joint Committee on Cancer (AJCC) staging manual supporting its implementation in clinical practice. Interobserver variation in pT1 diagnosis and the associated pitfalls in pT1 assessment are the critical pathological issues. The aim of this review paper is to provide the practicing pathologist with the state of the art of morphological and immunohistochemical features useful for the diagnosis of early invasive bladder carcinomas, including practical clues on how to avoid relevant interpretative pitfalls, and to summarize the current status of pT1 substaging.

Keywords Bladder cancer · Stage · pT1 sub-staging · Pitfalls,

Introduction

Pathologic stage is a critical determinant in bladder cancer management and prognostication. As in other hollow organs, tumor (T) stage categories in the bladder are defined by the extent of cancer invasion through its wall. Currently, pT1 tumors are malignant epithelial neoplasia invading the subepithelial connective tissue of the lamina propria, beyond the basement membrane, but not the muscularis propria (MP) [1, 2].

In the past, pT1 tumors have been grouped under the heading of superficially bladder carcinoma or non-muscle invasive bladder carcinoma (NMIBC) along with low-grade papillary tumors confined to the mucosa without invading the lamina propria (Ta LG) and flat high-grade (HG) tumors confined to the mucosa or carcinoma in situ (CIS), classified as Tis. However, clinical and molecular data have shown strong differences between the low-grade bladder cancer patients and high-grade carcinoma bladder patients [3–6]. At the current time, NMIBC and superficially bladder carcinoma are obsolete terms.

Different behavior has been reported within the pT1 bladder cancer group. The need to substage pT1 disease comes from clinical analysis since recurrence of disease occurs in about 50% of NMIBC patients and approximately 10% patients develop progression to muscle-invasive disease in 5 years after diagnosis [7]. Some studies have shown improved survivals in patients with pT1 lesions treated with immediate cystectomy, while others have reported no benefit [8–11]. Although pT1 bladder carcinomas are heterogeneous

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in terms of outcome, current guidelines for pT1 carcinoma do not discriminate between different levels of lamina propria invasion [12]. Thus, the identification of high-risk patients within the pT1 group is of great interest to both pathologists and clinicians [11, 13–17].

In this review paper, we aim to analyze the current criteria for a differential diagnosis in order to avoid the pitfalls in the early invasion assessment of bladder cancer and to increase the reproducibility of pT1 stage diagnosis between pathologists. An update of the substaging issue and the prognostic value of the variants that may be present in pT1 high-grade bladder cancers have been also discussed.

Current criteria for the diagnosis

The challenging assessment of lamina propria invasion of papillary urothelial carcinoma (UC) should be based on strict criteria. The evaluation of lamina propria-epithelial interface and the morphologic appearance of the basement membrane and the characteristics of the invading epithelium with the lamina propria modification are the key points in the assessment of pTa or pT1 stage bladder carcinoma; the knowledge of the specific features of the bladder tumors may help in the diagnosis (Table 1). Non-invasive papillary tumors show a plain and regular lamina propria-epithelial interface (Fig. 1). When the specimen includes tangential sections through non-invasive disease or in case of tumor involving the von Brunn's nests, the basement membrane shows a blunt border: high magnification permits to observe the presence of a continuous basement membrane. Contrarily, the invading epithelium may show single cells or irregularly shaped nests of tumor within the lamina propria with random arrangement without basement membrane, or the invasive front of the tumor may show finger-like extensions arising from the base of the papillary tumor (Fig. 2). In many cases, the invading nests appear with a higher degree of nuclear pleomorphism in comparison to the non-invasive tumor cells; in some cases, the invasive tumor cells may acquire abundant eosinophilic cytoplasm. Uncommon finding appears at low-to-medium power magnification, when the microinvasive cells seem to be more differentiated than the non-invasive component, a feature known as paradoxical differentiation (Fig. 3).

Unlike cases with extensive and unquestionable lamina propria invasion where single tumor cells and variable-sized and irregularly-shaped nests causing a stromal fibrous reaction are common features (Fig. 4), microinvasive diseases rarely show inflammatory, hypocellular stroma with myxoid background, or fibrous stromal reactions. In the majority of the cases, microinvasive UC does not cause a stromal response.

Early features of invasion into lamina propria may be a retraction artifact around superficially invasive tumor cells mimicking a lymphovascular space invasion (Fig. 5) and a strong proliferation of fibroblasts with no expansive features around the invasive component of the tumor.

Invasive tumor cells can be present at the base of the connective tissue, and also, in some cases only, in a papillary stalk. Stalk invasion is characterized by the presence of single cells or irregular tumor nests confined to the edematous connective tissue of a papillary stalk (Fig. 6).

UC with inverted growth is characterized by broad-front growth or broad bulbous tongues of urothelial tumor extending into lamina propria, with smooth basement membrane. Inverted growth should not be misdiagnosed as pT1. The invasion of lamina propria, both for papillary exophitic and inverted growth carcinomas, is assessed when there are irregularities of the basement membrane, or retraction artifact, or desmoplasia or other findings suggestive for lamina propria invasion.

Table 1 Histological features to recognize lamina propria invasion

Pathologic issues in diagnosis of lamina propria invasion
General features
Histologic grade
Stroma-epithelial interface (basement membrane)
Invading epithelium
Stromal response
Specific bladder tumors
CIS with microinvasion
Papillary urothelial carcinoma with microinvasion
Papillary urothelial carcinoma with invasion into stalk
Well-established invasion into underlying lamina propria

Pitfalls in early invasion assessment

The diagnosis of lamina propria invasion by UC is often difficult, as also attested by the numerous studies on the interobserver differences in staging of superficial UC [18–24] (Table 2).

This critical issue has been addressed in a study in which 63 tumors originally diagnosed as stage pT1 have been down-staged to pTa tumors in 56% of the cases and up-staged to pT2-pT3 tumors in 13% of the cases after a consensus diagnosis by experienced genitourinary pathologists. Disease progression was more common in the 20 cases with a consensus confirmed stage pT1 (25% progression diseases) when compared to the original pT1 cases (20% progression disease). In addition, tumors that were down-staged to pTa showed less frequent progression than the stage pT1 tumors confirmed when reviewed (17% versus 25%) [19].

