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Case Report

Urothelial carcinoma with oncocytic features: an extremely rare case presenting a diagnostic challenge in urine cytology

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Abstract: Recognizing histological variants in urothelial carcinoma (UC) is important because some may be associated with different clinical outcomes and/or therapeutic approaches; being aware of unusual histological variants may also be crucial in preventing diagnostic misinterpretations. Histological variants based on cytoplasmic features, such as clear-cell, plasmacytoid, rhabdoid, and lipoid-rich variants, are described in invasive UC; however, these cytoplasmic features are not formally defined and not usually encountered in non-invasive UC. Oncocytic cytoplasm has not been well described in either invasive or non-invasive UC. Herein, we report an exceedingly rare case of UC with oncocytic features arising in the right renal pelvis, which presented a diagnostic challenge in urine cytology due to the relatively low nuclear-to-cytoplasmic ratio; however, it could definitively be diagnosed using histological specimens. UC diagnosis is based on the presence of papillary architecture and widespread p53 nuclear accumulation, suggesting malignancy. An oncocytic tumor is generally considered to be not actively dividing, as shown by the low Ki-67 labeling index in this case. In spite of the low proliferative activity, the possibility of intravesicle recurrence (IVR) should be considered since positive preoperative cytology of upper tract UC is a risk factor for IVR after nephroureterectomy.

Keywords: Urothelial carcinoma, oncocytic features, renal pelvis, urine cytology, histology, immunohistochemistry

Introduction

The spectrum of morphology of urothelial carcinoma (UC) has been expanded to include several unusual histological variants. Recognizing histological variants in UC is important because some may be associated with different clinical outcomes and/or therapeutic approaches compared with conventional UC, and being aware of unusual histological variants may be crucial in preventing diagnostic misinterpretations [1]. Other than UC with squamous and/or glandular differentiation seen both in non-invasive and invasive components, histological variants are usually observed in invasive components, in which UC with unusual cytoplasmic features, such as clear-cell (glycogen rich), plasmacytoid, rhabdoid, and lipoid-rich features, are included [1]. With respect to non-invasive components, cytoplasmic features such as those described in invasive components are not formally defined and are not usually encountered.

Oncocytic carcinoma has been documented in many organs, including the salivary gland [2] and thyroid gland [3], where these tumors appear to be more common than in other organs. Oncocytes are large epithelial cells with a low nuclear-to-cytoplasmic ratio, a centrally situated round nucleus containing a prominent nucleolus and abundant eosinophilic granular cytoplasm that is ultrastructurally confirmed to harbor numerous mitochondria [2]. Tumor cells of oncocytic carcinoma show these same characteristics. In the urological region, oncocytic tumors, such as oncocytoma and hybrid oncocytic/chromophobe tumors of the kidney, have been established [4]. Under a name including both “oncocytic” and “carcinoma”, oncocytic papillary renal cell carcinoma (RCC) was first reported by Lefevre et al. in 2005 [5]. Several other entities, e.g. acquired cystic disease-associated RCC, can have oncocytic morphology [6]. However, oncocytic carcinoma of the urinary tract has not been well described.

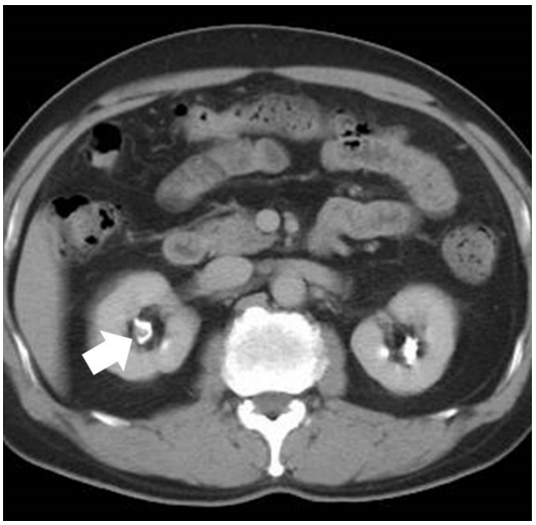

Figure 1. Computed tomography findings. A mass is seen as a defect within the background of contrast material filling the right renal pelvis (arrow).

