

# Apparent diffusion coefficient as a prognostic biomarker of upper urinary tract cancer: a preliminary report

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## Abstract

**Objectives** To investigate the role of apparent diffusion coefficient (ADC) as a biomarker reflecting the aggressiveness of upper urinary tract urothelial cell carcinoma (UUT-UCC).

**Methods** Thirty-four consecutive patients treated with nephroureterectomy for non-metastatic disease were prospectively enrolled in this study. ADC was compared with clinicopathological variables including Ki-67 labelling index (LI) and cancer-specific survival (CSS).

**Results** The overall 3-year CSS rate was 82 % (median follow-up, 36 months). ADC was significantly lower in grade 3 disease than in grades 1–2 disease ( $P=0.011$ ) and significantly and inversely correlated with Ki-67 LI ( $\rho=-0.59$ ,  $P=0.0002$ ). Low ADCs ( $<1.10 \times 10^{-3}$  mm<sup>2</sup>/s) were significantly associated with shorter CSS ( $P=0.039$ ). Multivariate analysis of preoperative variables identified ADC and MRI-based clinical T stage as independent indicators of shorter CSS; the patients were stratified into high-risk (8 patients with low ADC and at least clinical T3) and low-risk (26 patients with high ADC or not more than clinical T2) groups with 3-year CSS rates of 43 % and 93 % ( $P=0.0003$ ).

**Conclusions** Our preliminary data suggest the potential role of ADC as a quantitative biomarker reflecting the aggressiveness of UUT-UCC. ADC might be useful for preoperative risk stratification of UUT-UCC patients.

## Key Points

- Diffusion-weighted MRI offers new information about the aggressiveness of urinary tract cancers.
- The apparent diffusion coefficient acts as a quantitative biomarker for upper urinary tract cancers.
- ADC inversely correlates with immunohistochemical and histological grading of UUT-UCC.
- ADC offers a prognosis for UUT-UCC patients treated with nephroureterectomy.

**Keywords** Diffusion magnetic resonance imaging · Prognosis · Kidney pelvis · Ureter · Neoplasm

## Abbreviations

ADC	apparent diffusion coefficient
CSS	cancer-specific survival
DW-MRI	diffusion-weighted MRI
UUT-UCC	upper urinary tract urothelial cell carcinoma

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## Introduction

Upper urinary tract urothelial cell carcinoma (UUT-UCC) is a relatively uncommon malignancy, accounting for approximately 5–7 % of all urothelial cancers [1]. However, UUT-UCC is a potentially lethal and aggressive disease; 40 % of patients have muscle-invasive disease at diagnosis, and nearly one half of patients die within 5 years even though they undergo nephroureterectomy, the definitive surgical therapy for the disease [2–4]. Therefore, it is critical to identify high-risk patients and to give them effective

adjunctive therapy to improve their oncological outcomes [5]. Meanwhile, recent studies suggest nephron-sparing, conservative surgery including segmental resection and ureteroscopic ablation as viable options for carefully selected, low-risk UUT-UCC patients [6]. Thus, accurate risk stratification, both after and before surgical therapy, is requisite for decision making in the management of UUT-UCC patients.

Pathological parameters obtained through examination of surgical UUT-UCC specimens, including pathological tumour stage, histological grade, lymph node status and lymphovascular invasion, are well-known prognostic factors [6–10]. To date, however, accurate preoperative risk stratification still remains challenging. Although the introduction of flexible ureteroscopy has enabled tumour biopsy and pathological evaluation of UUT-UCC before surgical therapy, ureteroscopic biopsy grading alone is unlikely to be sufficient to characterise the tumour. Recent studies have demonstrated the limited accuracy of ureteroscopic biopsy grading in UUT-UCC patients, especially in patients with low-grade tumours who were diagnosed on the basis of biopsy [11, 12]. Ureteroscopic biopsy is likely to underestimate histological grade with an upgrading rate at approximately 60 % for ureteroscopic biopsy grade 1–2 tumours [12]. Favaretto et al. have suggested the combined use of the information obtained from ureteroscopy and imaging studies to predict locally advanced, non-organ-confined disease [13].

Application of functional imaging techniques would be another approach to characterising the tumour. Diffusion-weighted magnetic resonance imaging (DW-MRI) is a functional imaging technique that reveals physiological information by quantifying the diffusion of water molecules in tissues [14, 15]. This imaging sequence is a non-invasive technique that does not involve the use of a contrast agent or radiation exposure. The extent of the water molecule diffusion can be quantified as the apparent diffusion coefficient (ADC). Malignant tissues with a lower ADC tend to demonstrate higher signal intensity than the surrounding tissue on a DW-MRI image. This imaging sequence is increasingly used for both detection and qualitative assessment of tumour characteristics, including prediction of the therapeutic response [16]. Recently, the prognostic role of the ADC has been emerging among patients with lymphoma, glioblastoma, head and neck cancer, and non-small cell lung cancer [17–21].

