BMC Urology

RESEARCH

Open Access

Pitfalls of frozen section diagnosis in ureter margin evaluation of plasmacytoid urothelial carcinoma of urinary bladder

Ji Min Kim[1] and Sanghui Park[1*]

Abstract

Background Plasmacytoid urothelial carcinoma (PUC) is a rare and aggressive subtype that often presents at advanced stages with poor prognosis. This study investigated tumor invasion to better understand tumor behavior and potentially to improve management strategies by comparing the clinicopathologic characteristics of PUC with positive ureter resection margin (+URM) with PUC with negative URMs (-URM).

Methods This retrospective analysis used pathology reports from 2017 to 2023 for cases diagnosed with PUC during radical cystectomy (RC). All applicable H&E slides of RC specimens were reviewed. Cases with a plasmacytoid component greater than 25% in the RC specimens were analyzed. Frozen section analyses (FSAs) and permanent section analyses (PSAs) of ureter resection margins were performed.

Results Fifteen patients with a plasmacytoid component greater than 25% in their RC specimens were identified. Compared with -URM PUC cases, +URM PUC cases were located more frequently at the trigone or bladder neck, and all +URM cases exhibited ureter orifice involvement. Among 6 PSA-positive cases, three (50%) cases showed discrepancies with FSA. Three +URM cases exhibited PUC tumor cells along the submucosa and muscularis propria layer, and the 3 remaining cases showed PUC tumor cells along the adventitia. We observed a consistent adventitia invasion in all the discordant cases, with sectioning errors and misinterpretation identified as the primary causal factors.

Conclusion To the best of our knowledge, this is the first study to demonstrate two separate patterns of tumor infiltration along the ureter and to discuss the significance of comparing FSA with PSA in PUC. The significance of comprehensive management strategies for PUC patients, including a thorough evaluation of ureteral margins and accurate interpretation of periureteral fat tissue, is highlighted. Large, well-designed studies are needed to strengthen the evidence and to establish optimal management strategies for patients with PUC.

Keywords Urothelial carcinoma of bladder, Plasmacytoid subtype, Frozen section analysis, Ureter, Resection margin, Radical cystectomy

*Correspondence:
Sanghui Park
spark0430@ewha.ac.kr
[1]Department of Pathology, Ewha Womans University Mokdong Hospital, Ewha Womans University College of Medicine, Yangcheon-Gu, Seoul, Republic of Korea

Introduction

Plasmacytoid urothelial carcinoma (PUC) is a rare subtype that accounts for 1–3% of invasive UC [1–3]. PUC typically presents as advanced stage disease and is associated with an advanced tumor stage at radical cystectomy (RC), a high rate of lymph node (LN) metastasis, and poor prognosis [4–6]. Several studies have identified a notable preference for peritoneal spread and a unique spreading pattern along the fascial plane as the distinguishing characteristics of PUC [5–7].

Frozen section analyses (FSAs) on the ureters during RC help to identify malignant tissue and enables additional resection to decrease the recurrence risk [8]. On average, patients with the plasmacytoid subtype undergo 2.3 intraoperative surgical frozen margin resections for each affected ureter before negative margins are obtained, which is twice the number typical in conventional UC [5]. Furthermore, PUC had an 8.6 times higher risk of a positive ureter resection margin (+URM) in a permanent section analysis (PSA) than was found in conventional UC; even compared with the micropapillary subtype, PUC demonstrated a URM positivity rate about twice as high [5].

In this study, we compare the clinicopathologic characteristics of PUC with +URM with those of PUC with negative URM (-URM). Based on that assessment, we investigated tumor invasion to better understand tumor behavior from a pathological point of view and to explore improved management options for patients with the plasmacytoid subtype.

