

Original article

Prognostic significance of serum γ -glutamyltransferase in patients with advanced urothelial carcinoma

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Abstract

Objectives: Serum γ -glutamyltransferase (GGT) is reportedly associated with prognosis in patients with various malignancies. However, the prognostic role of GGT is unknown among patients with advanced urothelial carcinoma (aUC). This study was designed to examine the prognostic role of serum GGT in patients with aUC.

Materials and methods: Charts of 125 consecutive aUC patients (inoperable cT4 and/or metastasis to lymph nodes/distant organs) managed at a single cancer center between 2004 and 2016 were retrospectively reviewed. Variables collected included age, sex, body mass index, Karnofsky performance status, primary site, clinical tumor stage, lymph node/visceral metastasis, hepatic comorbidities, the presence of curative treatment before the diagnosis of aUC, white blood cell count, neutrophil-to-lymphocyte ratio, hemoglobin, albumin, lactate dehydrogenase, alkaline phosphatase, GGT, C-reactive protein, and treatments given after the diagnosis of aUC. Associations of variables with overall survival (OS) were analyzed using the Cox proportional hazard model.

Results: Serum GGT was elevated (≥ 60 U/l) at the diagnosis of aUC in 16 patients (13%). During follow-up period (median 12.1 months), 101 patients died (2-year OS rate, 32%). Patients with elevated GGT at the diagnosis of aUC had a significantly poorer prognosis than those with normal GGT with respective 2-year OS rates of 0% and 37% ($P < 0.001$). On multivariate analysis, elevated GGT was a significant and independent risk factor for shorter OS (hazard ratio, HR = 2.97; $P < 0.001$) as were poorer Karnofsky performance status (HR = 3.47; $P < 0.001$), elevated lactate dehydrogenase (HR = 1.86; $P = 0.033$), advanced age (HR = 1.82; $P = 0.013$), elevated neutrophil-to-lymphocyte ratio (HR = 1.80; $P = 0.015$), elevated C-reactive protein (HR = 1.73; $P = 0.018$), the absence of systemic chemotherapy (HR = 1.71; $P = 0.035$), and primary site of upper urinary tract (HR = 1.71; $P = 0.014$) in descending order by HR. The prognostic significance of elevated GGT was also observed in a subset of 101 patients who had been diagnosed with aUC at their first presentation.

Conclusion: The present study for the first time demonstrated that elevated serum GGT was an independent adverse prognostic factor in aUC patients. © 2018 Elsevier Inc. All rights reserved.

Keywords: Gamma-glutamyltransferase; Prognosis; Serologic tests; Transitional cell carcinoma; Urologic neoplasms

1. Introduction

Urothelial carcinoma (UC), including bladder UC (90%–95%) and upper urinary tract UC (5%–10%), are estimated to account for 85,000 new cancer cases and 18,000 deaths in the United States in 2018, making UC the fourth and

twelfth most common cancer in men and women, respectively [1]. Patients with advanced UC (aUC) generally show poor prognosis with median overall survival (OS) of 3 to 6 months without treatment and 13 to 16 months with systemic chemotherapy [2]. The first prognostic model predicting survival of patients with aUC was developed by Bajorin et al., which consisted of Karnofsky performance status (KPS) and the presence of visceral metastasis as dual independent predictors of OS [3]. After that, Apolo et al. and Galsky et al. developed prognostic models consisting of performance status, primary

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tumor site, lymph node/visceral metastasis, white blood cell (WBC) count, hemoglobin, and/or albumin [4,5]. Recently, inflammation markers such as neutrophil-to-lymphocyte ratio (NLR) and C-reactive protein (CRP) have garnered attention because these are informative biomarkers as well as significant prognostic factors in patients with UC [6,7]. The identification of other prognostic biomarkers, which are convenient and available worldwide, is desirable because more accurate risk stratification would help clinicians counsel patients about their prognoses and treatment strategies.