Since the publication of our first report demonstrating the feasibility of DW-MRI for detecting UUT-UCC [22], several studies have confirmed the diagnostic performance of DW-MRI in this disease [23–26]. DW-MRI exhibited excellent diagnostic accuracy for UUT-UCC in combination with T1- and T2-weighted (T1W and T2W) MRI; the sensitivity ranges from 92 to 98 % and the specificity ranges from 81 to 96 %, despite unconvincing diagnostic performance for flat carcinoma in situ (CIS) [23, 26]. ADCs of bladder urothelial

cancer (BUC) and UUT-UCC tissues were significantly lower than those of the surrounding normal tissues, accounting for the high signal of the tumour [26, 27]. In BUC, several investigators including us demonstrated a significant and inverse correlation between ADC and histological grade [28–31]. However, this correlation is controversial in the series of UUT-UCC; some reported the presence of significant inverse correlation [23, 26], whereas others did not [24, 32].

In the current prospective study, we investigated the potential associations of ADC with survival after nephroureterectomy and clinicopathological parameters including proliferative potential, which is assessed by evaluating Ki-67 labelling index (LI), in UUT-UCC patients. Our preliminary results suggest that the ADC is a quantitative biomarker reflecting the aggressiveness of UUT-UCC and that the ADC may be useful for preoperative risk stratification of UUT-UCC patients.

## Materials and methods

### Patients

At a single academic centre between January 2007 and September 2010, multi-sequence MRI including DW-MRI was prospectively performed before any intervention in 52 consecutive patients who were clinically diagnosed with UUT-UCC on the basis of urine cytology, ultrasound and/or CT findings. Fourteen patients were excluded from the study: 5 with metastatic disease, 4 who did not undergo nephroureterectomy, 4 who received neoadjuvant therapy before nephroureterectomy and 1 who received adjuvant therapy. Thus, 38 patients treated with nephroureterectomy alone for non-metastatic UUT-UCC were included in the analysis. An institutional review board approved this study, and written informed consent was obtained from each participant.

### MRI

Magnetic resonance imaging was performed using 1.5-T MR system (Intera Achieva; Philips, Best, Netherlands) with a four-channel sensitivity-encoding body coil without breath-holding sequences. The maximal gradient strength was 33 mT/m, and the slew rate was 160 T/m/s. The imaging parameters for DW-MRI with a single shot spin-echo planar imaging sequence were set as follows: repetition time, 5,000 ms; echo time, 65 ms; matrix, 128 × 126/256 × 256 zip; field of view, 35 cm; slice thickness, 5 mm; interslice gap, 0.5 mm; slice number, 24; number of excitations, 8; bandwidth, 1,880 Hz per pixel; 3 different diffusion gradient  $b$  values ( $b=0, 400$  or  $800$  s/mm<sup>2</sup>); number of motion probing direction gradients, 3; number of averages, 9; fat suppression, spectral attenuated inversion recovery;

and total acquisition time, approximately 3 min 40 s. The MRI protocols in this study consisted of routine T1W- and T2W-MRI and DW-MRI (Figs. 1a and 2a).

### Image analysis

The ADC was calculated using the following formula:  $ADC = -\ln(S/S_0)/(b-b_0)$ , where  $S_0$  and  $S$  are the signal intensities, obtained with two different diffusion gradient values ( $b_0$  and  $b=400$  and  $800$  s/mm<sup>2</sup>, respectively). The ADC maps were reconstructed at a workstation (View Forum R4.1, Philips Healthcare), and the ADC of the lesion corresponding to the tumour was calculated to quantitatively analyse the degree of diffusion. A region of interest (ROI) was manually drawn to maximally cover the index tumor on the transverse ADC map at the slice that showed maximal tumour diameter (Figs. 1b and 2b). The ADC of each pixel in the ROI was quantified. The distribution of each pixel's ADC was charted using a histogram (Figs. 1c and 2c), and the mean and standard deviation (SD) of the ADCs within the ROI were calculated. In this study, the ADC of a tumour represented the mean ADC within the ROI. In a patient with multiple tumours, the ADCs were calculated for the index tumour, which was defined as the tumour with the highest clinical T stage.

