Alpha-l-fucosidase: a novel serum biomarker to predict prognosis in early stage esophageal squamous cell carcinoma

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Background: Alpha-l-fucosidase (AFU) not only detects hepatocellular carcinoma (HCC) early but also is used as a clinical prognostic indicator of several malignant tumors. However, no study has investigated the prognostic significance of AFU in a cohort of patients with esophageal squamous cell carcinomas (ESCCs).

Methods: A retrospective dataset that included 160 consecutive patients with early stage (pT1N0) ESCC who received surgery between January 2005 and December 2012 was analyzed to identify the prognostic value of serum AFU for overall survival (OS) by using Kaplan-Meier analysis and Cox multivariate regression modeling.

Results: The level of serum AFU ranged from 6.2 to 77.0 U/L with a median of 19.9 U/L, and the best cutoff point for OS was 17.95 U/L. Analysis by Pearson's correlation showed that the levels of serum ALT and GGT were both positively correlated with the level of serum AFU (r=0.403, P<0.001 and r=0.264, P=0.001, respectively). After adjusting for significant factors identified by univariate analysis, the Cox multivariate regression model indicated that a young age (<65 years), no history of alcohol consumption, and a low AFU level (≤17.95 U/L) were still significantly associated with longer OS (P=0.008, 0.004 and 0.017, respectively). The 5-year and 10-year OS rates for patients with high AFU levels *vs.* low AFU levels were 76.2% *vs.* 86.0%, and, 46.7% *vs.* 83.4%, respectively.

Conclusions: Compared with other serum biomarkers, AFU showed a better prognostic value for long-term survival in patients with early stage ESCC.

Keywords: Esophageal squamous cell carcinoma (ESCC); alpha-l-fucosidase (AFU); prognosis

Introduction

Esophageal carcinoma (EC) is the ninth most common malignant tumor and causes the fifth highest number of cancer-related deaths worldwide (1). It is an endemic malignancy, particularly in the Asian esophageal cancer belt, and the incidence of EC varies in different countries and regions (1,2). In China, the estimated number of new EC cases in 2015 was 477,900, which accounted for more than half of new global cases (1). Despite tertiary prevention,

guideline–concordant treatment, and standardized follow-up for EC established by the Chinese Society of Clinical Oncology (CSCO), the survival outcomes are still not ideal, and 5-year overall survival (OS) rates ranged from 5% to 17% in recent reports (1,3,4). Early detection and treatment provide a great opportunity to reduce death from cancers, such as colorectal cancer, gastric cancer, and non-small cell lung cancer (3,5,6). However, the outcomes for some patients, even those with early stage EC (T1) treated with limited surgery, have not been favorable; in China, more than 15% of these patients died of cancer-related causes 5 years after diagnosis (compared with approximately 30% of those in the United States and 22.5% in Japan) (2,4,5,7). Therefore, finding independent predictors to distinguish patients with poor survival is particularly important (5,8). Individual multidisciplinary therapy (MDT) may be suitable for prolonging the survival of patients with early stage EC who also have high levels of other-risk factors (2,5,7).

Increased or altered alpha-l-fucose-containing fucoglycoconjugates, such as glycolipids, glycoproteins, and mucoopolysaccharides etc., on cell surface are not only correlated with oncogenic transformation, lymphatic and vascular invasion, but also associated with the immune escape or tolerance. However, alpha-l-fucosidase (AFU), a liposomal enzyme, is widely present in mammalian cells and associated with key aspects of the degradation of alpha-l-fucose. Consequently, the balance of alpha-l-fucose and AFU may affect the prognosis of malignancies. And theoretically, because AFU would be consumed during the degradation of alpha-l-fucose, patients with a low AFU level may have better survival (9-16). As a tumor biomarker, AFU was first introduced by Deugnier *et al.* in the diagnosis of hepatocellular carcinoma (HCC), and subsequent studies have also confirmed that serum AFU diagnosed HCC earlier than ultrasonography (9-11). Currently, the independent prognostic value of AFU for the postoperative survival of patients with HCC, colorectal carcinoma, intrahepatic cholangiocarcinoma and breast cancer has also been found (12-14). HCC patients with an elevated AFU level are more likely to die from cancer, but, possibly due to neoplastic heterogeneity, an elevated AFU level predicts a better OS rate among those with triple-negative breast cancer (12,15).

However, to our knowledge, no available evidence has shown the feasibility of AFU as a prognostic factor in esophageal squamous cell carcinoma (ESCC). Thus, the aim of our study was first to explore the association between AFU and long-term survival in Chinese patients with early stage ESCC (pathological stage T1N0).

