Original Article
Preoperative AST/ALT ratio predicts long-term survival after radical nephroureterectomy in patients with upper tract urothelial carcinoma

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Abstract: Purpose: We evaluated the association between preoperative AST/ALT ratio and postoperative survival in patients with upper tract urothelial carcinoma following radical nephroureterectomy. Materials and methods: We included 259 patients with upper tract urothelial carcinoma who underwent radical nephroureterectomy from March 2005 to August 2015. The potential effect of the preoperative AST/ALT ratio on overall survival, progression-free survival, cancer-specific survival, and bladder-recurrence-free survival was analyzed using the Kaplan-Meier method, univariate and multivariate Cox proportional regression models, and followed by competing-risk analysis. Results: The higher preoperative AST/ALT ratio (1.3 or greater) was significantly associated with several unfavorable parameters, including elderly age ($P < 0.05$), and pathologic T stage ($P < 0.05$). Multivariate analysis identified higher preoperative AST/ALT ratio as an independent risk factor for overall survival (HR = 1.972, $P = 0.005$), progression-free survival (HR = 2.286, $P = 0.002$) and cancer-specific survival (HR = 2.146, $P = 0.006$), while preoperative AST/ALT ratio was not statistically correlated with bladder-recurrence-free survival on univariate analysis (P = 0.668). The subdistribution HR (SHR) of higher AST/ALT ratio for progression-free survival and cancer-specific survival (competing event: death from another cause) was 2.118 ($P = 0.004$) and 2.069 ($P = 0.010$), respectively. Conclusions: Higher preoperative AST/ALT ratio was significantly associated with poor overall survival, progression-free survival, and cancer-specific survival, rather than bladder-recurrence-free survival after radical nephroureterectomy for upper tract urothelial carcinoma.

Keywords: Upper tract urothelial carcinoma, survival rate, biological markers, radical nephroureterectomy

Introduction

Upper tract urothelial carcinoma (UTUC) is relatively rare compared to urothelial carcinoma of the bladder, comprising only 5-10% of all urothelial carcinomas. Radical nephroureterectomy (RNU) with bladder cuff removal remains the gold standard treatment for non-metastatic UTUC patients with a normal contralateral kidney [1]. Conversely, conservative approaches, such as endoscopic ablation and segmental resection, were now considered for low-risk UTUC patients [2]. The current concerns were the determination of patients who may benefit from these treatments. While the lack of high-quality data together with heterogeneity of

UTUC biology, the decision-making of treatment options seems extremely challenging. In this regard, specific validation of risk predictors of oncologic outcomes for UTUC is essential to guide physicians and patients in the management of this disease.

Aminotransaminases, including aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT), are well-known liver enzymes to assess the liver function [3]. Now they have been demonstrated to be associated with several malignancies [4-7]. Moreover, the De Ritis ratio (AST/ALT), also a useful biomarker for other hepatic diseases, has been identified as an independent risk predictor for nonmetastat-

ic renal cell carcinoma [8, 9]. Previously, Nishikawa et al. reported that De Ritis ratio was a significant predictor of extravesical recurrence-free survival in 109 consecutive Japanese patients with UTUC following RNU [10]. In their study, De Ritis ratio was not significant correlated with overall survival (OS) (P = 0.37) and disease-specific survival (DSS) (P = 0.16) on univariate analysis, respectively due to their limited sample size, although there was a trend toward worse long-term prognosis in the patients with high De Ritis ratio. In addition, they did not evaluate the cancer-specific survival (CSS) and bladder tumor recurrence. Therefore, the objective of the current study was to evaluate the true prognostic value of AST/ALT in UTUC patients after RNU.

Materials and methods

Patients

From March 2015 to August 2015, 315 consecutive patients diagnosed as UTUC and underwent RNU at the First Affiliated Hospital of Wenzhou Medical University, China were included into this study. The exclusion criteria included patients who: (i) underwent palliative surgery instead of RNU; and (ii) received kidney transplantation before RNU; and (iii) had a presence of cancer metastasis that could not be cured by radical surgery; (iv) received preoperative chemotherapy; (V) had the presence of hepatitis or severe fatty liver. Overall, 259 patients were included into this study, and all of them agreed to take part in the study and signed the informed consent.