The pitfall in the assessment of bladder carcinoma between non-invasive and early invasive bladder cancer remains subject of considerable interobserver variation. Therefore, a

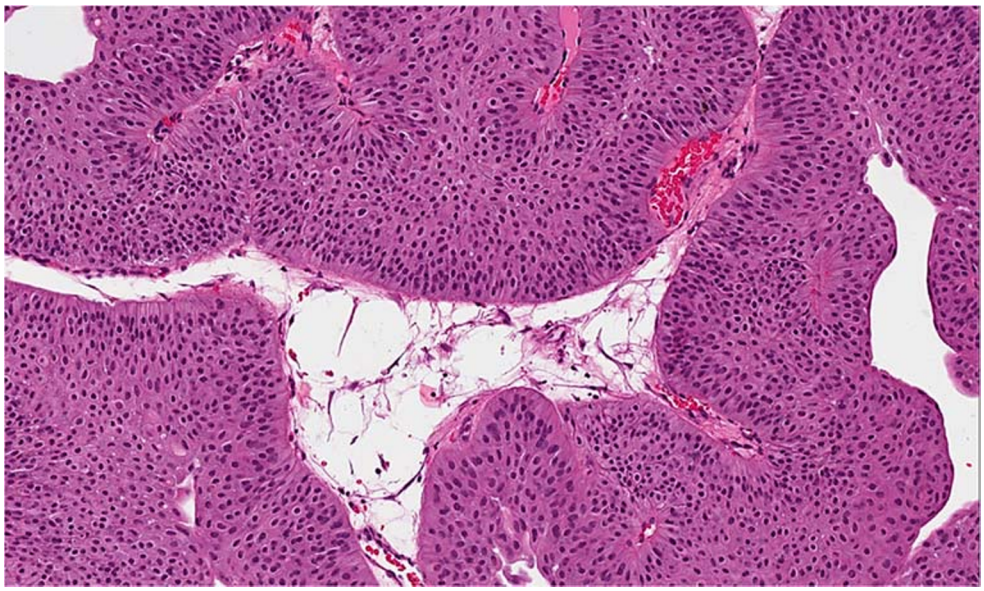

Fig. 1 Low-grade papillary without invasion of the lamina propria (pTa). The basement membrane has a blunt and continuous border

group of expert uropathologists has aimed to generate a teaching set of images [21].

Table 3 lists the more common pitfalls in early assessment of bladder carcinoma invasion: the knowledge of pitfalls in early assessment of lamina propria invasion may improve the concordance rates among pathologists.

Tangential sectioning and poor orientation

Papillary tumors, usually characterized by complex architecture, are usually excised in fragments and sectioned in multiple planes; consequently, they are poorly oriented during embedding. This may cause the presence of tumor nests within connective tissue of lamina propria. Diagnosis of stromal invasion should be only done in presence of single-tumor cells and nests with irregular shape and size, without the presence of basement membrane (Fig. 7). On the other hand, the presence of regular shape, with smooth and plain margins and the presence of basement membrane encourages the Ta stage diagnosis.

Obscuring inflammation

The presence of inflammatory infiltrate may obscure the presence of single-tumor cells infiltrating the lamina propria. In this case, the staining with anti-cytokeratin antibodies may

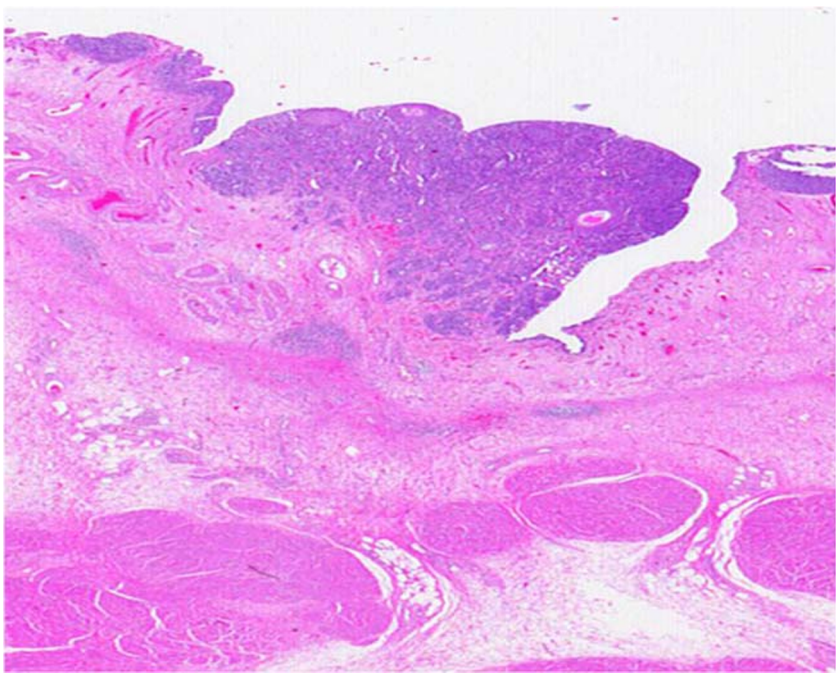

Fig. 2 Papillary urothelial carcinoma with irregularly-shaped nest of tumor within the lamina propria (pT1). The basement membrane is no longer visible