γ -Glutamyltransferase (GGT), which is expressed in various human cancer cells, is known as a membrane-bound enzyme implicated in neutralizing reactive oxygen compounds and free radicals [8]. GGT being a well-established serum marker for liver dysfunction, bile duct conditions, and alcohol consumption, its elevation is also linked to increased risk of a multitude of diseases and conditions including cardiovascular disease, diabetes, metabolic syndrome, and all-cause mortality [9]. Furthermore, elevated serum GGT level has been reported to be associated with decreased survival in patients with various malignancies including hepatocellular carcinoma, cervical cancer, endometrial cancer, cholangiocarcinoma, colorectal cancer, ovarian cancer, renal cell carcinoma, esophageal squamous cell carcinoma, gallbladder cancer, breast cancer, gastric cancer, and nasopharyngeal carcinoma [10–21]. So far, there has been no report on the prognostic role of GGT in UC. In the present study, we retrospectively evaluated the prognostic value of serum GGT in aUC patients.

2. Materials and methods

2.1. Patients

A total of 141 consecutive aUC patients (inoperable cT4 and/or metastasis to lymph nodes/distant organs) treated at a single cancer center between the years 2004 and 2016 were evaluated. Collected comprehensive clinical data were as follows: age, sex, body mass index (BMI), KPS, primary site, clinical tumor stage, lymph node/visceral metastasis (lung, liver, or bone), hepatic comorbidities, the presence of curative treatment before the diagnosis of aUC, WBC count, NLR, hemoglobin, albumin, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), GGT, CRP, and treatments given after the diagnosis of aUC. Of the 141 patients, 16 patients were excluded due to missing data. Finally, 125 patients were subjects of the present study. The Institutional Ethical Committee approved the present study protocol. BMI was calculated as follows: $BMI = (\text{weight [kg]} / (\text{height [m]})^2)$ whose cutoff value was set at 22 $[\text{kg}/\text{m}^2]$ based on a previous report [22]. Cutoff values of continuous variables followed those of previous reports: elevated WBC count $\geq 10,000$ cells/ μl [5], elevated NLR ≥ 3 [7], decreased hemoglobin < 13 and < 11.5 g/dl for men and women, respectively [4], elevated LDH ≥ 246 U/l [23], elevated ALP ≥ 220 U/l [24], decreased albumin < 4 g/dl [4], and elevated CRP ≥ 0.5 mg/dl [6].

2.2. Statistical analyses

OS was defined as the time from the diagnosis of aUC to either death or the last follow-up. The martingale residuals were used to assess the fitting of prognostic effect of GGT to a linear regression model and the most appropriate cutoff value for GGT was determined as previously described [25]. An optimal cutoff for patients' age was similarly determined. Two-tailed $P < 0.05$ was regarded as significant. The differences in variables between normal and elevated GGT groups were evaluated using the Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. The difference in the Kaplan–Meier curves was evaluated by the log-rank test. Associations of variables with OS were assessed using the Cox proportional hazard model. Variables showing statistical significance in the univariate analysis were included in the multivariate analysis. A reduced multivariate model was generated by backward elimination of the variable with the highest P value from each iteration of the multivariate analysis. All statistical analyses were conducted using JMP 13.0.0 (SAS Institute Inc., Cary, NC) and R 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Patient characteristics

The demographics of the 125 patients (85 males and 40 females) are shown in Table 1. The median (range) age was 70 (47–91) years. The primary site was bladder and upper urinary tract in 66 (53%) and 59 (47%) patients, respectively. Of the 125 patients, 24 (19%) patients developed aUC after curative treatment for their primary diseases; 22 (18%) patients had undergone curative surgery (radical cystectomy for 13, radical nephroureterectomy for 8 and both radical cystectomy and nephroureterectomy for 1) and the other 2 (2%) patients with muscle-invasive bladder cancer had received definitive chemoradiation. At the diagnosis of aUC, lymph node and visceral metastases were observed in 92 (74%) and 42 (34%) patients, respectively. A total of 93 (74%) patients received first-line platinum-based systemic chemotherapy. Thirty-nine (31%) patients finally received curative treatment after the diagnosis of aUC; 33 (26%) patients underwent curative surgery (radical cystectomy for 16 and radical nephroureterectomy for 17) and 6 (5%) patients received definitive radiation therapy (bladder for 3 and upper urinary tract for 3; radiation alone for 2 with bladder cancer and chemoradiation for the other 4).

3.2. Relationship between GGT and clinicopathological parameters

During follow-up period (median 12.1 months), 101 patients died (99 cancer deaths and 2 deaths of other causes; 2-year OS rate, 32%). Median (range) serum GGT level was 25 U/l (7–252 U/l) at the diagnosis of aUC. Plots of

Table 1
Demographics of 125 patients with advanced urothelial carcinoma.