### T staging and histological grading

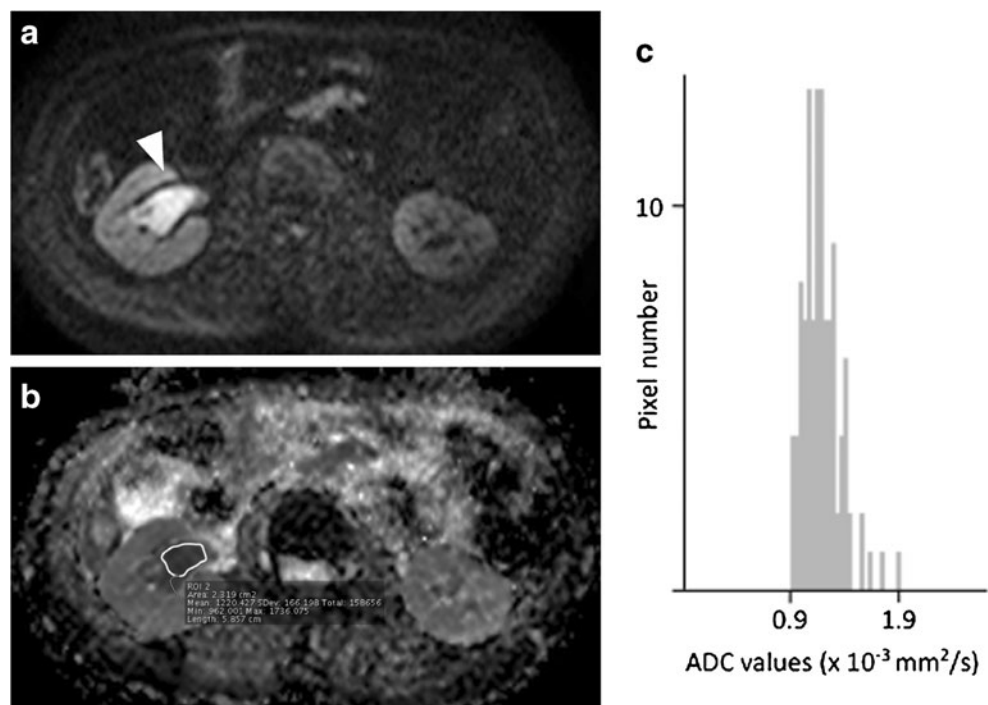
T stage was determined according to the 2002 TNM system [33]. For the clinical T stage, UUT-UCC was classified into organ-confined (not more than clinical T2) or locally

advanced (at least clinical T3) disease on the basis of T1W- and T2W-MRI findings according to the criteria reported previously [13]. Briefly, tumours radiologically confined within the muscle layer of the upper urinary tract were classified as not more than clinical T2 disease, whereas those extending into the renal sinus fat, renal parenchyma, perirenal fat or periureteral tissues were classified as at least clinical T3 disease. Tumour histological grading was evaluated according to the 1973 World Health Organisation classification [34].