Materials and methods

Patient selection

This study was approved by the Institutional Review Board at Ewha Womans University Medical Center (2024-03-006). We retrospectively searched our pathology reports from 2017 to 2023 and identified cases diagnosed with PUC during RC. Two genitourinary pathologists (J.M.K and S.P) reviewed all applicable H&E slides of RC specimens. Tumors were graded according to the 2022 World Health Organization Classification of the urinary and male genital tumors [9] and were staged according to the TNM system from the American Joint Committee on Cancer 8th Edition Cancer Staging Manual [10]. Cases with a plasmacytoid component greater than 25% in the RC specimens were included in the current analyses. Patient age, sex, tumor location, pathologic tumor stage, LN status, proportion of plasmacytoid component, neoadjuvant chemotherapy, and clinical outcome were recorded.

FSA and URM assessment

RC was performed following the standard protocol, with routine dissection of pelvic LNs, including the bilateral internal iliac, external iliac, and obturator LNs. The right and left URMs were sent for FSA prior to reconstruction of the ureterointestinal anastomosis for the neobladder. The URMs were snap-frozen, cut at 5 μm, and stained with a rapid H&E protocol. Two genitourinary pathologists performed histological assessments of those specimens to determine the presence of neoplastic changes. The URMs were trimmed, embedded in paraffin, and step-sectioned for the PSAs, which represent the final URMs. Pan cytokeratin (Novacastra, Buffalo Grove, IL) immunostaining was performed as needed to highlight PUC tumor cells.

Statistical analysis

Clinicopathologic variables were compared between groups using the Chi-square and two-sample t tests. Statistical analyses were performed using SPSS® software version 20 (SPSS Inc., Chicago, IL). A p-value < 0.05 was considered statistically significant.

Results

Clinicopathologic characteristics in the -URM and +URM groups

Fifteen patients with a plasmacytoid component greater than 25% in their RC specimens were identified during the study period. All 15 patients were male and presented at an advanced tumor stage (≥pT2) during RC. Nine of the 15 (60%) cases showed -URM, and 6 (40%) showed+URM. Notably, the +URM PUC cases were located more frequently at the trigone or bladder neck than the -URM PUC cases ($p=0.005$), and all +URM cases exhibited ureter orifice involvement ($p=0.003$). The +URM PUC patients displayed a higher proportion of plasmacytoid component, along with increased incidences of LN involvement, distant metastasis, and mortality. However, those parameters did not exhibit significant differences from those of the -URM PUC patients. The clinicopathologic characteristics of the two groups are summarized in Table 1.

Concordance between FSA and PSA

URM PUC involvement was confirmed in 6 of the 15 specimens by PSA (40%). The FSA was positive in 3 of the 15 specimens. The FSA was negative in all 9 of the PSA-negative specimens, but 3 (50%) of the 6 PSA-positive cases had FSA-negative results. Upon examining the URM involvement pattern in the 6+URM cases, 3 cases exhibited PUC tumor cells along the submucosa and muscularis propria layer (Fig. 1a, c), and the remaining 3 cases showed PUC tumor cells along the adventitia (Fig. 1b, d). These patterns extended into the longitudinal section of the ureter in the RC specimens (Fig. 2). Notably, in all 3 of the discordant cases between FSA and PSA, only the adventitia invasion of tumor was observed.

Table 1 The clinicopathologic characteristics of the negative ureter resection margin patients and the positive ureter resection margin patients

Clinicopathological variables	Ureter RM (-) (*n* = 9, 60%)	Ureter RM (+) (*n* = 6, 40%)	*P* value
Median age (years, range)	74 (68–78)	67 (51–76)	0.127
Gender			
Male	9 (100%)	6 (100%)	
Female	0	0	
Tumor location			0.005
Trigone or Bladder neck	1 (11%)	5 (83%)	
Other	8 (89%)	1 (17%)	
Tumor stage on RC with PLND			0.324
≤pT2	1 (11%)	0	
pT3a	7 (78%)	3 (50%)	
pT3b	1 (11%)	2 (33%)	
pT4	0	1 (17%)	
LN stage on RC with PLND			0.435
pN0	6 (67%)	2 (33%)	
pN1	1 (11%)	1 (17%)	
pN2	2 (22%)	3 (50%)	
Ureter orifice involvement			0.003
Absent	7 (78%)	0	
Present	2 (22%)	6 (100%)	
Proportion of PUC component			0.132
30–65	5 (56%)	1 (17%)	
66–100	4 (44%)	5 (83%)	
NAC			0.264
Absent	7 (78%)	3 (50%)	
Present	2 (22%)	3 (50%)	
Progression (Metastasis)			0.205
Absent	6 (67%)	2 (33%)	
Present	3 (33%)	4 (67%)	
Death			0.634
No	7 (78%)	4 (67%)	
Yes	2 (22%)	2 (33%)	