Variables	No. patient (%)			P value
	Total (n = 125)	Normal GGT (n = 109)	Elevated GGT (n = 16)	
Age [years], median (IQR)	70 (64–74)	70 (64–74)	69 (65–74)	0.909
Sex				0.089
Male	85 (68)	71 (65)	14 (88)	
Female	40 (32)	38 (35)	2 (12)	
KPS				0.388
≥80	111 (89)	98 (90)	13 (81)	
<80	14 (11)	11 (10)	3 (19)	
BMI [kg/m ²], median (IQR)	22.2 (20.1–24.5)	22.2 (20.1–24.6)	22.1 (19.7–23.8)	0.634
Primary site				0.192
Bladder	66 (53)	55 (50)	11 (69)	
UUT	59 (47)	54 (50)	5 (31)	
Clinical T stage				0.690 ^a
T1	1 (1)	1 (1)	0 (0)	
T2	7 (6)	6 (6)	1 (6)	
T3	53 (43)	45 (41)	8 (50)	
T4	42 (34)	38 (35)	4 (25)	
Clinical N stage				0.603
N0	33 (26)	29 (27)	4 (25)	
N1	35 (28)	30 (28)	5 (31)	
N2	54 (43)	48 (44)	6 (38)	
N3	3 (2)	2 (2)	1 (6)	
The number of visceral metastasis site (lung, liver, or bone)				0.021*
0	83 (66)	77 (71)	6 (38)	
1	32 (26)	25 (23)	7 (44)	
2	10 (8)	7 (6)	3 (19)	
Metastatic site (% of total visceral metastasis)				0.577
Liver	5 (12)	3 (9)	2 (20)	
Nonliver	37 (88)	29 (91)	8 (80)	
Hepatic comorbidities	16 (13)	13 (12)	3 (19)	0.701
HBV carrier	3 (2)	3 (3)	0 (0)	
HCV carrier	5 (4)	5 (5)	0 (0)	
Fatty liver	5 (4)	4 (4)	1 (6)	
Others	5 (4)	3 (3)	2 (13)	
Curative treatment before the diagnosis of aUC	24 (19)	21 (19)	3 (19)	1.000
Surgery	22 ^b (18)	19 ^b (17)	3 (19)	1.000
Radical cystectomy	14 (11)	11 (10)	3 (19)	
Radical nephroureterectomy	9 (7)	9 (8)	0 (0)	
Pathological T stage (% of patients who underwent curative surgical therapy)	22 (18)	19 (17)	3 (19)	1.000
T2	8 (36)	7 (37)	1 (33)	
T3	11 (50)	9 (47)	2 (67)	
T4	3 (14)	3 (16)	0 (0)	
Pathological N stage (% of patients who underwent curative surgical therapy)				0.743
N0	12 (55)	9 (47)	3 (100)	
N2	3 (14)	3 (16)	0 (0)	
N3	1 (5)	1 (5)	0 (0)	
NX	6 (27)	6 (32)	0 (0)	
Chemoradiation	2 (2)	2 (2)	0 (0)	1.000
Clinical T stage (% of patients who underwent curative chemoradiation)				N/A
T2	1 (50)	1 (50)	0 (0)	
T4	1 (50)	1 (50)	0 (0)	
Clinical N stage (% of patients who underwent chemoradiation therapy)				N/A
N0	2 (100)	2 (100)	0 (0)	
Pretreatment laboratory parameters, median (IQR)				
WBC count [cells/ μ l]	7,400 (5,700–9,400)	7,100 (5,600–8,900)	9,700 (7,200–11,100)	0.006*
NLR	3.4 (2.5–5.2)	3.3 (2.3–4.8)	4.8 (3.9–6.2)	0.013*
Hemoglobin [g/dl]	12.3 (10.9–13.4)	12.4 (11.2–13.4)	12.0 (10.3–12.9)	0.352
Albumin [g/dl]	4.1 (3.8–4.3)	4.1 (3.9–4.3)	3.8 (3.6–3.8)	<0.001*
LDH [U/l]	188 (165–227)	188 (165–223)	209 (174–272)	0.152
ALP [U/l]	255 (215–307)	251 (209–291)	450 (273–601)	<0.001*
GGT [U/l]	25 (18–46)	22 (16–34)	86 (71–152)	<0.001*