Herein, we report an exceedingly rare case of UC with oncocytic features arising in the right renal pelvis, which presented a diagnostic challenge in urine cytology but was definitively diagnosed using histological specimens. In addition, we extensively analyzed the immunohistochemical characteristics of this case.

Clinical summary

A 68-year-old man presented with hematuria. Physical examination and laboratory tests revealed no abnormalities. Urinalysis revealed atypical cells along with numerous red blood cells. Urine cytology was conducted and a cytological diagnosis of a suspected tumoral lesion but equivocal for malignancy was rendered. Ultrasonography did not identify any lesion in the urinary tract. Subsequently, contrast-enhanced computed tomography (CT) was performed, revealing a mass in the right renal pelvis. The mass appeared as a defect area within the background of contrast material filling the right renal pelvis (**Figure 1**). Right nephroureterectomy was conducted for suspected UC in the right renal pelvis. UC of the right renal pelvis, evaluated as pTa, was confirmed by pathological examination. The patient has been recurrence-free for 3 years.

Pathological findings

Analysis of cytological specimens revealed many cellular clusters composed of atypical cells and abundant scattered atypical cells with

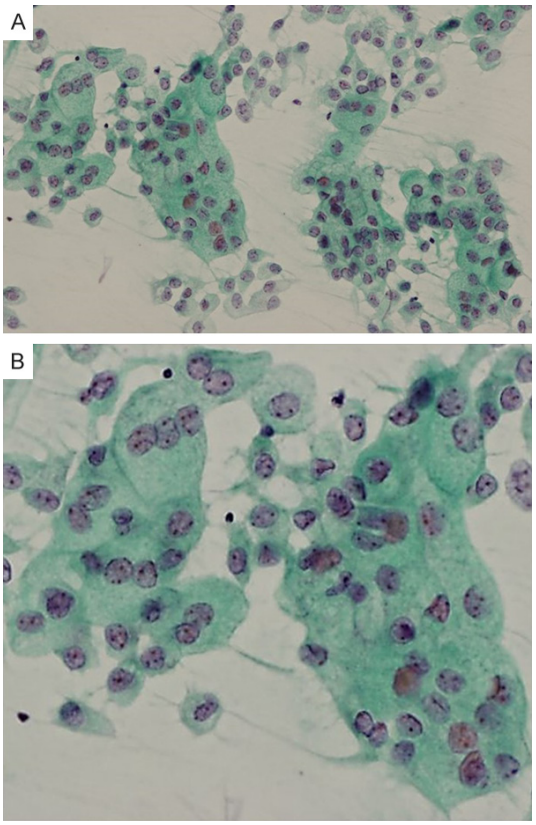

Figure 2. Cytological findings with Papanicolaou (Pap) staining. A. There are cellular clusters composed of atypical cells and abundant scattered cells of the same basic morphology. The presence of many scattered cells indicates loosened cellular cohesiveness (Pap, ×200). B. Atypical cells have abundant eosinophilic cytoplasm with some granularity, and their nuclei are enlarged and hyperchromatic (Pap, ×600).

basically the same morphology. The cellular composition of the cytological specimens was monotonous, and the atypical cells were almost exclusively observed. The presence of many scattered atypical cells indicates loosened cellular cohesiveness (**Figure 2A**). The atypical cells had abundant eosinophilic cytoplasm with some granularity and their nuclei were enlarged and hyperchromatic (**Figure 2B**). Due to the abundant cytoplasm, the nuclear-to-cytoplasmic ratio was relatively low compared with that seen in UC in general. Being unable to reach a diagnosis of a malignant lesion, a diagnosis of a tumoral lesion equivocal for malignancy was rendered.

Gross examination of the surgically resected specimen revealed a 1.3 × 0.8-cm papillary tumor located in the right renal pelvis. Invasion of the renal pelvis was not apparent.