Data collection

Referring to our prospectively maintained computer database, the following data were analyzed retrospectively: (i) clinicopathological features, including age, sex, ASA grade, AST, ALT, De Ritis ratio (AST/ALT), TNM stage of tumor, tumor grade, lymphovascular invasion, tumor history (pure urothelial vs. mixed type), and Sarcomatoid differentiation; and (ii) treatment details and postoperative outcomes, including laparoscopic surgery, mortality, overall survival (OS), progression-free survival (PFS), cancer-specific survival (CSS), and bladder tumor recurrence. In this study, the upper reference level of AST and ALT were 35 and 45 U/L, respectively. All the laboratory data were col-

lected within a week before surgery. Tumors were staged according to 2002 TNM classification system. Tumor grading was assessed according to the World Health Organization (WHO) 2004 grading system. Study endpoint was OS, defined as the time from the date of surgery to death from any cause (event) or alive at last follow-up (censored). CSS was defined as death from UTUC. PFS was defined as the time from the date of surgery until local recurrence, evidence of distant metastasis, or death from UTUC or still alive at last follow-up (censored). This study was approved by the ethics committee of The First Affiliated Hospital of Wenzhou Medical University, China.

Follow-up

The routine follow-up consisted of blood and urine tests, cystoscopy, and chest and abdominal CT or magnetic resonance imaging four times per year for the first year after RNU, twice from the second year to fifth year, and annually thereafter. The death cause was determined by the treating clinician or reviewing death certificates.

Statistical analysis

Statistical analyses were performed by the SPSS software package version 22.0 (IBM, Armonk, NY). All tests were two-sided and considered statistically significant at $P < 0.05$. To determine the AST/ALT cutoff at which the difference between survival and all-cause death was most significant, we used optimum stratification and log-rank Chi-square statistics to find the most significant p value. This method has been previously applied to solve the threshold value of the continuous covariable at which sarcopenic and non-sarcopenic patients are best separated with respect to time to OS [11]. Normally distributed continuous variables are presented as mean ± standard deviation, and non-normally distributed continuous variables are presented as median and interquartile ranges. Categorical variables are presented as counts and percentages. Clinical variables were compared using Student's t test (normally distributed variables), Pearson's chi-square test, or Fisher's exact test (categorical variables), and the Mann-Whitney U test (non-normally distributed continuous variables and ranked data) as appropriate. Effect of preoperative AST/ALT on several parameters was com-

Table 1. Patient characteristics

Factors	Total (n = 259)	Low ASL/ALT < 1.3 (n = 115)	High ASL/ALT ≥ 1.3 (n = 144)	P value
Age, year, mean (SD)	67.53 (10.43)	64.07 (10.56)	70.28 (9.50)	0.005*
Sex				0.266
Female, n (%)	72 (28)	26 (23)	46 (32)	
Male, n (%)	187 (72)	89 (77)	98 (68)	
ASA grade				0.141
≤ II, n (%)	222 (86)	103 (90)	119 (83)	
III, n (%)	37 (14)	12 (10)	25 (17)	
Laparoscopic surgery				0.007*
No, n (%)	80 (31)	46 (40)	34 (24)	
Yes, n (%)	179 (69)	69 (60)	110 (76)	
Pathological T stage				0.008*
≤ pT2, n (%)	171 (66)	85 (74)	86 (60)	
≥ pT3, n (%)	88 (34)	30 (26)	58 (40)	
Pathological N stage				0.862
N0 or Nx, n (%)	235 (91)	105 (91)	130 (90)	
N1-3, n (%)	24 (9)	10 (9)	14 (10)	
Grade				0.507
G1, n (%)	59 (23)	29 (25)	30 (19)	
G2/3, n (%)	200 (77)	86 (75)	114 (79)	
Histology				0.253
Pure urothelial, n (%)	236 (91)	106 (92)	130 (90)	
Mixed type[a], n (%)	23 (9)	9 (8)	14 (10)	
Sarcomatoid differentiation				0.324
No, n (%)	249 (96)	112 (97)	137 (95)	
Yes, n (%)	10 (4)	3 (3)	7 (5)	
Lymphovascular invasion				0.119
No, n (%)	217 (84)	101 (88)	116 (81)	
Yes, n (%)	42 (16)	14 (12)	28 (19)	