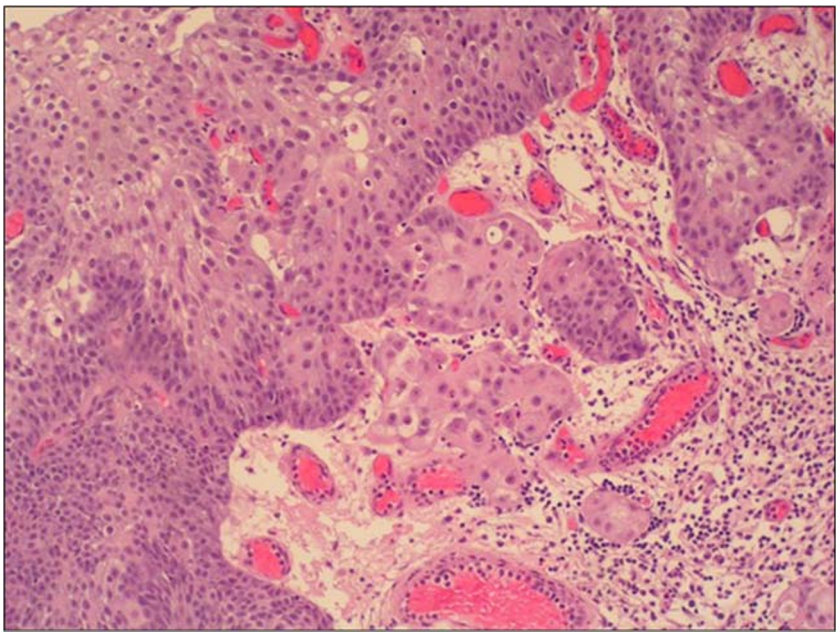

Fig. 3 Microinvasive cells show paradoxical differentiation

help in identifying the presence of single cells or small tumor nests (Fig. 8).

Cautery artifacts

Cautery artifacts produce distorted morphology in TURBT specimens, which may cause a potential pitfall for the pathologist, especially when tumor involves the von Brunn's nests. In some cases, the use of immunohistochemistry with anti-cytokeratin antibodies may be of help in the diagnosis. CIS involving von Brunn's nests may mimic lamina propria invasion. This is especially problematic when von Brunn's nests are prominent or when they have been distorted by inflammatory or cautery artifact.

Type of muscle invasion

The involvement of muscle fibers by invasive tumors may be a critical source of pitfalls in the staging of bladder tumor. The presence of a desmoplastic response, inflammation, and hypertrophy of the muscle fibers may make difficult the differential diagnosis between the muscle fibers from muscularis mucosa (MM) or MP. This distinction is critical, as MP invasion is currently regarded as the cutoff for being out conservative management and aggressive therapy. MM bundles hypertrophy can be difficult to distinguish from MP, especially in TURBT specimens. Hyperplastic MM is seen in about 50% of bladder sections, being most abundant in the dome (70%) and least in the trigone [25, 26]. MM is often not discernible at the trigone [15]. In addition, the problems in diagnosis come out from the matter that hyperplastic MM, which frequently has random outline distinguishable from MP, may have compact parallel muscle fibers and regular outline closely resembling the MP [15, 26].

Moreover, invasive tumors may cause hypertrophy of stromal myofibroblasts. Cellular stroma with spindled fibroblasts and desmoplastic response may resemble muscle fibers. These features may be the cause of over-staging to T2. On the other hand, an exuberant proliferation of fibroblasts, which may display alarming cellular atypia, should not be mistaken for the spindle cell component of a sarcomatoid urothelial carcinoma. The proliferating stroma is limited to areas around the neoplasm and is composed of cells which have a predominant degenerate or smudged appearance [27].

Immunostaining with smoothelin has been proposed to distinguish MM and MP: several studies have demonstrated the unequal staining in MM (usually weak or absent) versus MP (usually strong) with smoothelin [28–34]. In addition, smoothelin does not stain subepithelial myofibroblasts and desmoplasia, and MP staining is preserved in cauterized tissue in most of the cases [33, 34].

However, overlap in staining of MM and MP may occur sometimes and discrimination in TURB specimens becomes problematic, particularly when staining is modest and without both muscle types present as internal reference [32]. Staining intensity may also vary with titration and antigen retrieval techniques [30, 35]. Other muscle markers such as desmin and caldesmon do not show unequal staining in MM and MP although they may be used to highlight the muscle and allow the differential diagnosis with subepithelial myofibroblasts and desmoplastic response [31, 34]. The 2013 International Society of Urological Pathology (ISUP) conference on best practice recommendation in the application of immunohistochemistry recognized the limitations of smoothelin precluding its routine use [28]. According to our