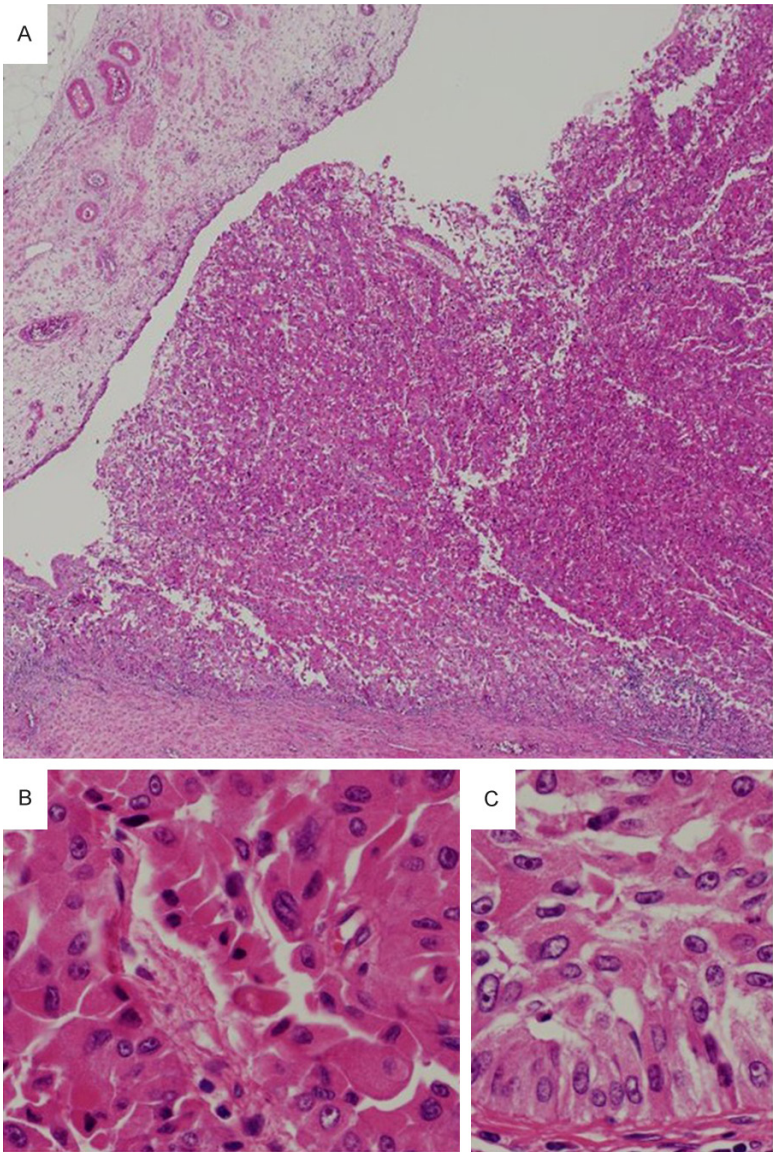

Figure 3. Histological findings with hematoxylin and eosin (H&E) staining. A. A papillary tumor appears as a strongly eosinophilic lesion (H&E, ×40). B. Tumor cells have abundant eosinophilic and granular cytoplasm with enlarged nuclei containing distinct nucleoli, findings considered to represent oncocytic morphology (H&E, ×400). C. At the base of the papillary tumor, some tumor cells without oncocytic morphology are seen (H&E, ×400).

The surgically resected specimen was fixed with 10% buffered formalin, embedded in paraffin, and was then sliced into sections. Formalin-fixed paraffin-embedded sections were used for hematoxylin and eosin (H&E) staining (2.5 μm-thick) and immunohistochemistry (IHC) (4 μm-thick). An automated slide stainer (Bench-Mark GX; Ventana Medical Systems, Tucson, AZ, USA) was used to perform IHC. For electron microscopy, formalin-fixed tumor sections were retrieved, fixed again with 2.5% glutaraldehyde, and post-fixed with 0.8% osmium tetroxide. They were dehydrated in graded ethanol and embedded in Epon. The ultrathin sections for EM were stained with uranyl acetate and lead citrate.