*Indicates statistically significant. [a]Mixed type carcinomas include sarcomatoid differentiated carcinoma and lymphoepithelioma-like carcinoma.

pared using Student's t test and chi-square test. OS, PFS and CSS rates were estimated by Kaplan-Meier method, and the log-rank test was used to assess the differences. Variables with a $P < 0.05$ in the univariate analysis, as well as variables with known prognostic value were included into the subsequent multivariate analysis (Cox proportional hazards regression). We used the competing-risk analysis for PFS and CSS because the risk of UTUC progression and death from UTUC may be biased estimated if patients' death from another cause were not taken into consideration [12, 13]. The competing event for PFS and CSS was death from a cause other than UTUC in the competing-risk analysis. Univariate analysis using competing risk regression was performed to demonstrate the risk factors, followed by stepwise multivariate analysis for determining significant factors based on a significant level of 0.2. After verifying the interaction of each significant variable, the final model comprised only those factors with a significance level of 0.05.

Results

Grouping

The mean age of all patients was 67.53 (10.43), the median follow-up duration was 33.3 (15.5-64.2) months), 82 (32%) patients experienced local recurrence or *distant metastasis,* 93
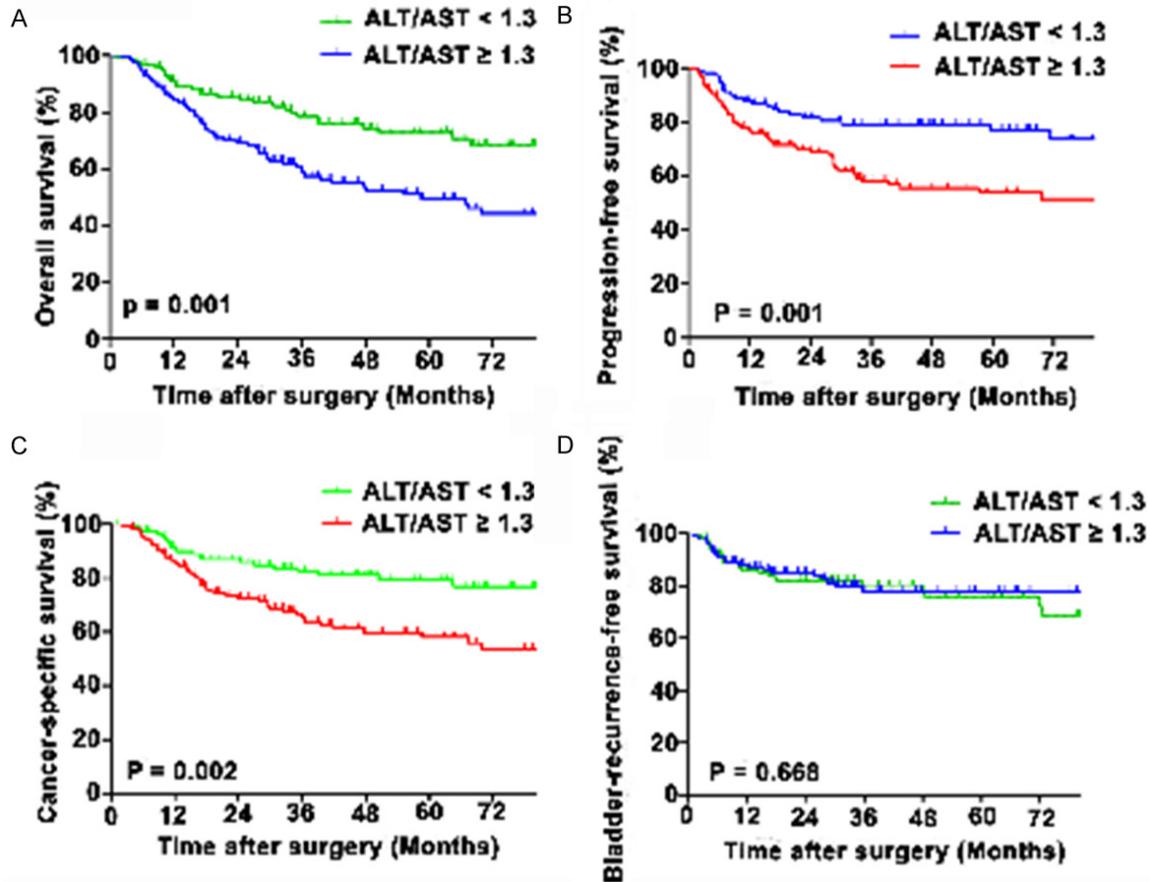
Figure 1. Kaplan-Meier survival curves for OS, PFS, CSS, and BRFS groups categorized by preoperative AST/ALT ratio.