(continued)

Table 1 (Continued)

Variables	No. patient (%)			P value
	Total (n = 125)	Normal GGT (n = 109)	Elevated GGT (n = 16)	
CRP [mg/dl]	0.9 (0.2–3.1)	0.7 (0.2–2.3)	3.5 (2.4–5.7)	0.002*
Systemic chemotherapy	93 (74)	83 (76)	10 (63)	0.357
First-line regimen (% of total chemotherapy)				0.186
MVAC	20 (22)	19 (23)	1 (10)	
GC	61 (66)	53 (64)	8 (80)	
G-CBDCA	10 (11)	10 (12)	0 (0)	
Others	2 (2)	1 (1)	1 (10)	
Curative treatment for the primary site after the diagnosis of aUC	39 (31)	37 (34)	2 (13)	0.146
Surgery	33 (26)	33 (30)	0 (0)	0.006*
Radical cystectomy	16 (13)	16 (15)	0 (0)	
Radical nephroureterectomy	17 (14)	17 (16)	0 (0)	
Radiation	6 (5)	4 (4)	2 (13)	0.142

ALP = alkaline phosphatase; aUC = advanced urothelial carcinoma; BMI = body mass index; CRP = C-reactive protein; GC = gemcitabine/cisplatin; GGT = γ -glutamyltransferase; G-CBDCA = gemcitabine/carboplatin; HBV = hepatitis B virus; HCV = hepatitis C virus; IQR = interquartile range; KPS = Karnofsky performance status; LDH = lactate dehydrogenase; MVAC = methotrexate/vinblastine/doxorubicin/cisplatin; NLR = neutrophil-to-lymphocyte ratio; N/A = not available; UUT = upper urinary tract; WBC = white blood cell.

* Statistically significant.

^a Calculated for patients who had not undergone prior surgical therapy before the diagnosis of aUC.

^b One case underwent both radical cystectomy and radical nephroureterectomy.

the martingale residuals demonstrated that the prognostic effect of GGT fitted a linear model and that patients were classified into 2 groups at an optimal cutoff GGT value of 60 U/l, where OS curves of the 2 groups were separated most greatly with the lowest *P* values for both males and females. Likewise, patients were categorized into 2 groups at the age of 67 years with the lowest *P* value. Elevated GGT was significantly associated with the number of visceral metastasis site (lung, liver, or bone; *P* = 0.021), elevated WBC count (*P* = 0.006), elevated NLR (*P* = 0.013), decreased albumin (*P* < 0.001), elevated ALP (*P* < 0.001), elevated CRP (*P* = 0.002), and the absence of curative surgical therapy for the primary site after the diagnosis of aUC (*P* = 0.006). Five (4%) patients had liver metastasis of whom 3 and 2 were classified into the normal and elevated GGT group, respectively (*P* = 0.577). Sixteen (13%) patients had hepatic comorbidities (e.g., hepatitis virus carrier and fatty liver) of whom 13 and 3 were classified into the normal and elevated GGT group, respectively (*P* = 0.701).

3.3. Relationship between GGT or other prognostic factors and OS

Fig. 1 shows OS curves of the normal GGT (<60 U/l) and elevated GGT (\geq 60 U/l) groups, which had 2-year OS rates of 37% and 0%, respectively (*P* < 0.001). The association of each variable with OS is shown in Table 2. On univariate analysis, elevated GGT was significantly associated with shorter OS (hazard ratio, HR = 3.25; *P* < 0.001). Additionally, the following variables were significantly associated with worse OS: advanced age (HR = 2.09; *P* < 0.001), poorer KPS (HR = 3.04; *P* < 0.001), primary site of the upper

urinary tract (HR = 1.61; *P* = 0.018), the presence of visceral metastasis (HR = 1.83; *P* = 0.003), elevated NLR (HR = 2.02; *P* < 0.001), decreased albumin (HR = 2.10; *P* < 0.001), elevated LDH (HR = 1.82; *P* = 0.030), elevated CRP (HR = 1.86; *P* = 0.004), the absence of systemic chemotherapy (HR = 1.83; *P* = 0.010), and the absence of curative treatment for the primary site after the diagnosis of aUC (HR = 2.39; *P* < 0.001). Multivariate analysis demonstrated that elevated GGT (HR = 2.97; *P* < 0.001) and following factors were independently associated with shorter OS: poorer KPS (HR = 3.47; *P* < 0.001), elevated LDH (HR = 1.86; *P* = 0.033), advanced age (HR = 1.82; *P* = 0.013), elevated