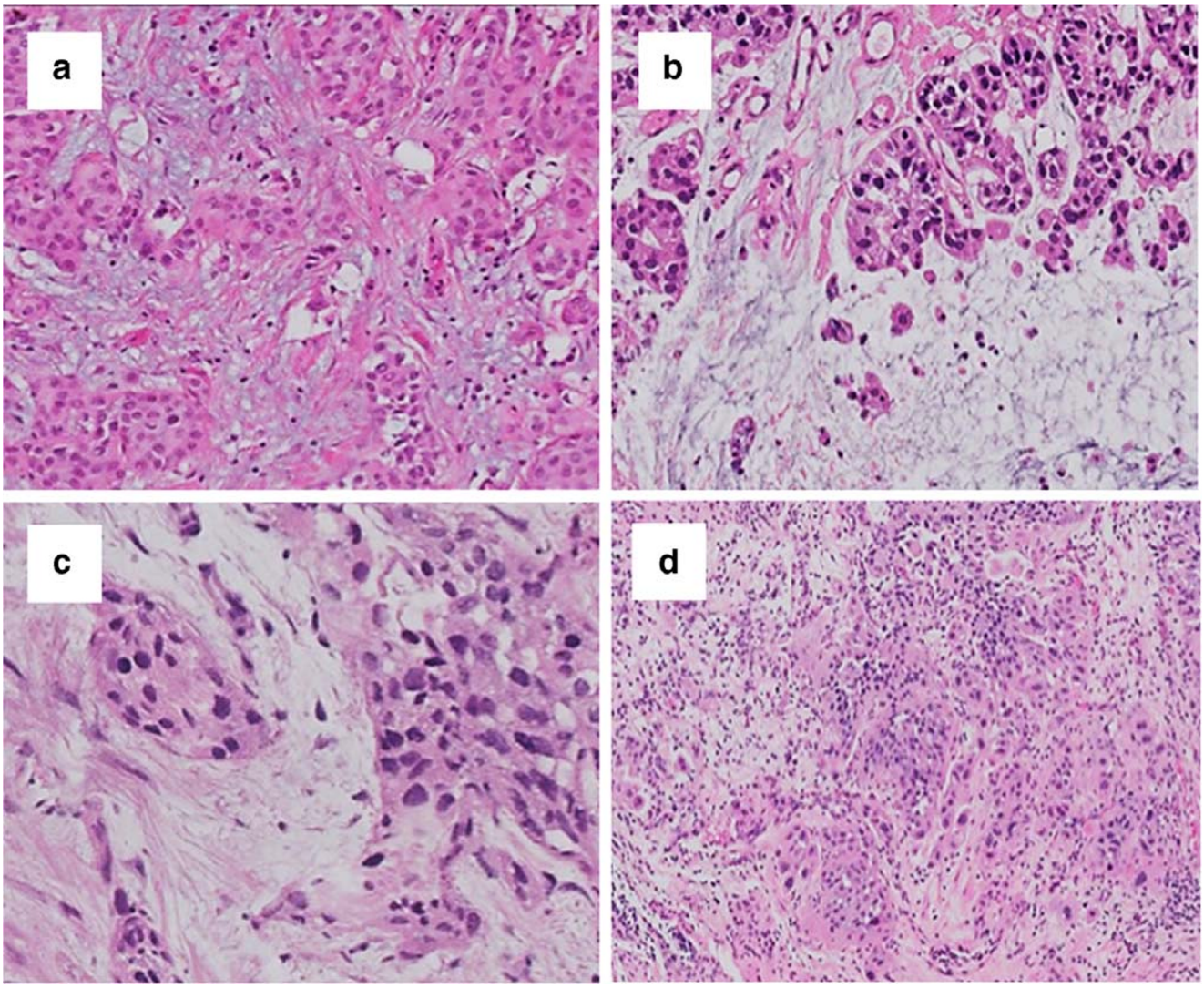

Fig. 4 Stromal fibrous reactions: mucoid stroma (**a**), colloid stroma (**b**), fibro-mixoid stroma (**c**), and inflammatory stroma (**d**)

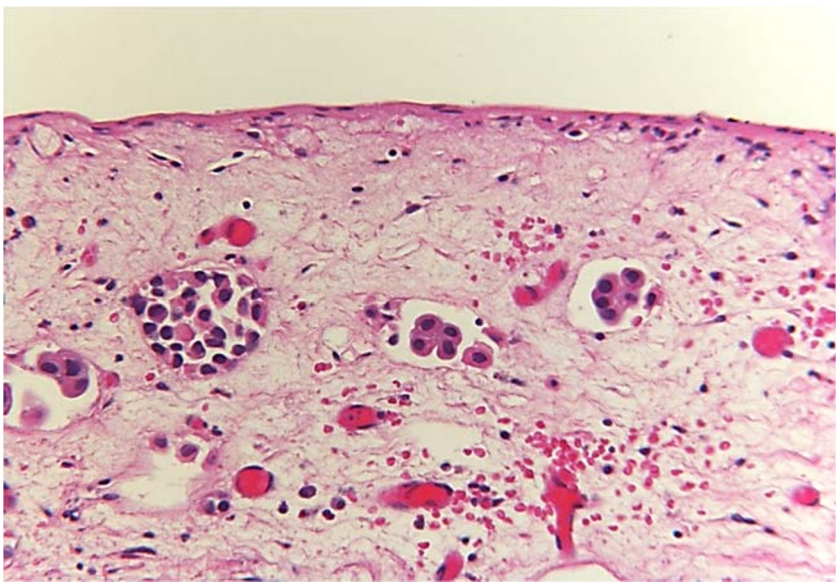

Fig. 5 Retraction artifact around invasive tumor cells mimicking a lymphovascular space invasion

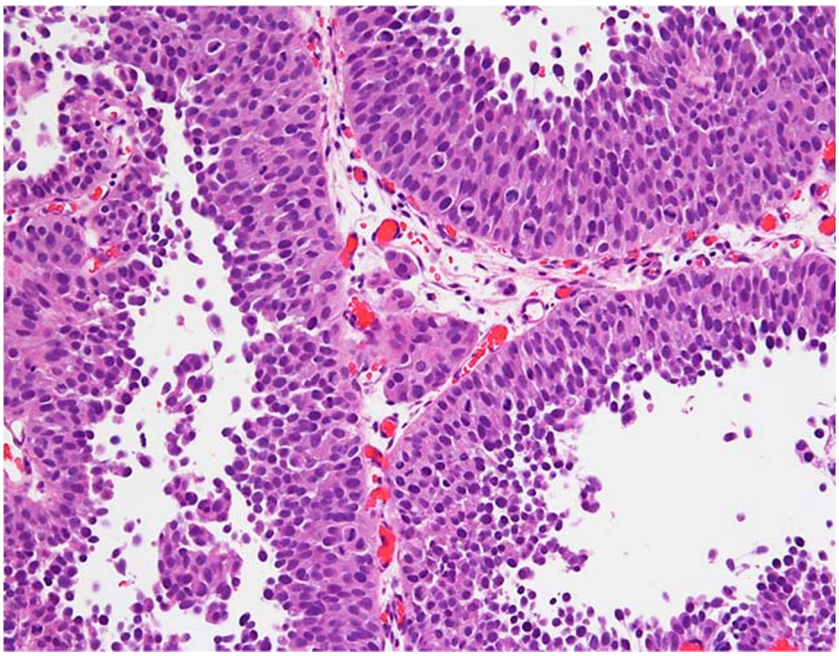

Fig. 6 Connective tissue of papillary stalk invaded by irregular tumor nests

experience, smoothelin stain can differentiate MM from MP, while desmin may give an important help in the differential diagnosis between myofibroblasts/desmoplastic reaction and MM/MP, even though it is not useful in the distinction between MM and MP (Fig. 9). The clinical information of the exact site of the TURB specimen associated with the knowledge of the topographic variation may further implement the correct sub-staging [36, 37].

Diagnosis of pT1 bladder carcinoma arising in a diverticulum is made when tumor involves the connective tissue underneath urothelium (Fig. 10). In the majority of the cases of acquired diverticula, few muscle fascicles may be identified in the connective tissue; however, in some cases, they become hyperplastic until they look like a continuous layer of smooth muscle. In acquired diverticula, a true MM is lacking. Presence of tumor cells beyond the connective tissue, invading the peri-diverticular soft tissue, should be staged as pT3.