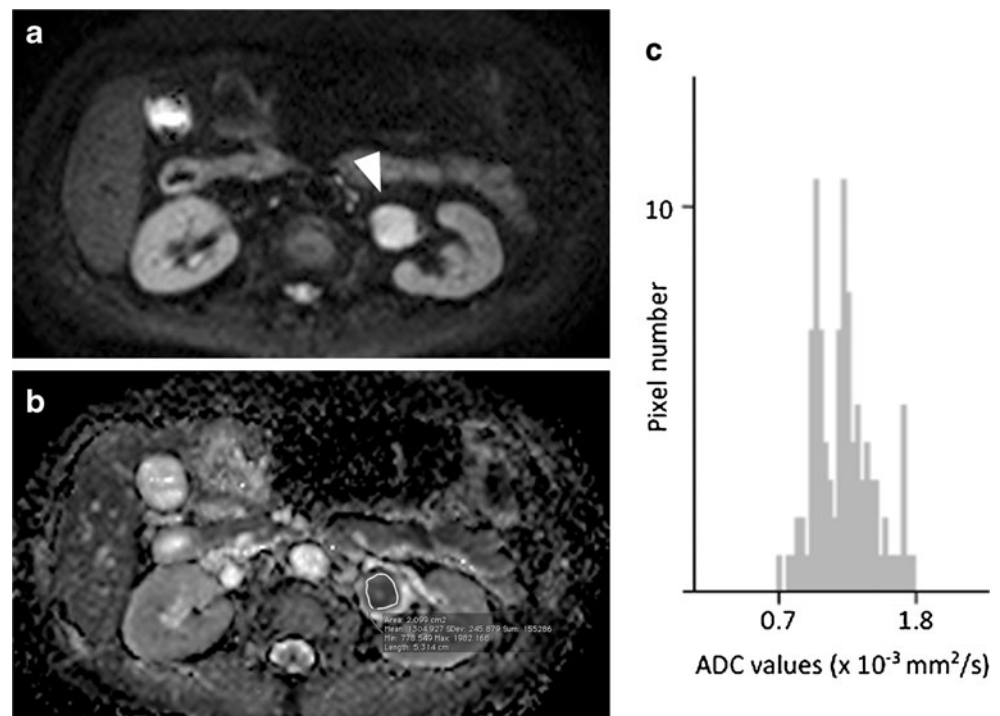
### Immunohistochemistry

Paraffin-embedded tissue sections obtained from nephroureterectomy were subjected to immunostaining for Ki-67 in each patient. Immunostaining was performed as previously described [16]. Briefly, after inhibition of endogenous peroxidase, the slides were autoclaved and incubated with Ki-67 antibody (clone MIB-1, Dako, Denmark). After incubation with a horseradish peroxidase-labelled secondary antibody (HISTOFINE Simple Stain MAX PO, Nichirei, Japan), colour was developed using 3,3-diaminobenzidine (Nichirei). The sections were counterstained with haematoxylin. Negative controls were included by replacing the primary antibody with phosphate-buffered saline. The Ki-67 LI was calculated as the percentage of tumour cells that were positive for nuclear Ki-67 expression when more than 1,000 tumour cells ( $\times 400$  magnification) were counted by one of the authors (S.K.), who was blinded to the clinicopathological data.

**Fig. 1** Diffusion-weighted (DW)-MRI, apparent diffusion coefficient (ADC) map and histogram of the ADC within a region of interest (ROI) of a 76-year-old man with renal pelvic cancer. **a** Tumour shows homogeneously high signal intensity (white arrowhead) using DW-MRI with a  $b$  value of  $800$  s/mm<sup>2</sup>. **b** An ROI was positioned not extending over the tumour on the ADC map. **c** Histogram for distribution of the ADC of each pixel within the ROI shows a single prominent peak



**Fig. 2** Diffusion-weighted (DW)-MRI, apparent diffusion coefficient (ADC) map and histogram of the ADC within a region of interest (ROI) of a 64-year-old woman with renal pelvic cancer. **a** Tumour shows homogeneously high signal intensity (*white arrowhead*) using DW-MRI with a *b* value of 800 s/mm<sup>2</sup>. **b** An ROI is positioned not extending over the tumour on the ADC map. **c** Histogram for distribution of the ADC of each pixel within the ROI shows two peaks



## Follow-up

Patients were followed essentially every 3 months over the first 2 years, every 6 months over the next 3 years, and annually thereafter. Basically, evaluations included complete blood counts, blood chemistry, urinalysis, urine cytology, cystoscopy and CT of the chest, abdomen and pelvis. The median follow-up period was 33 months (range, 7–65 months).

## Statistical analysis

Differences between groups were assessed using the Wilcoxon test for continuous variables. Concordance between clinical and pathological T staging was calculated using kappa statistics. Correlations between the mean ADC and the Ki-67 LI were analysed using the Spearman's rank correlation test. Martingale residual analysis was performed to evaluate the functional form of mean ADC to be used in a Cox proportional hazards model.

Cancer-specific survival (CSS) was estimated and analysed using the Kaplan–Meier method and log-rank test. The prognostic role of ADC was investigated by examining associations of ADC with CSS. Factors related to CSS were analysed using the Cox proportional hazards model, wherein age was divided into two groups at the median of the patients. Tumour location was categorised into renal pelvis or ureter. A cut-off ADC was determined to separate CSS curves of the low and high ADC groups most significantly.

Ki-67 LI was classified into high and low groups according to a previously reported cut-off value of 30 % [35]. The CSS duration was defined as the interval from the date of UUT-UCC diagnosis to the date of last follow-up or cancer-specific death. These statistical analyses were performed using JMP 7.0 statistical software (SAS Institute, Cary, NC, USA) and the R statistical package 2.15.0 (The R Foundation for Statistical Computing, 2012, Vienna, Austria), and *P* values less than 0.05 were considered statistically significant.

## Results

### Patient and tumour characteristics

Of the 38 patients, 26 (68 %) and 12 patients (32 %) were diagnosed as having not more than clinical T2 and at least clinical T3 disease, respectively. In 4 patients with not more than clinical T2 disease, cancerous lesions did not form a mass and were pathologically diagnosed as primary CIS on the basis of nephroureterectomy specimens. These 4 patients were excluded from the analysis because it was not possible to position an ROI to measure the ADC. The patient and tumour characteristics of the 34 eligible patients are described in Table 1. In all of the patients, histopathological diagnosis was urothelial carcinoma. Of the 17 patients with pathological T3–4 disease, 6 patients were understaged as having not more than clinical T2 disease, whereas 1 of the 17 patients with pathological Ta-2 was overstaged as having