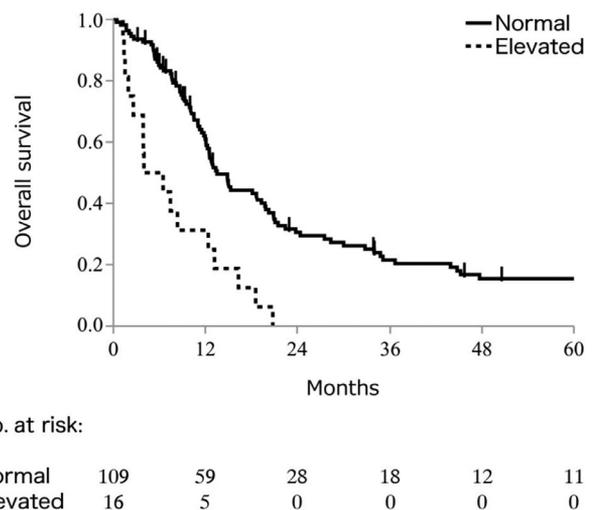


Fig. 1. The Kaplan–Meier estimates of overall survival for 125 patients with advanced urothelial carcinoma according to normal (*n* = 109) and elevated (*n* = 16) serum GGT levels (*P* < 0.001).

Table 2
Univariate and multivariate analysis for variables predicting overall survival in 125 patients.

Variables	Univariate			Multivariate		
	HR	(95% CI)	P value	HR	(95% CI)	P value
Age						
≥67 (vs. <67 [years])	2.09	(1.36–3.20)	<0.001*	1.82	(1.14–2.92)	0.013*
Sex						
Female (vs. male)	1.07	(0.70–1.64)	0.744			
KPS						
<80 (vs. ≥80)	3.04	(1.69–5.48)	<0.001*	3.47	(1.79–6.75)	<0.001*
BMI						
<22 (vs. ≥22 [kg/m ²])	1.41	(0.95–2.09)	0.091			
Primary site						
UUT (vs. bladder)	1.61	(1.09–2.39)	0.018*	1.71	(1.11–2.62)	0.014*
Clinical T stage						
3, 4 (vs. ≤2)	1.76	(0.91–3.39)	0.092			
Clinical N stage						
≥1 (vs. 0)	1.38	(0.85–2.23)	0.194			
The number of visceral metastasis site (lung, liver, or bone)						
≥1 (vs. 0)	1.83	(1.22–2.76)	0.003*			
Curative treatment before the diagnosis of aUC						
No (vs. Yes)	1.27	(0.75–2.14)	0.370			
WBC count						
Elevated (vs. normal ^a)	1.24	(0.77–1.98)	0.378			
NLR						
Elevated (vs. normal ^b)	2.02	(1.33–3.07)	<0.001*	1.80	(1.12–2.89)	0.015*
Hemoglobin						
Decreased (vs. normal ^c)	1.45	(0.98–2.12)	0.064			
Albumin						
Decreased (vs. normal ^d)	2.10	(1.40–3.15)	<0.001*			
LDH						
Elevated (vs. normal ^e)	1.82	(1.06–3.14)	0.030*	1.86	(1.05–3.27)	0.033*
ALP						
Elevated (vs. normal ^f)	1.50	(0.96–2.35)	0.075			
GGT						
Elevated (vs. normal ^g)	3.25	(1.87–5.65)	<0.001*	2.97	(1.62–5.44)	<0.001*
CRP						
Elevated (vs. normal ^h)	1.86	(1.23–2.82)	0.004*	1.73	(1.10–2.74)	0.018*
Systemic chemotherapy						
No (vs. Yes)	1.83	(1.16–2.90)	0.010*	1.71	(1.04–2.81)	0.035*
Curative surgery for the primary site after the diagnosis of aUC						
No (vs. Yes)	2.39	(1.48–3.84)	<0.001*			

ALP = alkaline phosphatase; aUC = advanced urothelial carcinoma; BMI = body mass index; CI = confidence interval; CRP = C-reactive protein; GGT = γ -glutamyltransferase; HR = hazard ratio; KPS = Karnofsky performance status; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; UUT = upper urinary tract; WBC = white blood cell.