LN Lymph node, NAC Neoadjuvant chemotherapy, PLND Pelvic lymph node dissection, PUC Plasmacytoid urothelial carcinoma, RC Radical cystectomy, RM Resection margin

Upon investigating the cause of the discordant cases, we found that 2 cases were due to sectioning errors (Fig. 3), and 1 case was due to misinterpretation by the surgical pathologist.

Discussion

To the best of our knowledge, we are the first to compare FSA and PSA results from the URM of PUC cases and to demonstrate two separate patterns of tumor infiltration. In this study, we found a notable 50% discrepancy rate between FSA and PSA results for the URM. Moreover, we observed a consistent periureteral fat infiltrative pattern (adventitia invasion) in all the discordant cases, with sectioning errors and misinterpretation identified as the primary causal factors.

Submission of URM for intraoperative pathology consultation is common for bladder cancer patients undergoing RC [11]. However, studies about the significance of FSA of the URM in PUC are rare. Kaimakliotis et al. [5] reported that a 32% URM-positive status in PUC, which aligns closely with our results of a 40% URM positivity rate. In the study of Kaimakliotis et al. [5], the URM positivity rate in PUC was 8.6 times higher than that in conventional UC (2.3%). Furthermore, PUC showed a much higher URM positivity rate than that (17.9%) in micropapillary UC (MPC), another aggressive subtype [5]. Notably, all +URM in conventional UC and MPC involved only carcinoma in situ (CIS) on the endoluminal URM, whereas 5 of the 8+URM cases in PUC showed tumor cell invasion without concurrent CIS [5]. In accordance with the literature, all +URM cases in our cohort exhibited only invasion of PUC tumor cells. These findings are consistent with previous studies indicating that PUC has a propensity to manifest disease on