(36%) died, and 73 (28%) suffered from cancer-specific death. The clinicopathologic characteristics of UTUC patients according to the AST/ALT ratio are summarized in **Table 1**.

Preoperative AST/ALT ratio cut-off values

With respect to overall mortality, 1.3 was identified as optimal threshold value for the AST/ALT ratio associated with OS using optimum stratification. Median value (range) of AST/ALT ratio was 1.4 (1.0-1.8). The high AST/ALT ratio (≥ 1.3) was correlated with elderly age, laparoscopic surgery, high pathologic T stage (P < 0.05). Sex, ASA grade, pathologic N stage, grade, tumor histology, and sarcomatoid differentiation were not associated with AST/ALT ratio.

Association of AST/ALT ratio with survival

The 1-, 3-, 5-year survival rate were 87%, 69% and 60% for OS, 82%, 68% and 65% for PFS, and 88%, 73%, and 68% for CSS, respectively.

The Kaplan-Meier curves of patients' OS, PFS, and CSS revealed that increased AST/ALT ratio was correlated with poor prognosis (**Figure 1**). Univariate analysis identified the AST/ALT ratio as poor predictor for OS (HR = 2.097, 95% CI, 1.351-3.254, *P* = 0.001), PFS (HR = 2.145, 95% CI, 1.339-3.436, *P* = 0.001), and CSS (HR = 2.167, 95% CI, 1.313-3.576, *P* = 0.002). On multivariate analysis including age, ASA grade, laparoscopic surgery, pathological T and N stage, tumor grade and history, sarcomatoid differentiation, lymphovascular invasion, and preoperative AST/ALT ratio, the results indicated that AST/ALT ratio was an independent risk factor for OS (HR = 1.972, *P = 0.005*), PFS (HR = 2.286, *P = 0.002*) and CSS (HR = 2.146, *P = 0.006*). Moreover, pathological T and N stage, and sarcomatoid differentiation were also independent risk predictor for OS and CSS, tumor grade and lymphovascular invasion were statistically independent risk factor for PFS (**Table 2**). After adjusting for competing risk of non-UTUC

Table 2. Multivariate analysis using Cox regression analysis of possible factors associated with OS, PFS, and CSS

Factors	Overall survival HR (95% CI), *p* value	Progression-free survival HR (95% CI), *p* value	Cancer-specific survival HR (95% CI), *p* value
Age (≥ 75)	1.487 (0.922-2.398), 0.104	1.149 (0.683-1.935), 0.600	1.087 (0.617-1.914), 0.773
ASA grade (III)	1.423 (0.791-2.558), 0.239	1.308 (0.668-2.560), 0.434	1.498 (0.755-2.976), 0.248
Laparoscopic surgery	1.227 (0.693-2.172), 0.482	1.839 (1.047-3.230), 0.340	1.651 (0.882-3.088), 0.117
AST/ALT (≥ 1.3)	1.972 (1.227-3.168), 0.005*	2.286 (1.370-3.813), 0.002*	2.146 (1.252-3.681), 0.006*
Pathological T stage (≥ pT3)	2.255 (1.356-3.752), 0.002*	1.982 (1.140-3.445), 0.015*	2.392 (1.316-4.347), 0.004*
Pathological N stage (N1-3)	4.417 (1.994-9.783), < 0.001*	3.930 (1.786-8.647), 0.001*	4.465 (1.965-10.159), < 0.001*
Grade (≥ G2)	1.469 (0.682-3.167), 0.326	2.726 (1.055-7.046), 0.038*	2.337 (0.813-6.717), 0.115
Histology	0.559 (0.215-1.457), 0.234	0.905 (0.337-2.432), 0.844	0.469 (0.151-1.463), 0.192
Sarcomatoid differentiation	4.860 (1.546-15.277), 0.007*	3.038 (0.909-10.153), 0.071	7.079 (1.947-25.741), 0.003*
Lymphovascular invasion	1.459 (0.763-2.790), 0.253	1.987 (1.018-3.879), 0.044*	1.955 (0.989-3.863), 0.054