* Statistically significant.

^a The normal range for WBC count was set at <10,000 [cells/ μ l].

^b The normal range for NLR was set at <3.

^c The normal ranges for hemoglobin were set at ≥ 13 [g/dl] for men and ≥ 11.5 [g/dl] for women.

^d The normal range for albumin was set at ≥ 4 [g/dl].

^e The normal range for LDH was set at <246 [U/l].

^f The normal range for ALP was set at <220 [U/l].

^g The normal range for GGT was set at <60 [U/l].

^h The normal range for CRP was set at <0.5 [mg/dl].

NLR (HR = 1.80; $P = 0.015$), elevated CRP (HR = 1.73; $P = 0.018$), the absence of systemic chemotherapy (HR = 1.71; $P = 0.035$), and primary site of upper urinary tract (HR = 1.71; $P = 0.014$) in descending order by HR.

To address the prognostic role of elevated GGT in patients diagnosed with aUC at their first presentation, we conducted subgroup analysis in such 101 patients,

excluding 24 who had received prior curative treatment for the primary site from the original patient cohort (Table 3). In this subset, elevated GGT (HR = 2.97; $P = 0.001$) was still a significant and independent risk factor for shorter OS as were poorer KPS (HR = 2.80; $P = 0.003$), the absence of systemic chemotherapy (HR = 2.51; $P < 0.001$), the absence of curative surgery for the primary site (HR = 2.10;

Table 3
Subgroup analysis for variables predicting overall survival in 101 patients who had not received prior curative treatment.

Variables	Univariate			Multivariate		
	HR	(95% CI)	<i>P</i> value	HR	(95% CI)	<i>P</i> value
Age						
≥67 (vs. <67 [years])	2.11	(1.32–3.38)	0.002*			
KPS						
<80 (vs. ≥80)	3.12	(1.68–5.81)	<0.001*	2.80	(1.44–5.47)	0.003*
Primary site						
UUT (vs. bladder)	1.69	(1.09–2.61)	0.019*	1.84	(1.17–2.91)	0.009*
The number of visceral metastasis site (lung, liver, or bone)						
≥1 (vs. 0)	1.94	(1.24–3.06)	0.004*			
NLR						
Elevated (vs. normal ^a)	1.89	(1.21–2.95)	0.005*			
Albumin						
Decreased (vs. normal ^b)	2.47	(1.58–3.86)	<0.001*			
GGT						
Elevated (vs. normal ^c)	3.76	(2.02–6.99)	<0.001*	2.97	(1.52–5.81)	0.001*
CRP						
Elevated (vs. normal ^d)	2.26	(1.43–3.59)	<0.001*	2.09	(1.27–3.45)	0.004*
Systemic chemotherapy						
No (vs. Yes)	2.20	(1.35–3.58)	0.002*	2.51	(1.51–4.18)	<0.001*
Curative surgery for the primary site after the diagnosis of aUC						
No (vs. Yes)	2.72	(1.64–4.53)	<0.001*	2.10	(1.21–3.66)	0.009*

aUC = advanced urothelial carcinoma; CI = confidence interval; CRP = C-reactive protein; GGT = γ -glutamyltransferase; HR = hazard ratio; KPS = Karnofsky performance status; NLR = neutrophil-to-lymphocyte ratio; UUT = upper urinary tract.

* Statistically significant.

^a The normal range for NLR was set at <3.

^b The normal range for albumin was set at ≥ 4 [g/dl].

^c The normal range for GGT was set at <60 [U/l].

^d The normal range for CRP was set at <0.5 [mg/dl].

$P = 0.009$), elevated CRP (HR = 2.09; $P = 0.004$), and primary site of upper urinary tract (HR = 1.84; $P = 0.009$) in descending order by HR.