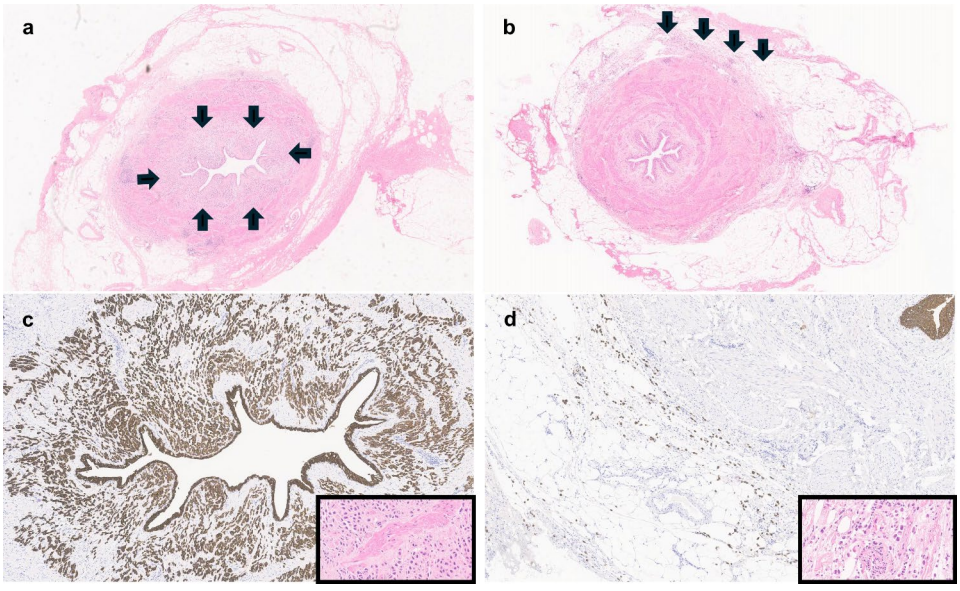

Fig. 1 Two involvement patterns of plasmacytoid urothelial carcinoma in the ureter resection margin. **a** Tumor cells are exhibited along the submucosa and the muscularis propria layer (arrows) (H&E, x4). **b** Tumor cells are noted along the adventitia (arrows) (H&E, x4). **c** Magnified (H&E, x10) of **a**, pan cytokeratin staining highlights tumor cells, inset (H&E, x40). **d** Magnified (H&E, x10) of **b**, pan cytokeratin staining highlights tumor cells, inset (H&E, x40)

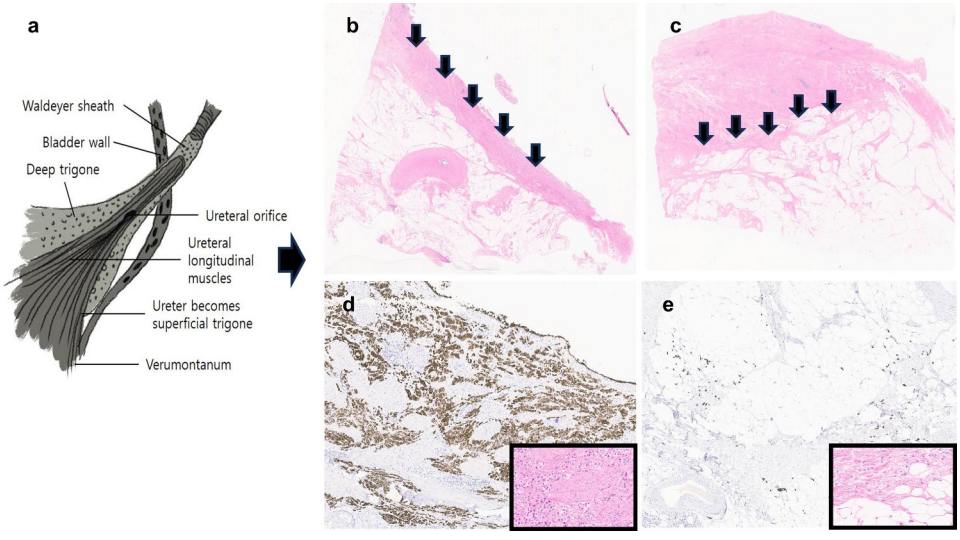

Fig. 2 **a** Illustrated anatomy of the ureterovesical junction. **b** Plasmacytoid urothelial carcinoma tumor cells extend into the muscularis propria of the ureter in radical cystectomy specimen (arrows) (H&E, x4). **c** Plasmacytoid urothelial carcinoma tumor cells extend into the adventitia of the ureter in radical cystectomy specimen (arrows) (H&E, x4). **d** Magnified (H&E, x10) of **b**, pan cytokeratin staining highlights tumor cells, inset (H&E, x20). **e** Magnified (H&E, x10) of **c**, pan cytokeratin staining highlights tumor cells, inset (H&E, x20)

peritoneal surfaces, leading to common+URM and surgical upstaging [6, 7]. These results suggest a significant likelihood that residual tumor will remain at the operative site even after radical surgery. These observations are associated with the independent effect of plasmacytoid subtype histology on survival outcomes. It remains unknown whether +URM in PUC is independently associated with disease-specific or overall survival, but our findings in this study and those in the literature imply that a thorough assessment of intraoperative URMs is essential in PUC.