**Table 1** Patient and tumour characteristics of 34 eligible upper urinary tract urothelial cell carcinoma (UUT-UCC) patients

Variables	n (%)
Age, years <sup>a</sup>	71 (55–85)
Gender	
Female	13 (38)
Male	21 (62)
Clinical T stage	
≤T2	22 (65)
≥T3	12 (35)
Pathological T stage	
Ta	9 (26)
T1	4 (12)
T2	4 (12)
T3	17 (50)
Histological grade	
Grade 1–2	18 (53)
Grade 3	16 (47)
Pathological N stage	
pN0	14 (41)
pNx	20 (59)
Tumour location	
Renal pelvis	23 (68)
Ureter	11 (32)
ADC ( $\times 10^{-3}$ mm <sup>2</sup> /s) <sup>a</sup>	0.98 (0.67–1.59)
SD of ADC ( $\times 10^{-3}$ mm <sup>2</sup> /s) <sup>a</sup>	0.13 (0.04–0.41)
No. of peaks in ADC histogram	
One	28 (82)
Two or more	6 (18)
Ki-67 LI, % <sup>a</sup>	28.7 (4.1–44.2)

ADC apparent diffusion coefficient, SD standard deviation, Ki-67 LI Ki-67 labelling index

<sup>a</sup>Median (range)

at least clinical T3 disease. The concordance between clinical and pathological T staging was moderate ( $\kappa=0.69$ ). The surgical margin was negative in all of the patients. Regional lymph node dissection was performed in 14 patients and no metastasis was found histologically.

#### Characteristics of the ADC in UUT-UCC

In most cases, UUT-UCC appeared as a homogeneous mass with high signal intensity in DW-MRI. Homogeneity of ADC values was assessed on histograms. We objectively evaluated homogeneity of ADC in UUT-UCC. Histogram analyses of intratumoral ADC showed a single prominent peak in 28 patients (82 %, Fig. 1c) and two or more peaks in 6 patients (18 %, Fig. 2c). The mean UUT-UCC ADC ranged from  $0.67 \times 10^{-3}$  to  $1.59 \times 10^{-3}$  mm<sup>2</sup>/s (median,

$0.98 \times 10^{-3}$  mm<sup>2</sup>/s) and the ADC SD for each UUT-UCC ranged from  $0.04 \times 10^{-3}$  to  $0.41 \times 10^{-3}$  mm<sup>2</sup>/s (median,  $0.13 \times 10^{-3}$  mm<sup>2</sup>/s).

Of age, gender, tumour location, clinical and pathological T stage, and histological grade, the ADC was significantly associated with the histological grade (Table 2); the ADC was significantly lower in grade 3 disease than grades 1–2 disease (median,  $0.90 \times 10^{-3}$  mm<sup>2</sup>/s vs.  $1.20 \times 10^{-3}$  mm<sup>2</sup>/s;  $P=0.011$ ).

#### Association between ADC and tumour proliferative activity

We evaluated the association between the ADC and UUT-UCC proliferative activity. The Ki-67 LI of UUT-UCC ranged from 4.1 to 44.2 % (median, 28.7 %) and 14 (41 %) and 20 (59 %) patients were classified into high and low Ki-67 LI groups, respectively. The ADC was significantly lower in the high Ki-67 LI group (median,  $0.86 \times 10^{-3}$  mm<sup>2</sup>/s) than in the low Ki-67 LI group (median,  $1.29 \times 10^{-3}$  mm<sup>2</sup>/s;  $P=0.0009$ ; Table 2). As shown in Fig. 3, the ADC was significantly and inversely correlated with the Ki-67 LI ( $\rho=-0.59$ ,  $P=0.0002$ ).