4. Discussion

GGT has been investigated as a possible cancer biomarker in a variety of cancers [10–21]. GGT is a well-established serum marker for a multitude of diseases and conditions and is widely used throughout the world [9]. The present study, for the first time, demonstrated the prognostic significance of serum GGT in aUC patients. In the present study, elevated GGT was an independent adverse prognostic factor along with advanced age, poorer KPS, primary site of upper urinary tract, elevated NLR, elevated LDH, elevated CRP, and the absence of systemic chemotherapy, supporting clinical relevance of GGT as a prognostic biomarker.

There were several background clinical (e.g., visceral metastasis and curative surgery for the primary site) and laboratory (e.g., WBC count, NLR, albumin, ALP, and CRP) parameters showing significant differences between normal- and elevated-GGT groups (Table 1). All of those background parameters were worse in (the) elevated-GGT group. Notably, no patients with elevated serum GGT received surgical therapy after the diagnosis of aUC in our

cohort. Given that curative surgery was only given to patients in good shape with good response to multidisciplinary therapy, the population with elevated serum GGT as a whole was composed of less healthy patients, who were unlikely to be surgical candidates. Curative surgery for the primary site after the diagnosis of aUC was not carried into the final model for whole 125 patients (Table 2), whereas it served as an independent adverse prognostic factor in the subanalysis for 101 patients without prior curative treatment (Table 3).

GGT is a key enzyme involved in glutathione (γ -glutamyl-cysteinyl-glycine, GSH) metabolism and is expressed by a wide range of cell types [26]. GGT has been traditionally regarded to play an important protective role against oxidative stress because GGT is important to maintain adequate intracellular GSH, a main intracellular water-soluble antioxidant [27]. GGT also provides the cell with amino acids necessary for protein synthesis and maintenance of intracellular GSH re-synthesis, promoting cell proliferation. Indeed, in vitro and in vivo studies have shown that GGT-overexpressing cells utilize extracellular GSH as a source of cysteine more efficiently, resulting in a selective growth advantage both at physiological and at limiting cysteine concentrations [8]. Moreover, expression of GGT could allow tumors to resist the effects of chemotherapy since a number of drugs are conjugated with GSH by glutathione-S-transferases [27].

Although GGT is an insoluble ectoenzyme primarily located on the outside of cell membranes, the low level of GGT activity found in normal human serum is attributed to the presence of a hydrophilic form of the enzyme. It is notable that the source of the soluble GGT in normal serum is not known [8]; however, the release of a GGT-containing soluble complex from cancer cells and a specific GGT fraction found in the plasma of healthy individuals have been described [28]. Although it has also been proposed that serum GGT is increased due to quantitative changes in GGT expression and necrotic alterations within the patients with metastatic disease [16], the site of metastasis (liver vs. nonliver) did not differ between aUC patients with normal and elevated serum GGT in the present study ($P=0.577$). Even though recent studies have indicated that elevated serum and tumor GGT levels have a joint effect on the poor outcome in ovarian and gastric cancer patients [15,20], studies on GGT expression in UC tissues are very limited [29,30]. Further investigations on human serum GGT, local expression, and clinical outcomes are needed to elucidate its roles in UC progression.

This study has several limitations. First, our finding is preliminary since it is from a retrospective single institutional study with a relatively small patient cohort. Thus, external validations on completely independent patient cohorts, preferably multicentric cohorts, would enhance the generality of our findings according to the REMARK guidelines [25]. Second, no patient of the present cohort received immune-oncology agents, which have recently been approved for aUC patients [31]. Further investigation on the prognostic role of GGT in aUC patients treated with these agents is needed in the future. Third, localization of GGT in tumor cells was not investigated in this study. Whether elevated serum GGT accompanies GGT overexpression in UC tissues is of great interest. We are planning an immunohistochemical study using surgically resected materials to examine this question. Fourth, although serum GGT levels depend not only on clinicopathological parameters but also on life style factors, these factors are not considered in the present study. Nonetheless, a previous study on GGT and life style factors (e.g., obesity, hypertension, and diabetes mellitus) showed that none of the investigated parameters were associated with GGT levels [12].

5. Conclusion

Our study suggests that serum GGT is a prognostic biomarker in aUC patients. Validation of multicentric larger patient cohorts is needed to confirm this finding.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.urolonc.2018.11.002](https://doi.org/10.1016/j.urolonc.2018.11.002).

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