Iatrogenic change of the urinary bladder: radiation therapy

Radiation therapy for pelvic cancers may cause variable morphologic alterations in the bladder wall such as acute and chronic radiation cystitis, mucosal ulceration and denudation, and late fibrosis with bladder contracture. In addition, it induces cellular and nuclear enlargement, frequent multinucleation, and vacuolization with low nuclear-cytoplasmic ratio bringing on difficulties in differential diagnosis with pseudo-tumoral findings (Fig. 11). Histological features such as proliferative and pseudo-infiltrative urothelial nests within the stroma, called pseudo-carcinomatous hyperplasia, may be related to a previous history of radiation or other chronic injuries. Pseudo-carcinomatous hyperplasia should be differentiated from invasive urothelial carcinoma and the nested variant of urothelial carcinoma. The presence

Table 2 Review of the interobserver differences in the pT1 stage assessment of bladder carcinoma

Year	Author (ref)	Cases number	% of consensus
2000	Tosoni [23]	301	62
2000	Cheng [20]	105	56
2000	Van Der Meijden [24]	1400	53
2001	Bayraktar [18]	127	25
2003	Miladi [22]	Literature analysis	9–49
2003	Bol [19]	63	31
2013	Comperat [21]	25	44%

Table 3 List of the pitfalls in early assessment of pT1 stage bladder carcinoma

Tangential sectioning/poor orientation
Obscuring inflammation
Thermal injury
Muscle invasion indeterminate for type of muscle
Iatrogenic changes of the urinary bladder (reactive atypia with pseudo-invasion of the lamina propria)
CIS involving von Brunn's nests
Variants of urothelial carcinoma with deceptively bland cytology
Pseudo-invasive nests of benign proliferative urothelial lesions and/or reactive lesions

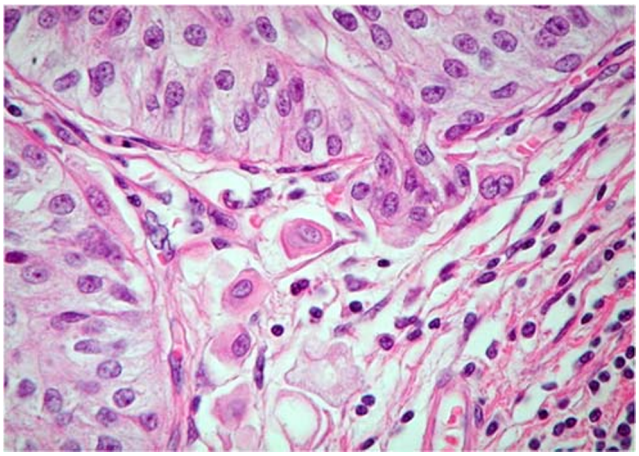

Fig. 7 Single cells invading beyond basement membrane

of background therapy-related changes, such as dilated thrombosed vessels, edema, reactive-appearing endothelial and stromal cells, and hemorrhage, can help in distinguishing pseudo-carcinomatous hyperplasia from carcinoma. The known previous history of radiation therapy is essential. The expression of p53, CD44, and CK20 could be of help since it is similar to what is seen in reactive urothelium [38, 39].

Variants of urothelial carcinoma with misleading features

The recognition of variants in pT1 samples may be more difficult compared to the advanced cases, not only because some variants may be under-recognized due to the frequent interobserver variability but also for the pitfalls of early assessment of some uncommon variants such as nested-type carcinoma, micropapillary carcinoma, large nested type, or microcystic carcinoma. Additionally, the differential diagnosis between these unusual variants and benign proliferative urothelial