We found that +URM cases in PUC showed 2 distinct patterns of tumor infiltration. In our cohort, PUC tumor cells infiltrate through the submucosa and muscularis propria layer of the distal ureter, or tumor cells were noted along the adventitia over a longitudinal section of the ureter in the RC specimen. Kaimakliotis et al. [5] also reported that all +URM involving invasive tumor cells in PUC showed an infiltrative pattern of the subserosal

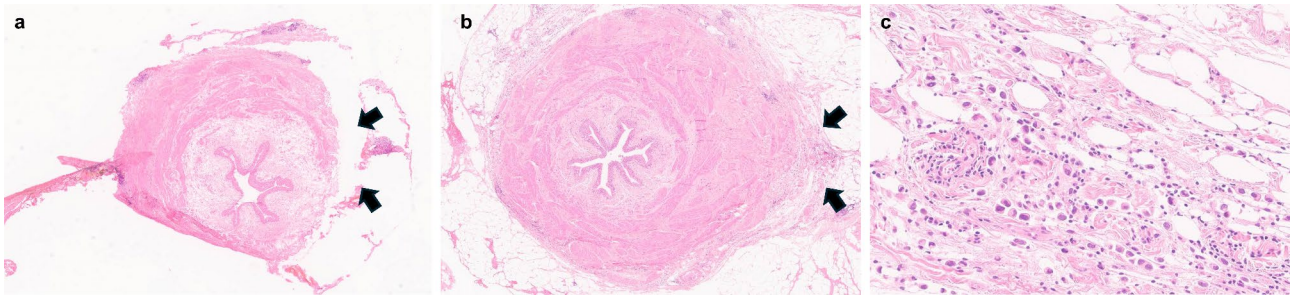

Fig. 3 Representative H&E slides from a discordant case. **a** Periureteral fat tissue loss is observed in the frozen section of the ureter resection margin (arrows) (H&E, x4). **b** Tumor cells are noted in periureteral fat tissue in the permanent section of the ureter resection margin (arrows) (H&E, x4). **c** Magnified (H&E, x10)

and adventitia ureteral plane, and PUC tumor cells were also observed along the subserosal surface and ureteral adventitia on multiple, sequential surgical excisions. These findings explain that PUC tumor cells that penetrate beyond the detrusor muscle could potentially move along the bladder serosa beneath the peritoneal reflection or the endopelvic fascia. Once within Waldeyer's space, they can be tracked along the distal ureteral subserosa and adventitia [5]. On the other hand, the mechanism of intramuscular invasion was elucidated by the study of Beunk et al. [12]. They explained that smooth muscle tumor invasion in the bladder involves multiple steps, beginning with epithelial cancer cells infiltrating the connective tissue that separates the epithelial and muscle layers, followed by muscle invasion. Here, bladder tumor cells exhibit chain-like invasion into smooth muscle tissue, showing characteristics of jammed or collective migration aligned with the linear endomysium [12]. They also reported that muscle invasion in cancer patients is frequently correlated with destructive growth and a worse prognosis [12]. Anatomically, the inner smooth muscle layer of the ureter merges with that of the contralateral ureter to form the superficial trigone [13]. The distal ureter is accompanied by a separate external smooth muscle layer (Waldeyer sheath) to the bladder [13]. As it traverses the bladder wall, the Waldeyer sheath merges with detrusor muscle fibers to form the deep trigone of the bladder, which is enclosed by the 2 ureter orifices [13]. These distinctive anatomical structures between the ureter and bladder can provide additional support for the 2 PUC tumor cell infiltration patterns highlighted in this study, as well as the observation that these 2 infiltration patterns align with the longitudinal ureter muscle. These explanations are in line with the findings in our cohort that PUC with +URM was significantly more common in the trigone or bladder neck, and all PUC cases with +URM showed tumor involvement in the ureteral orifice, tended to exhibit higher tumor and LN stages, and showed slightly more progressive behavior. The aggressiveness of PUC can be explained by the loss of E-cadherin. Several studies have demonstrated that decreased expression of E-cadherin, a membrane connective tissue protein, is associated with more aggressive pathology, loss of cell differentiation, increased cell invasion, and poor prognosis in UC [14–16]. Loss of membranous E-cadherin expression and its nuclear accumulation are highly associated with PUC [14, 15] and could allow PUC tumor cells to easily invade surrounding tissues, ultimately leading to an advanced stage and rapid progression.