Histological specimen indicated that the papillary tumor was a strongly eosinophilic lesion (**Figure 3A**). Tumor cells had abundant eosinophilic and granular cytoplasm with enlarged nuclei containing distinct nucleoli, a pattern that was recognized as oncocytic morphology (**Figure 3B**). At the base of the papillary tumor, some tumor cells without oncocytic morphology were present, which accounted for approximately 5% of all the tumor cells (**Figure 3C**). Mitotic figures were not apparent, and tumor necrosis was absent. Stromal invasion was not identified, which corresponded to the pTa. The surgical margin was negative for the tumor.

Upon examination with IHC, CK7 (OV-TL 12/30, 1:100; Dako, Glostrup, Denmark) was diffusely and strongly positive in the tumor cells (**Figure 4A**). CK20 (Ks20.8, 1:100; Dako) was weekly positive in approximately 30% of tumor cells (**Figure 4B**), and high molecular weight cytokeratin (34βE12, 1:100; Dako) was completely negative (**Figure 4C**). P63 (4A4, 1:100; Dako) was positive in approximately 70% of tumor cells (**Figure 4D**). GATA binding protein 3 (GATA3) (HG3-31, 1:50; Santa Cruz Biotechnology, Santa Cruz, CA) was positive in approximately 80% of tumor cells (**Figure 4E**). Uroplakin II (BC21, prediluted; Biocare Medical, Concord, CA) was positive in approximately 70% of tumor cells (**Figure 4F**). A marker for mitochondria (MTC02, 1:100; Epitomics, Burlingame, CA) was diffusely and strongly pos-