HR, hazards ratio; CI, confidence interval. *Indicates statistically significant.
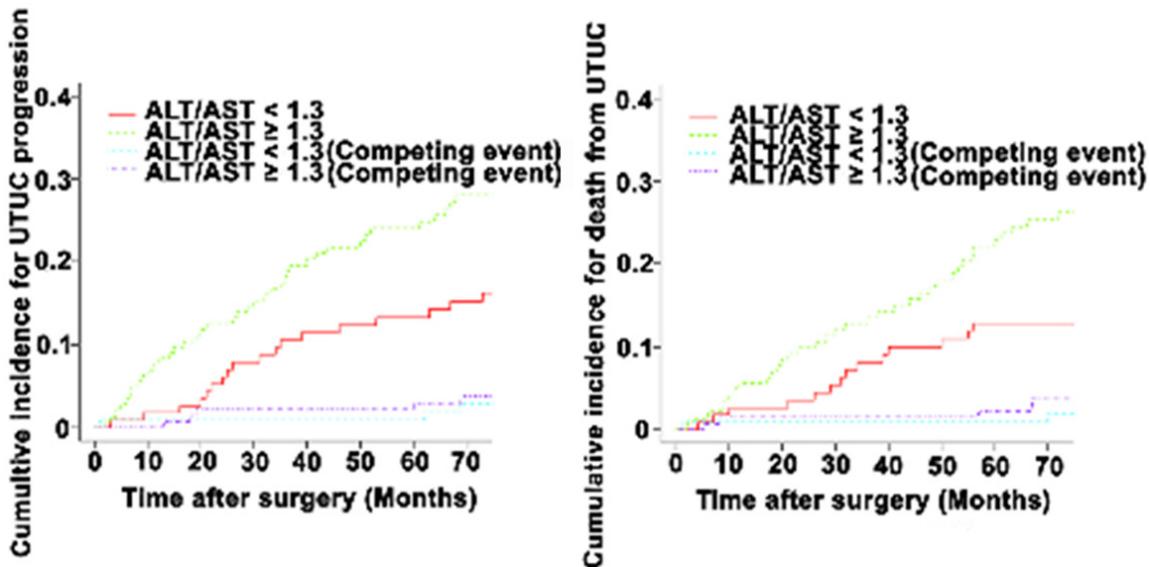

Figure 2. Cumulative incidence of UTUC progression and death from UTUC in patients categorized by preoperative AST/ALT ratio, using competing-risk analysis. The competing event was death from another cause.

death, higher AST/ALT ratio remained predictive ability of PFS (HR = 2.118, *P* = 0.004) and CSS (HR = 2.069, *P* = 0.010). The cumulative incidence of UTUC progression and death from UTUC in patients was shown in **Figure 2**. In addition, the observed PFS rates or CSS rates and the competing risk-adjusted rates as stratified by AST/ALT ratio were summarized in **Table 3**. In addition, we also analyzed relationship between the AST/ALT ratio and the bladder recurrence in patients, while there was no significant association (*P* = 0.668) (**Figure 1**).

Discussion

In the present study, high AST/ALT ratio was identified as independent risk predictor for

long-term outcomes in UTUC patients following RNU. The high AST/ALT ratio was independently correlated with OS, PFS, and CSS rather than bladder tumor recurrence in UTUC patients. Additionally, to minimize the biased estimated for PFS and CSS, the competing-risk analysis was performed.