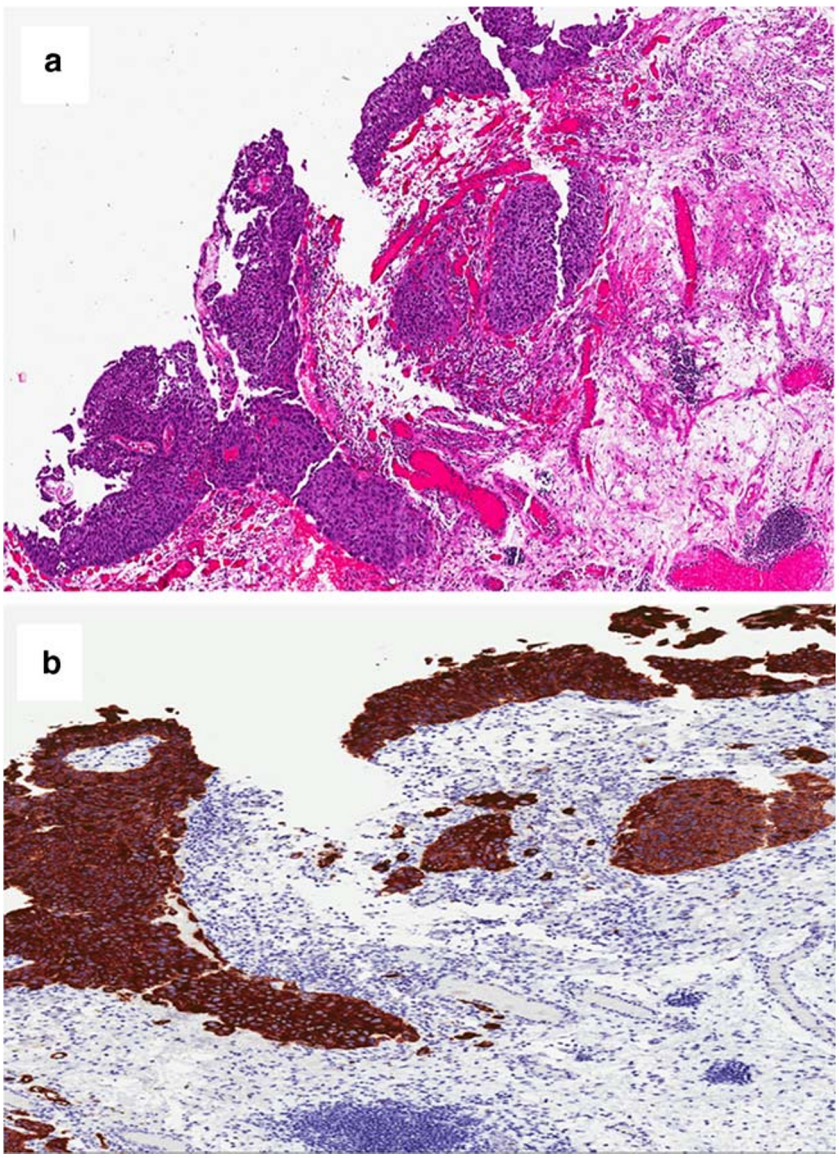

Fig. 8 Papillary urothelial carcinoma with inflammatory infiltrate of the lamina propria (H&E stain) (**a**). Anti-cytokeratin antibodies show the presence of the neoplastic cells infiltrating the lamina propria (CK20 stain) (**b**)

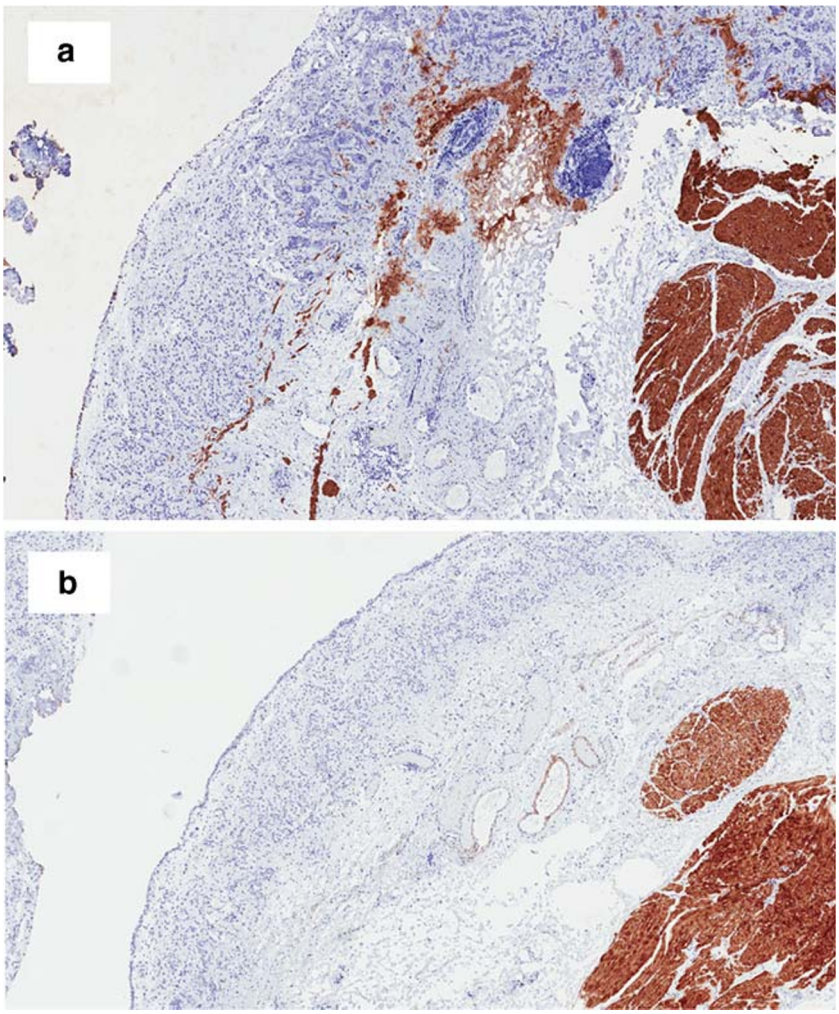

Fig. 9 Desmin stain used to highlight the muscle in both MM and MP (**a**). Smoothelin stain used to distinguish MM (absent stain) and MP (strong stain) (**b**)

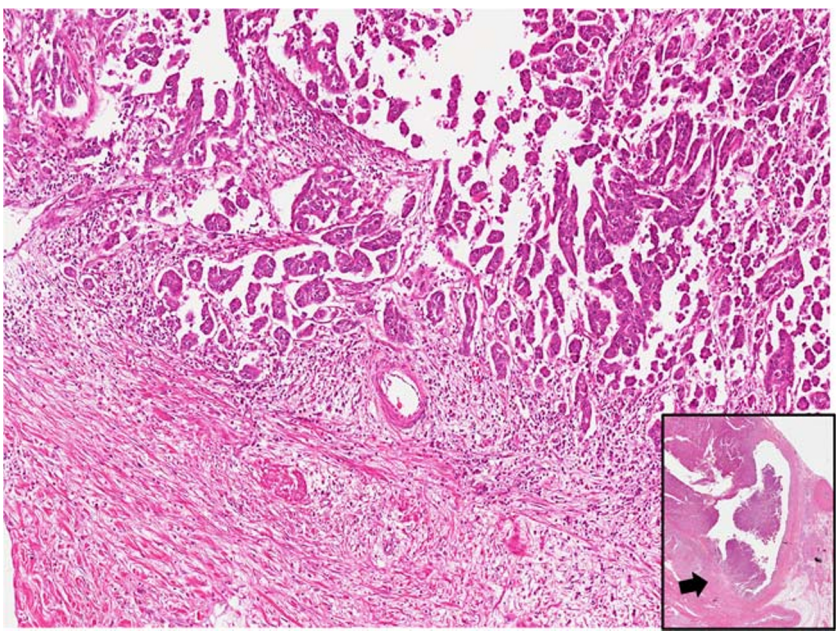

Fig. 10 pT1 micropapillary carcinoma originating in a diverticulum (insert; diverticulum with an arrow indicates the tumor area). The tumor is infiltrating the subepithelial connective tissue. There is no muscularis propria