In this study, discordance between FSA and PSA was observed in 3 of the 6 (50%)+URM cases in the histopathological examination of the permanent URM tissues. Interestingly, all the discordant cases exhibited the periureteral fat infiltrative pattern (adventitia invasion), and the causes of the discordance between FSA and PSA were sectioning errors and misinterpretation by the pathologist of tumor cells as inflammatory cells. These findings indicate that a thorough evaluation of the ureteral margin should be conducted, with a focus on the periureteral fat tissue. The freezing and thawing processes used for frozen sectioning can cause tissue damage, with fatty tissues being especially vulnerable, potentially resulting in tissue loss and sectioning challenges. When preparing frozen sections, it is important to include periureteral fat tissue in the embedding process. Additionally, if the periureteral fat tissue is not visible on the slide or in any suspicious cases, additional frozen section examinations should be conducted. Pathologists should also pay close attention to the periureteral fat tissue when interpreting frozen slides. Ultimately, this thorough assessment of intraoperative URM should be applicable to all UCs that include PUC. In addition to this, it is recommended that pathologists include detailed information on PUC in every pathological report, as lack of awareness regarding the plasmacytoid subtype during FSA appears to pose greater challenges. Given that PUC tumor cells show morphological resemblance to plasma cells, it can be suggested that including detailed information about PUC in pathological reports may assist pathologists in minimizing the risk of misinterpretation during FSA.

This study has several limitations. It had a retrospective design that could not avoid inevitable limitations such as selection bias. The small sample size caused by the rarity of this entity and our focus on PUC with a plasmacytoid subtype component greater than 25%, and inclusion of patients who received neoadjuvant chemotherapy could affect the overall data quality. Also, statistically significant differences were not found in recurrence and survival between +URM patients and -URM patients, although +URM patients showed slightly more progressive behavior in the present study. Larger cohorts and further well-designed multicenter studies are needed to clarify the clinical significance of frozen assessment in PUC and to establish strong recommendations. Despite those shortcomings, this study offers valuable insights based on currently available data. These findings can aid clinicians and pathologists in shared decision-making discussions about the management and prognosis of patients with PUC.

Conclusion

To the best of our knowledge, this is the first study to demonstrate 2 separate patterns of tumor infiltration along the ureter and to discuss the significance of FSA compared with PSA in PUC. Based on an understanding of the two unique infiltration patterns of PUC, we emphasize the importance of thoroughly embedding and sectioning periureteral fat tissue during the frozen sectioning process and accurately evaluating periureteral fat tissue during frozen slide interpretation. Large prospective studies are warranted to clarify the importance of a comprehensive management strategy in patients with PUC.

Abbreviations

CIS	Carcinoma in situ
FSA	Frozen section analysis
LN	Lymph node
MPC	Micropapillary UC
PSA	Permanent section analysis
PUC	Plasmacytoid urothelial carcinoma
RC	Radical cystectomy
-URM	Negative ureter resection margin
+URM	Positive ureter resection margin

Acknowledgements

Not applicable.

Author contributions

"J.M.K. contributed to the data acquisition, analysis, and interpretation of this work and participated in drafting and revising the contents. S.P. contributed to the data analysis and the concept of this work and approved the submission of the final version. All authors have read and approved the manuscript."

Funding

This study was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI21C0977). This study was supported by the Ewha Medical Center Research Promoting Grant Program.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The Institutional Review Board at Ewha Womans University Medical Center (2024-03-006) approved this retrospective study. All methods were performed in accordance with the relevant guidelines and regulations, and the ethical standards of the 1964 Helsinki declaration. The requirement for informed consent was waived by the Institutional Review Board due to the retrospective nature of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number

Not applicable.