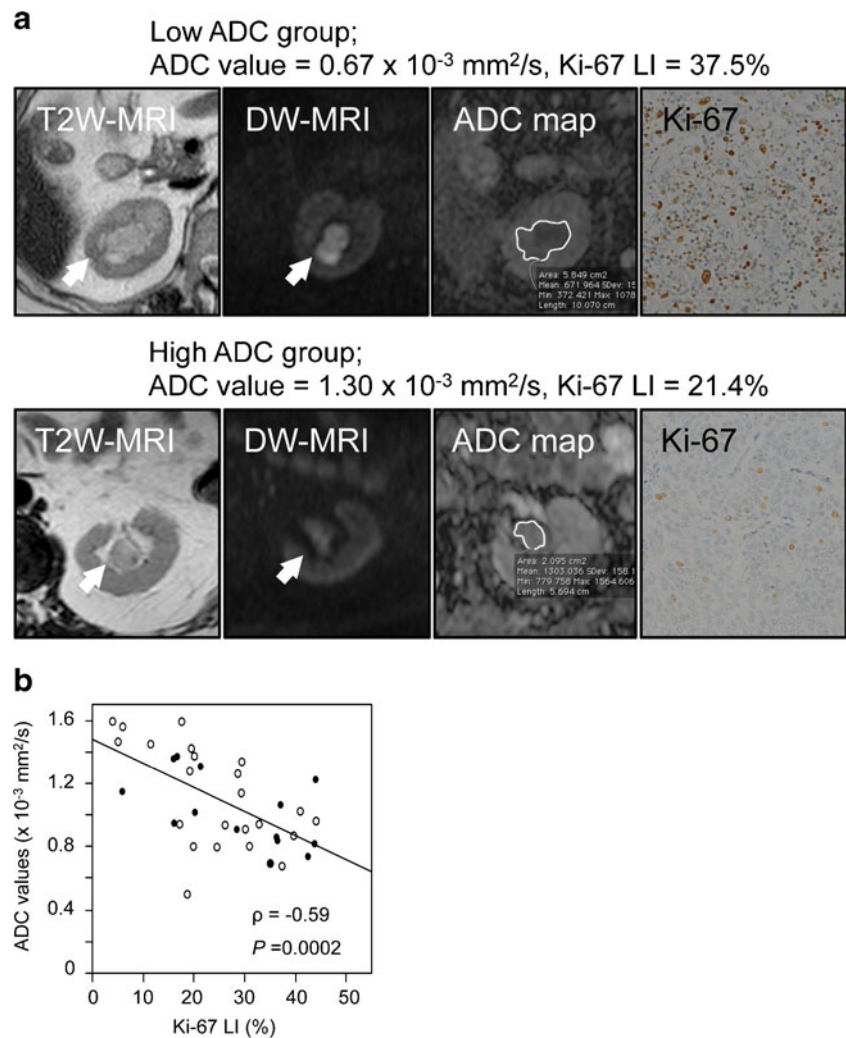
**Table 2** ADC according to clinicopathological characteristics in 34 eligible UUT-UCC patients

Variables	ADC ( $\times 10^{-3}$ mm <sup>2</sup> /s) <sup>a</sup>	P
Age, years		0.82
<71	1.01 (0.67–1.59)	
≥71	0.96 (0.69–1.55)	
Gender		0.58
Female	1.02 (0.67–1.59)	
Male	0.93 (0.69–1.59)	
Tumour location		0.70
Renal pelvis	1.02 (0.67–1.59)	
Ureter	0.96 (0.69–1.59)	
Clinical T stage		0.39
≤T2	0.98 (0.67–1.59)	
≥T3	0.98 (0.69–1.37)	
Pathological T stage		0.15
Ta-2	1.13 (0.69–1.59)	
T3	0.94 (0.67–1.41)	
Histological grade		0.011
Grade 1–2	1.20 (0.73–1.59)	
Grade 3	0.90 (0.67–1.42)	
Ki-67 LI, %		0.0009
≥30	0.86 (0.67–1.22)	
<30	1.29 (0.79–1.59)	

ADC apparent diffusion coefficient, Ki-67 LI Ki-67 labelling index

<sup>a</sup>Median (range)

**Fig. 3** Correlation between the ADC and Ki-67 labelling index (LI). **a** T2W-MRI, DW-MRI, ADC map and Ki-67 immunostaining of upper urinary tract urothelial cell carcinoma (UUT-UCC) in the low (*upper panels*) and high (*lower panels*) ADC groups. The cut-off ADC was set at  $1.1 \times 10^{-3} \text{ mm}^2/\text{s}$ . Both tumours show similar signal intensity on T2W-MRI. The DW-MRI signal, the ADC of these tumours and Ki-67 LI are different in these tumours. Original magnification for Ki-67 immunostaining,  $\times 200$ . **b** ADC inversely correlated with Ki-67 LI. *Open and closed circles* indicate patients with Ta-2 and T3-4 tumours, respectively



### Cancer-specific survival

Six patients (15 %) died of UUT-UCC during follow-up. The median time to cancer-specific death was 21 months (range, 16–33 months). The median follow-up period for survivors was 36 months (range, 7–65 months). The overall 3-year CSS rate was 82 %.

Cancer-specific survival was significantly shorter in patients in the high Ki-67 LI group than in those in the low Ki-67 LI group (3-year CSS rate, 61 % vs. 100 %, respectively;  $P=0.0080$ ).