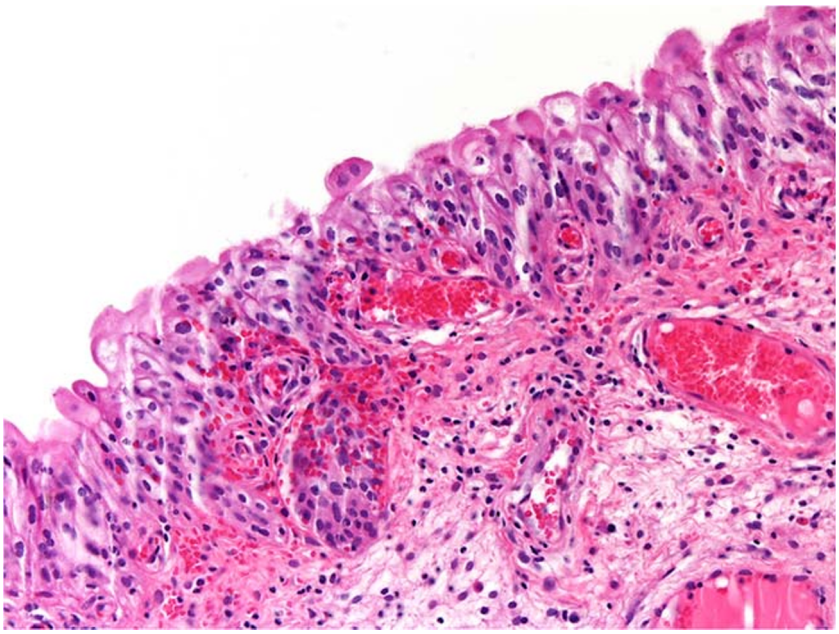

Fig. 11 Morphological changes in urothelial mucosa caused by radiation therapy with pseudo-invasion of the lamina propria

lesions, such as von Brunn's nests, inverted papilloma, and nephrogenic metaplasia which manifest as pseudo-invasive nests of urothelium within the lamina propria, can be a diagnostic challenge [40] (Fig. 12).

pT1 substaging

Known prognostic factors in pT1 tumors include grade, tumor size, CIS, multiplicity, and recurrence [12]. Substaging helps further stratify pT1 tumors, and its use is gaining reception in pathology practice, although the approach still has to be standardized. In a 2008 European Network of Uropathology (ENUP) survey, 37% of pathologists performed substaging mostly (68%) by using the MM [41]. While the 2016 8th edition AJCC recognizes that pT1 substaging is not yet optimized, it nevertheless strongly recommends attempting its use [1, 15–17]. The 2016 WHO "blue book" also suggested that pT1 substaging is clinically relevant [2]. The 2012 International Consultation on Urological Diseases (ICUD)-European Association of Urology (EAU) International Consultation on Bladder Cancer panel recommended pathologists to provide an estimate of LP involvement [42]. Most recently, the International Collaboration on Cancer Reporting (ICCR) suggests using either depth or dimension of invasion or using MM for pT1 subcategorization [43].

The description of the presence of the MM in the bladder led to consider it as a landmark for more precise staging of bladder carcinoma [44]. When there is lamina propria invasion, the tumor may be within the muscularis mucosae or to overcome the muscularis mucosae and lap (or lick) the MP. In every case, the presence or absence of MP within the transurethral resection specimen should be reported.

In 1990, Younes and coworkers suggested to substage the pT1 bladder carcinoma according to the level of invasion of the lamina propria, defining pT1a stage when the invasion of connective tissue was superficial to the level of MM, pT1b when the invasion was at the level of MM, and pT1c when the invasion of the tumor was throughout the level of the MM but above the MP. The authors found a 75% all-cause 5-year survival for patients with pT1a and pT1b tumors invading above and into the MM, compared to an 11% survival for those tumors invading below the MM (pT1c) [45]. This substaging has been also encouraged by other authors [46–54].

However, the main problem with this method of substaging pT1 urothelial carcinomas is that the MM is not a consistent histologic finding in all bladder specimens. Most authors have at least partially overcome this problem by using the large blood vessels in the submucosa as a substitute anatomic landmark when MM bundles are not present [37]. But, in many cases, substaging cannot be performed, because the vessels are not identified in the TURB specimen.

Carcinoma in situ with microinvasion was firstly defined by Farrow et al. in case of invasive component measuring less than 5 mm in depth [55]. Later, Amin and coworkers have reduced the cut-off for this diagnosis to 2 mm [56]. A proposal based on the Ancona International Consultation by Lopez-Beltran has suggested a cut-off of no more than 20 invading cells, measured from the lamina propria-epithelial interface, for the diagnosis of flat microinvasive disease [57]. In the last decade, several pathologists tried to formulate new histologic