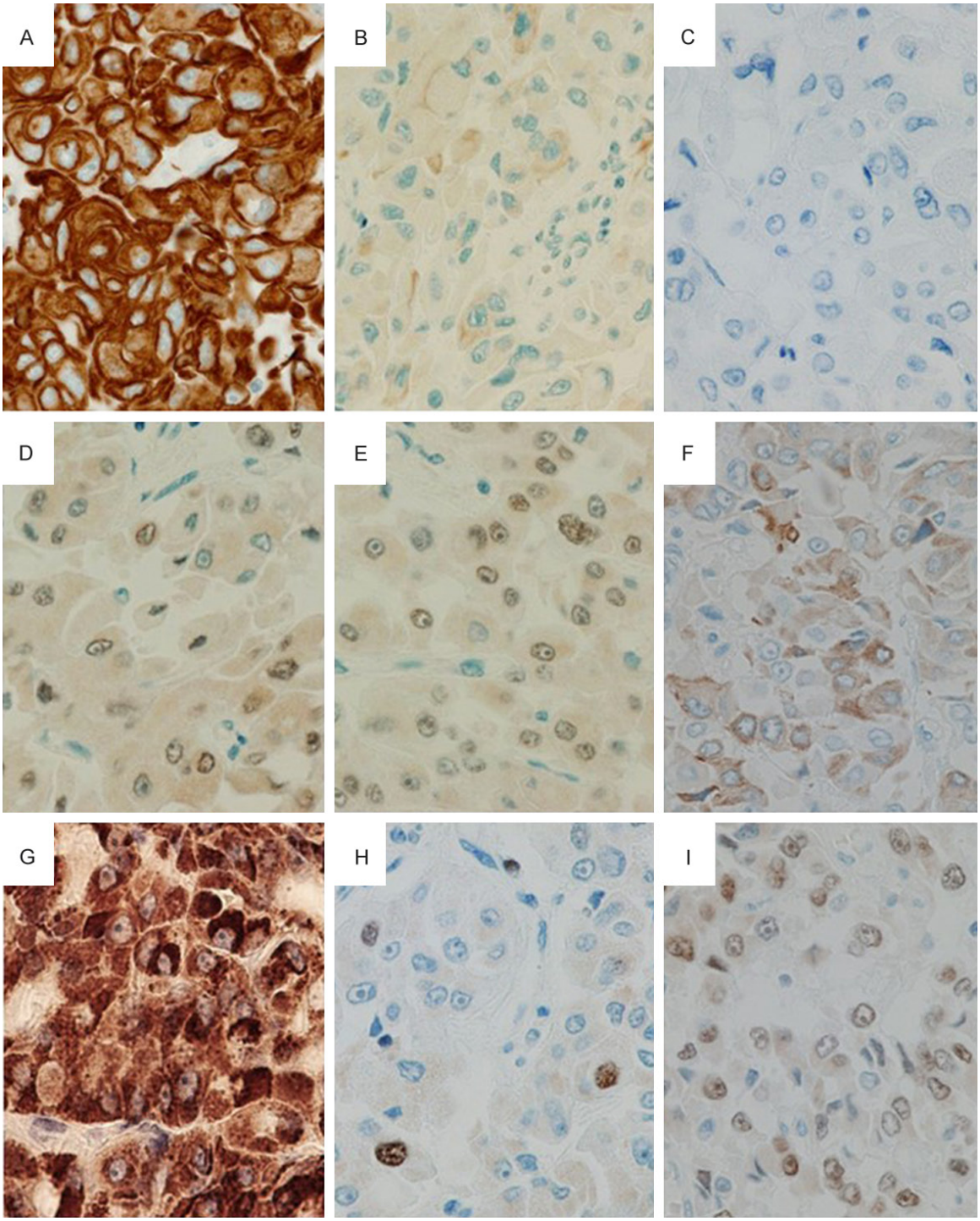

Figure 4. Immunohistochemical findings. A. CK7 is diffusely and strongly positive in tumor cells (×400). B. CK20 is weakly positive in approximately 30% of tumor cells (×400). C. High molecular weight cytokeratin is completely negative (×400). D. P63 is positive in approximately 70% of tumor cells (×400). E. GATA binding protein 3 is positive in approximately 80% of tumor cells (×400). F. Uroplakin II is positive in approximately 70% of tumor cells (×400). G. Mitochondria are diffusely and strongly positive in tumor cells (×400). H. Ki-67 immunostaining is seen in a few cells; this is one of the fields with the most Ki-67-positive cells within areas composed of tumor cells with oncocytic morphology (×400). I. Nuclear accumulation of p53 is observed in 80% of tumor cells (×400).

itive in tumor cells (**Figure 4G**), though to a lesser extent in the tumor cells without oncocytic morphology. The Ki-67 (MIB-1, 1:100; Dako) labeling index was 5.2%, and it was higher in

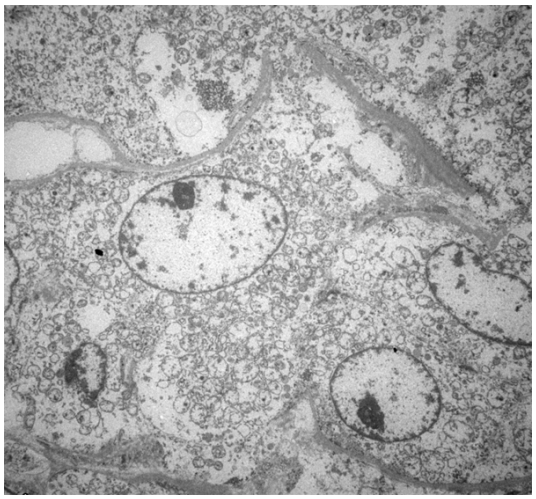

Figure 5. Electron microscopy findings. Tumor cells are filled with abundant mitochondria.

tumor cells without oncocytic morphology than in those with oncocytic morphology (**Figure 4H**). Nuclear accumulation of p53 (DO-7, 1:100, Dako) was observed in 80% of tumor cells (**Figure 4I**).

Electron microscopy was performed using formalin-fixed, paraffin-embedded blocks. It revealed tumor cells with abundant mitochondria in their cytoplasm (**Figure 5**).

The tumor's final pathological diagnosis was UC with oncocytic features. Nuclear accumulation of p53 indicates a malignant tumor, although the Ki-67 labeling index was relatively low for a malignant tumor.