Received: 31 May 2024 / Accepted: 4 November 2024
Published online: 16 November 2024

References

1. Monn MF, Kaimakliotis HZ, Pedrosa JA, Cary KC, Bihrle R, Cheng L, et al. Contemporary bladder cancer: variant histology may be a significant driver of disease. Urol Oncol. 2015;33(1):18. e5-.e20.
2. Fritsche HM, Burger M, Denzinger S, Legal W, Goebell PJ, Hartmann A. Plasmacytoid urothelial carcinoma of the bladder: histological and clinical features of 5 cases. J Urol. 2008;180(5):1923–7.
3. Mai KT, Park PC, Yazdi HM, Saltel E, Erdogan S, Stinson WA, et al. Plasmacytoid urothelial carcinoma of the urinary bladder report of seven new cases. Eur Urol. 2006;50(5):1111–4.
4. Kim DK, Kim JW, Ro JY, Lee HS, Park JY, Ahn HK, et al. Plasmacytoid variant Urothelial Carcinoma of the bladder: a systematic review and Meta-analysis of Clinicopathological features and survival outcomes. J Urol. 2020;204(2):215–23.
5. Kaimakliotis HZ, Monn MF, Cheng L, Masterson TA, Cary KC, Pedrosa JA, et al. Plasmacytoid bladder cancer: variant histology with aggressive behavior and a new mode of invasion along fascial planes. Urology. 2014;83(5):1112–6.
6. Dayyani F, Czerniak BA, Sircar K, Munsell MF, Millikan RE, Dinney CP, et al. Plasmacytoid urothelial carcinoma, a chemosensitive cancer with poor prognosis, and peritoneal carcinomatosis. J Urol. 2013;189(5):1656–61.
7. Ricardo-Gonzalez RR, Nguyen M, Gokden N, Sangoi AR, Presti JC Jr., McKenney JK. Plasmacytoid carcinoma of the bladder: a urothelial carcinoma variant with a predilection for intraperitoneal spread. J Urol. 2012;187(3):852–5.
8. Schumacher MC, Scholz M, Weise ES, Fleischmann A, Thalmann GN, Studer UE. Is there an indication for frozen section examination of the ureteral margins during cystectomy for transitional cell carcinoma of the bladder? J Urol. 2006;176(6 Pt 1):2409–13. discussion 13.
9. WHO classification of. Tumours: urinary and male genital tumours. Volume 8, 5 ed. International Agency for Research on Cancer; 2022.
10. Bochner B, Hansel D, Efstathiou J, Konety B, Lee C, McKiernan J et al. Urinary Bladder. 2017. pp. 757 – 65.
11. Miyamoto H. Clinical benefits of frozen section assessment during urological surgery: does it contribute to improving surgical margin status and patient outcomes as previously thought? Int J Urology: Official J Japanese Urol Association. 2017;24(1):25–31.
12. Beunk L, Brown K, Nagtegaal I, Friedl P, Wolf K. Cancer invasion into musculature: mechanics, molecules and implications. Semin Cell Dev Biol. 2019;93:36–45.
13. Fröber R. Surgical anatomy of the ureter. BJU international. 12007;100(4):949 – 65.
14. Lim MG, Adsay NV, Grignon DJ, Osunkoya AO. E-cadherin expression in plasmacytoid, signet ring cell and micropapillary variants of urothelial carcinoma:

comparison with usual-type high-grade urothelial carcinoma. Mod Pathology: Official J United States Can Acad Pathol Inc. 2011;24(2):241–7.
15. Keck B, Wach S, Kunath F, Bertz S, Taubert H, Lehmann J, et al. Nuclear E-cadherin expression is associated with the loss of membranous E-cadherin, plasmacytoid differentiation and reduced overall survival in urothelial carcinoma of the bladder. Ann Surg Oncol. 2013;20(7):2440–5.
16. Erdemir F, Ozcan F, Kilicaslan I, Parlaktas BS, Uluocak N, Gokce O. The relationship between the expression of E-cadherin and tumor recurrence and progression in high-grade stage T1 bladder urothelial carcinoma. Int Urol Nephrol. 2007;39(4):1031–7.

Publisher's note