### Role of ADC as a preoperative prognostic biomarker

The ADC was fitted to a Cox regression model without the covariate of interest, and the functional shape was checked using a smoothed Martingale residual plot. The Martingale residual analysis revealed that the prognostic effect of the ADC on CSS fitted a linear model. There was no abrupt increase in the hazard ratio for CSS. At the cut-off ADC determined at  $1.10 \times 10^{-3} \text{ mm}^2/\text{s}$ , 20 (59 %) and 14 patients

(41 %) were classified into the low and high ADC groups, respectively. Patients in the low ADC group showed significantly higher Ki-67 LI than those in the high ADC group ( $P=0.0008$ ). In the low ADC group there was more grade 3 disease and patients were older than in the high ADC group, although the difference was not statistically significant ( $P=0.071$  and  $0.082$ , respectively; Table 3). As shown in Fig. 4a, CSS curves were stratified according to the ADC; the 3-year CSS rate was 69 % for the low ADC group and 100 % for the high ADC group ( $P=0.039$ ).

On univariate analysis, the ADC and clinical T stage were identified as significant prognostic factors among preoperative variables that included age, sex, tumour location, clinical T stage, and ADC ( $P=0.013$  and  $0.024$ , respectively; Table 4). Multivariate analysis revealed that a low ADC ( $<1.10 \times 10^{-3} \text{ mm}^2/\text{s}$ ) and at least clinical T3 disease were significant and independent risk factors for cancer-specific death with respective hazard ratios (HR) of  $8.5 \times 10^6$  ( $P=0.017$ ) and  $8.1$  ( $P=0.031$ ), respectively (Table 4). When these two preoperative risk factors were combined, the 34 patients were stratified into high-risk (low ADC and clinical

**Table 3** Clinicopathological characteristics of the high and low ADC groups

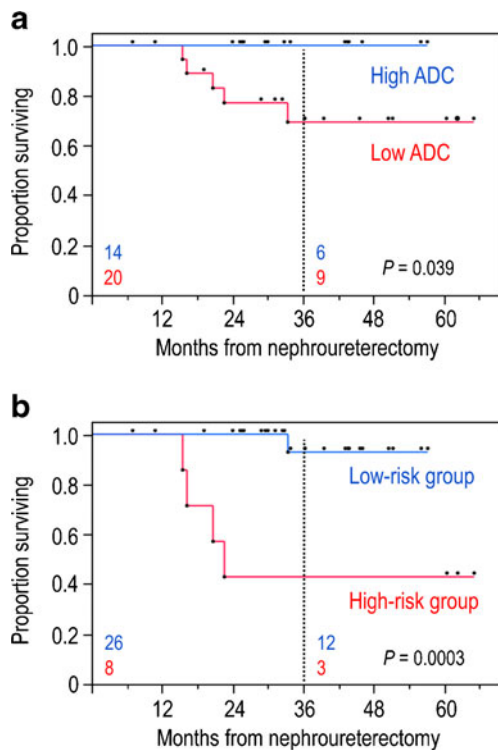
Variables	Low ADC group <i>n</i> (%)	High ADC group <i>n</i> (%)	<i>P</i>
Age, years <sup>a</sup>	74 (59–85)	68 (55–76)	0.082
Gender			0.46
Female	7 (20.6)	6 (17.7)	
Male	13 (38.2)	8 (23.5)	
Tumour location			0.77
Renal pelvis	14 (41.2)	9 (26.5)	
Ureter	6 (17.7)	5 (14.7)	
Clinical T stage			0.38
≤T2	12 (35.3)	10 (29.4)	
≥T3	8 (23.5)	4 (11.8)	
Pathological T stage			0.15
Ta-2	8 (23.5)	9 (26.5)	
T3	12 (35.3)	5 (14.7)	
Histological grade			0.071
Grade 1/2	8 (23.5)	10 (29.4)	
Grade 3	12 (35.3)	4 (11.8)	
Ki-67 LI, % <sup>a</sup>	34.1 (16.2–44.2)	18.7 (4.1–44.1)	0.0021

ADC apparent diffusion coefficient, Ki-67 LI Ki-67 labelling index

<sup>a</sup>Median (range)

T3–4, *n*=8) and low-risk (high ADC or clinical Ta-2, *n*=26) groups for CSS; patients in the high-risk group had a 3-year

CSS of 43 %, whereas those in the low-risk group had a 3-year CSS of 93 % (*P*=0.0003, Fig. 4b).