To date, increasing studies reported that amino transaminases served as independent prognostic indicators in various malignancies regardless of liver-specific cancer-, including breast, lung, colorectal, and pancreatic [4-7]. For example, a retrospective study of 312 consecutive patients with liver metastases from breast cancer was performed. Multivariate analysis identified increased level of AST was

Table 3. Progression-free survival and cancer-specific survival for UTUC patients: observed and competing risk-adjusted rates

ASL/ALT ratio	n	Progression-free survival rates					
		Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
< 1.3	115	88; 87 (91)	82; 80 (77)	79; 78 (61)	79; 78 (45)	77; 76 (36)	74; 73 (21)
≥ 1.3	144	77; 75 (101)	69; 66 (74)	58; 56 (46)	55; 52 (35)	54; 51 (28)	52; 49 (22)
ASL/ALT ratio	n	Cancer-specific survival rates					
		Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
< 1.3	115	92; 91 (96)	87; 86 (83)	82; 81 (64)	81; 80 (48)	79; 78 (39)	77; 76 (23)
≥ 1.3	144	86; 84 (113)	73; 70 (81)	65; 62 (55)	60; 57 (40)	58; 55 (32)	54; 51 (23)

Two rates are presented. The first is the PFS or CSS rate estimated by Kaplan-Meier analysis; the second is the rate after accounting for the competing event (death from non-UTUC causes). Number of patients at risk is presented in parentheses. Total = 259; 20 patients excluded for unknown cause of death.

the single most important prognostic factor for survival after diagnosis of liver metastasis [6]. Recently Stocken et al. also concluded that AST was correlated with CSS in patients with advanced pancreatic cancer [7]. In general, ALT was considered more liver specific or enrich, while AST was widely expressed in different tissue types [14]. Therefore, pathological conditions leading to a higher proliferative status, high tumor cell turnover, and tissue damage were more likely to increase AST, while ALT was not increased, which making the AST/ALT ratio an attractive potential biomarker. Bezan et al. and Lee et al. demonstrated that increased AST/ALT was significantly associated with worse postoperative survival in patients surgically treated for localized renal cell carcinoma one after another [8, 9].

The prognostic effect of AST/ALT ratio on UTUC patients after treated with RNU was firstly introduced by Nishikawa et al. [10]. In their study, 109 patients were retrospectively analyzed and reported AST/ALT as a significant predictor for extravesical recurrence-free survival (HR = 4.21, 95% CI, 1.95-9.08, P < 0.001) rather than OS (P = 0.37) and DSS (P = 0.16). Although their study was limited by a comparatively small number of patients, lack of evaluation of effect of AST/ALT ratio on CSS and bladder tumor recurrence, it was valuable for introducing a novel prognostic factor for UTUC patients. To our knowledge, no further study was performed to analyze the impact of AST/ALT ratio on long-term survival in UTUC patients.

It might be still difficult to clearly explain the relationship between AST/ALT ratio and long-

term survival in UTUC patients, while the association of AST/ALT ratio with elderly age and high pathologic T stage could strengthen the assumptions. In addition, Fantin et al. recently reported that lactate dehydrogenase and high ratio of NADH/NAD+ might play a pivotal role in maintaining glucose catabolism [15]. And AST had been shown to have crucial role in glycolysis by relocation of NADH into mitochondria through malate-aspartate shuttle pathway [16]. Therefore, these assumptions might be theoretically explained, at least in part. To date, various molecular markers had been proposed as prognostic factor for clinical outcomes of UTUC [1, 2, 17], while the majority of these predictors were tissue-based so that they were unable to be obtained before surgery to evaluate the patients in clinical practice. Moreover, tissue-based predictors were usually nonreproducible due to intratumor heterogeneity [18]. In addition, the assays for these biomarkers were expensive and time-consuming due to lack of standardization [19]. Taking all of these above into consideration, it was essential to further investigate the prognostic value of the AST/ALT ratio.

The present study also has several limitations. It is a retrospective study of a single-institutional database, and we did not include several important parameters with prognostic value, such as tumor location and distal ureter management. The follow-up period was relatively short. Moreover, although no significant association between AST/ALT ratio and the bladder recurrence, there seemed a trend and it was essential to be validated in large, independent cohort subsequently.

Conclusions

The preoperative AST/ALT ratio was identified as independent risk predictor for OS, PFS, and CSS in UTUC patients following RNU. There was no significant correlation between preoperative AST/ALT ratio and the bladder recurrence. These findings were needed to be validated before applying its results.

Disclosure of conflict of interest

None.

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