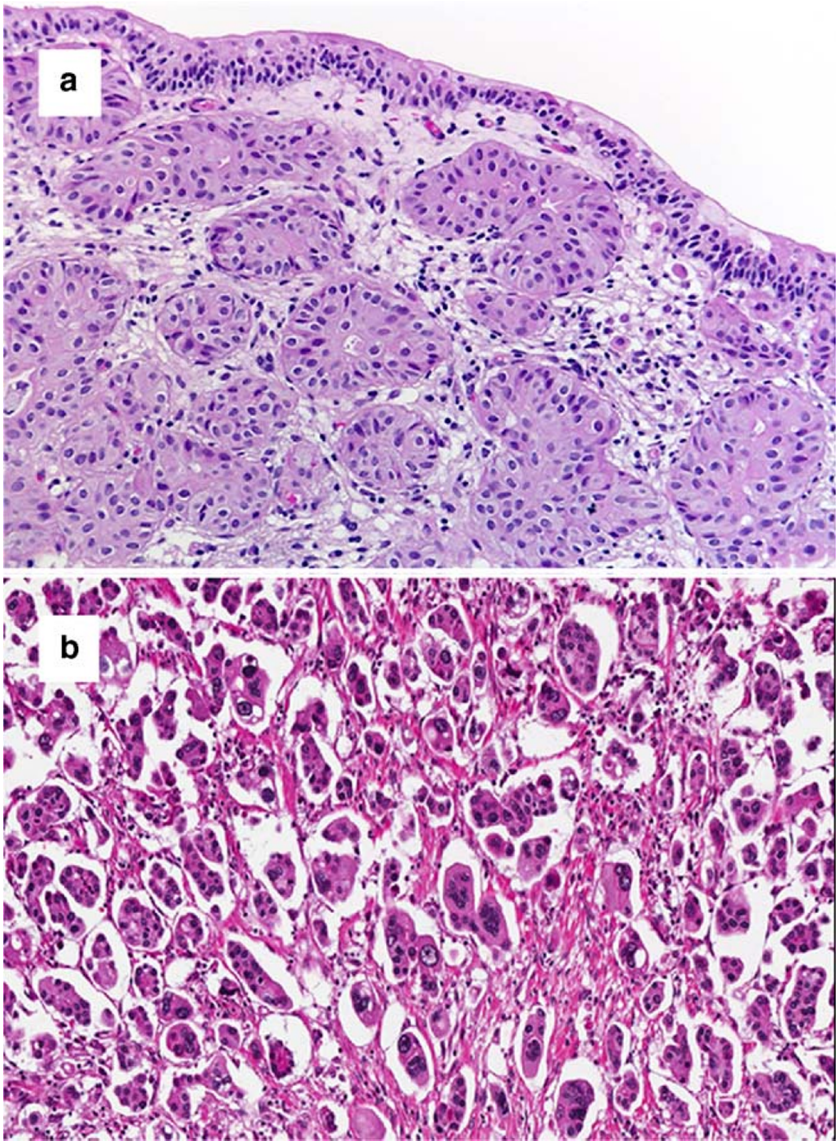

Fig. 12 Nested type carcinoma (**a**). Micropapillary carcinoma (**b**)

criteria to better define the bladder tumor staging particularly the pT1 sub-staging.

Table 4 reviews previous reports on depth of lamina propria involvement as a prognostic factor for disease progression in pT1 bladder carcinoma [15–17, 25, 45–54, 58–67].

At the moment, some open questions remain. In fact, the maximum length cut-off of micrometric approach and/or the type of approaches have not been established yet. In order to correctly evaluate a TURB specimen, we suggest to perform serial sections in high-grade UC cases, when staging diagnosis is critical, keeping in mind all the clinical data including the position (dome, walls, trigone) of the resected bladder neoplasms. In order to avoid differences in substaging diagnosis, the type of approach to substage the pT1 tumor should be easy to perform. The micrometric approach with a small length cut-off may be useful to stratify, within the pT1 category, two groups of patients. Different length cut-off such as 0.5 mm for the trigone area, characterized by a thin LP, and 1 mm for the dome area, characterized by a thick LP, could be applied.

pT1 interobserver reproducibility

The reproducibility of pT1 substaging, particularly the micrometric approach, remains to be fully investigated. Using the 0.5 mm cut-off, the interobserver agreement between two pathologists was of 81% (58). Overall, interobserver variation in pT1 diagnosis is considerable, more in terms of downstaging (15%–56%) than upstaging (3%–13%) [19, 24, 68, 69]. Given the significant downstaging in pT1 cancer with its inherent prognostic ramification, substaging is beneficial in a way of separating tumors with unequivocal small foci of invasion staged as pT1 from frankly extensively invasive pT1, which may be almost akin to pT2 tumors.

Table 4 Review of previous reports on depth of lamina propria involvement as a prognostic factor for disease progression in pT1 bladder carcinoma

Year	Author (Ref)	Staging system	Number of cases	Progression (%)
1990	Younes [45]	T1a (lamina propria)	15	NA
		T1b (into MM)	3	
		T1c (across MM)	14	
1994	Hasui [46]	T1a (Younes T1a)	60	6.7
		T1b (Younes T1b and c)	28	53.5
1995	Angulo [47]	T1a (Younes T1a and b)	50	NA
		T1b (Younes T1c)	49	NA
1997	Holmang [50]	T1a (Younes T1a)	26	36
		T1b (Younes T1b and T1c)	38	58
1998	Smits [54]	T1a	119	6
		T1b		33
		T1c		55
1998	Hermann [49]	T1a	31	NA
		T1b	60	NA
		T1c	52	NA
1999	Cheng [25]	T1 above MM	7	NA
		T1 into or below MM	3	
2000	Kondylis [51]	T1a into MM	32	22
		T1b beyond MM	17	29
2001	Bernardini [48]	T1a (Younes T1a)	54	
		T1b (Younes T1b and c)	40	
2005	Van der Aa [58]	pT1 mic	24	31
		pT1 ext. (> 0.5 mm)	29	50
2005	Orsola [53]	T1a (lamina propria)	38	8
		T1b (into MM)	10	34
		T1c (across MM)	37	34
2011	Bertz [59]	HPF $\leq$ 1	118	8
		HPF > 1	191	28
2012	Lee [52]	T1a (lamina propria)	119	5.8
		T1b (into MM)	57	21.9
		T1c (across MM)	7	21.9
2012	Chang [60]	T1 $\leq$ 1 mm	213	27
		T1 > 1 mm	193	51
2012	Van Rhijn [61]	T1 m	40	17
		T1 e	94	45
2012	Van Rhijn [17]	T1 m < 0.5 mm	40	7 (with CIS 35)
		T1 e	89	45
2012	Roupret [62]	T1a into MM	388	22
		T1b beyond MM	199	41
2013	Olsson [63]	T1a (lamina propria)	75	31
		T1b (into MM)	81	44
		T1c (across MM)	55	42
2013	Brimo [15]	Depth < 3 mm	86	
		Depth > 3 mm		
2014	Hu [16]	Focality (focal, multifocal, < 1%)	23	
		Invasion percentage (1–5%, 5–10%, > 10%		
		Aggregate length of invasion)		
2014	De Marco [64]	pT1 mic	40	No correlation with disease progression
		pT1 ext. (> 0.5 mm)		
2015	Patriarca [65]	ROL 1 $\leq$ 1 mm	152	8
		ROL 2 > 1 mm	162	19
2017	Lawless [66]	Invasion < 1 mm		Correlation with disease progression
		Invasion > 1 mm		
2018	Leivo [67]	ALLICA >		Correlation with disease progression

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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