**Fig. 4** Kaplan–Meier analysis in UUT-UCC patients treated with nephroureterectomy. **a** Cancer-specific survival (CSS) curves stratified by ADC at the cut-off value of  $1.1 \times 10^{-3} \text{ mm}^2/\text{s}$ . **b** CSS curves stratified by the ADC combined with clinical T stage. The number of patients at risk is shown on the horizontal axis at 0 and 36 months

## Discussion

The current study demonstrated significant and inverse correlations of ADC with Ki-67 LI as well as histological grade in UUT-UCC. Although the results were preliminary, we demonstrated that the patients with UUT-UCC of lower ADC value are at higher risk of cancer-specific death after nephroureterectomy. These results suggest that ADC is a quantitative biomarker reflecting the aggressiveness of

**Table 4** Univariate and multivariate analyses of preoperative variables predicting cancer-specific survival

Variables	Univariate <i>P</i>	Multivariate HR	<i>P</i>
Age			
≥71 vs. <71	0.12	–	
Sex			
Male vs. female	0.35	–	
Tumour location			
Renal pelvis vs. ureter	0.59	–	
Clinical T stage			
≥T3 vs. ≤T2	0.024	8.1	0.031
ADC			
Low vs. high	0.013	$8.5 \times 10^6$	0.017

HR hazard ratio, ADC apparent diffusion coefficient

UUT-UCC. In this study, UUT-UCC patients were stratified into high-risk and low-risk groups according to a combination of ADC and MRI-based clinical T stage. Although confirmatory studies are mandatory, ADC might be useful for preoperative risk stratification of UUT-UCC.

Malignant tumours have higher cellularity and tissue disorganisation compared with normal tissue, which is believed to be the underlying mechanism for the low ADC observed in malignant tumours compared with normal tissue. However, some recent reports including ours have shown that the functional and biological properties of malignant tumours play an important role in the restricted diffusion of water molecules [15, 16, 26, 29]. We have shown that ADC is inversely correlated with histological grade in BUC and UUT-UCC [26, 29], T stage in BUC [29] and Ki-67 LI in muscle-invasive BUC [16]. The present study confirmed the inverse correlation between the ADC and both the Ki-67 LI and histological grade in UUT-UCC, strengthening the proposal that the ADC is a potential biomarker that reflects the tumour proliferative activity and histological grade. Moreover, a lower ADC was associated with a shorter CSS in UUT-UCC patients treated with nephroureterectomy. Overall, ADC is considered to be a quantitative biomarker that reflects the aggressiveness of urothelial carcinomas.

Although the results were preliminary, the present study demonstrated that ADC and clinical T stage were independent prognostic indicators of UUT-UCC, and suggested that a subset of patients at high risk may be preoperatively identified by the combined use of these two variables. Because these variables are assessed at the same time in one MRI examination, multi-sequence MRI might be a promising tool for preoperative risk stratification of UUT-UCC patients. Incorporation of histopathological information obtained from a ureteroscopic biopsy into the MRI-based variables might further improve the preoperative risk stratification of UUT-UCC patients.

Although multidetector computed tomography using radiocontrast agents is widely used to evaluate UUT-UCC, it is ideal for minimising the use of potentially nephrotoxic, ionising contrast agents for UUT-UCC patients who often have renal insufficiency because of obstructive uropathy. DW-MRI does not require contrast agents and thus it can be used in patients with renal insufficiency. In addition, DW-MRI requires only a few minutes to complete during a routine MRI examination when performed using the most current clinical MRI systems. DW-MRI can be also applied to various phases of urothelial carcinoma patient management including diagnosis, and predicting and monitoring treatment outcomes [16, 36]. Its methodological simplicity, non-invasiveness and objectivity strengthen the routine clinical use of DW-MRI with the quantitative ADC assessment for urothelial cancer patients.

The present study has some limitations. First, this study has a small sample size with relatively short follow-up periods. To validate the current results, further prospective studies using larger patient cohorts with longer follow-up periods are mandatory. Second, the cut-off ADC proposed in this study is not universally applicable because the ADC depends on the MRI systems and imaging conditions used, which is an intrinsic matter in the ADC measurement. Thus, standardisation of the imaging systems and conditions is required. To begin standardising the ADC, we propose to use the mean ADC calculated on one representative slice as an index parameter of the diffusion environment in the whole UUT-UCC. The single prominent peak histogram pattern in most cases and the relatively small SD for each lesion indicated that the degrees of diffusion were homogeneous in most UUT-UCC tissues. The intratumoral homogeneity of ADC may justify using the mean ADC within an ROI as an index parameter for the diffusion of UUT-UCC tissues. Lack of confirmation of reliability and reproducibility of measuring ADC is another limitation of this study. Intra- and interobserver variability in ADC measurement needs to be evaluated in future studies.

In conclusion, the present preliminary data suggest that the ADC serves as a quantitative biomarker reflecting the aggressiveness of upper urinary tract urothelial cell carcinoma. Although confirmatory studies are mandatory, ADC might be useful for preoperative risk stratification.

**Conflict of interest** None of the authors has any potential conflicts of interest.

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