Discussion

The UC tumor cells in this case were similar to those in other oncocytic tumors in that they were filled with mitochondria [2]. The amount of mitochondria varied among tumor cells in this case, according to oncocytic morphology; the number of mitochondria in tumor cells with oncocytic morphology exceeded that in tumor cells without oncocytic morphology. Although the role of the mitochondrial accumulation is unclear, it is thought that an increase in mitochondria is a consequence of alterations in the mitochondrial DNA or in the DNA encoding mitochondrial proteins [7]. Under different physiologic conditions, the degree of oncocytic changes varies in the same lineage of cells; in addition, the development of oncocytic changes has been postulated to be secondary to cell aging, the degenerative process, and inflammation [8, 9]. As mitochondria are thought to accumulate only in tumor cells that are not actively dividing, recognizing oncocytic tumors as having low malignant potential seems plausible [10, 11]. The aforementioned hybrid oncocytic/chromophobe tumor, oncocytic papillary RCC, and acquired cystic disease-associated RCC are all considered to be more indolent tumors than conventional RCC [4, 5, 12], which would reflect the nature of oncocytic tumors in general. In our case of UC with oncocytic features, the Ki-67 labeling index was especially low in the tumor cells with oncocytic morphology corresponding to the observation that mitochondria do not accumulate in actively dividing cells.

In spite of the relatively low Ki-67 labeling index, the tumor in our case could be diagnosed as UC. This is because widespread nuclear accumulation of p53 probably reflects *TP53* mutation, suggesting malignancy, as well as the papillary architecture of the tumor showing positive IHC results for the relatively urothelial-specific markers, GATA3 and uroplakin II [13]. Typical UC expression patterns for other markers were observed, with the exception of the lack of 34βE12 expression [14]. In urothelial papilloma, *TP53* mutations are not expected, but fibroblast growth factor receptor 3 mutations are expected [15]. The degree of p53 nuclear accumulation and the Ki-67 labeling index on IHC, both of which were strongly associated, were found to be independent predictive factors of the biological behavior of UC [16]. It is thus difficult to predict the biological behavior of UC in our case on the basis of pathological examination due to the discrepancy between the degree of p53 nuclear accumulation and the Ki-67 labeling index.

Oncocytic tumors are difficult to diagnose on urine cytology. Cytological examination often does not allow for the discrimination between benign and malignant oncocytic tumors [17]. In our case, a cytological finding of loose cohesiveness of monotonously appearing atypical cells alone could suggest the possibility of malignancy; it is well known that cellular atypia is often augmented in urinary cytology and should be evaluated carefully, which could lead to false-positive results [18]. After confirmation of the histological diagnosis, atypical cells were ascertained to correspond to tumor cells of UC

with oncocytic features judging from their morphological resemblance.

The origination of synchronous and/or metachronous multifocal UC is due to the field effect and/or implantation following intraluminal seeding [19]. It has been documented that intravesicle recurrence (IVR) could occur after radical nephroureterectomy of the upper tract UC [20]. A significantly increased risk of IVR has been noted in patients with positive preoperative urinary cytology, which has been shown to be a greater risk factor for IVR than invasive pT stage [20]. Therefore, careful follow-up is required, even for our patient with non-invasive UC, owing to positive preoperative urinary cytology, since IVR might occur during the patient's clinical course after nephroureterectomy.

In conclusion, this is an extremely rare case of UC with oncocytic features. This case was readily diagnosed as UC because of the presence of papillary architecture along with widespread p53 nuclear accumulation, suggesting malignancy, and the expression of relatively urothelial specific markers, such as GATA3 and uroplakin II. This tumor is difficult to diagnose by preoperative urine cytology due to the relatively low nuclear-to-cytoplasmic ratio as compared with UC in general. Oncocytic tumors are generally considered to not be actively dividing, which was shown in this case by the low Ki-67 index. In spite of the low proliferative activity, the possibility of IVR should be kept in mind since positive preoperative cytology of upper tract UC is a risk factor for IVR after nephroureterectomy.

Disclosure of conflict of interest

None.

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