Methods

Patient selection

We retrospectively reviewed all patients who received surgery for pathological stage T1N0M0 (according to the 8th edition of the American Joint Committee on Cancer staging manual) ESCC at Sun Yat-sen University Cancer Center from January 2005 to December 2012. Then, patients were excluded from the preliminary database according to the following criteria: (I) received preoperative chemotherapy or chemoradiotherapy; (II) was diagnosed with secondary primary malignancy before or after the diagnosis of EC; (III) had a diagnosis of EC not otherwise specified (NOS); (IV) was diagnosed with other histology subtype(s), such as adenocarcinoma, small cell carcinoma or carcinosarcoma; (V) had no preoperative serum AFU examination; (VI) died within 30 days of their operation; (VII) were lost to follow-up. Finally, a total of 160 consecutive patients were retained in our study.

Serum AFU measurement

Serum AFU levels were measured within 1 week before the operation as previously described (9,11,12). Fasting venous blood samples were taken from the patients and serum was obtained by centrifugation. A Hitachi Clinical Analyzer 7600 (Tokyo, Japan) was used to assess the AFU content in the serum within 24 hours after each blood sample was collected. During the interpretation, the normal reference range for serum AFU levels in our center was 5–40 U/L.

Statistical analysis

First, X-Tile software version 3.6.1 (Copyright Yale University 2003) was used to determine the best cutoff values for tumor length, resected lymph node count, alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, gamma-glutamyl transferase (GGT) level, ALT/AST ratio, lactate dehydrogenase (LDH) level, and AFU level by the minimal P value approach. Second, all statistical analyses in our study were performed using the Statistical Product and Service Solutions software package version 23.0 (SPSS Inc., Chicago, IL, USA). Among these, the Pearson Chi-square test was applied to the categorical variables to compare baseline characteristics

between the low AFU group and the high AFU group, and an independent t test was used to compare continuous variables between the two groups. In addition, correlations from Pearson's linear correlation analysis are shown as scatter plots.

OS time was calculated from the day of ESCC diagnosis to death or the last follow-up (1 June 2018), but only patient occurred death was recorded as uncensored. In addition, the Kaplan-Meier method was used to plot the OS curve and for univariate analysis; all the statistically significant variables identified by univariate analysis were subsequently included in the COX multivariate regression model to explore potential prognostic indicators.

In addition, to ensure the consistency of the data and facilitate subsequent data processing and analysis, the alcohol use status was converted into digital data in our study based on the World Health Organization (WHO) Risk Drinking Levels, as shown in *Table S1*. Individuals with medium risk drinking or above were defined as alcohol consumption.

Following the well-known statistical principle, a P value less than 0.05 indicated statistical significance.

Ethics statement

The Research Data Deposit (RDD) Management Committee at Sun Yat-sen University Cancer Center has approved of our deposited research data, and the RDD number for this dataset is RDDA2018000855. In addition, our research was approved by the Institute Research Medical Ethics Committee at Sun Yat-sen University Cancer Center (approval number: B2014-110), and informed consent forms were signed by all patients.

Results

The baseline characteristics and relationships between the two AFU groups

From January 2005 to December 2012, a total of 160 consecutive patients with a pathologically confirmed diagnosis of pathological stage T1N0 ESCC were included in the final analysis. Their clinicopathological characteristics and hematological indexes are shown in *Table 1*. In our cohort, male patients comprised a large majority (n=105, 65.6%), and the median age at diagnosis was 59 years old (range, 32–82 years old). Interestingly, more than half of the patients (n=86, 53.8%) reported tobacco exposure, but only

22 patients (13.8%) had a history of alcohol consumption. In addition, primary carcinomas were located in the middle and lower thoracic esophagus at a high frequency (65.0% and 18.7%, respectively), and the Sweet procedure (left thoracic incision) was often chosen for the excision of esophageal neoplasms (n=102, 63.8%).

The levels of AFU ranged from 6.2 to 77.0 U/L with a median of 19.9 U/L. The best cutoff point for OS was found to be 17.95 U/L by X-Tile software, and the area under the curve (AUC) was 0.591 in the receiver operating characteristic (ROC) curve (*Figure 1A*). The baseline characteristics in the patients with low AFU levels (≤17.95 U/L) and high AFU levels (>17.95 U/L) were compared (*Table 2*). A high AFU level (>17.95 U/L) was associated with a history of smoking (P=0.019), a high level of ALT (P=0.012), and a high level of GGT (P=0.005). Additionally, the levels of ALT (*Figure 1B*) and GGT (*Figure 1C*) were positively associated with the level of AFU using Pearson's linear correlation analysis (r=0.403 and 0.264, respectively). However, there was no statistically significant difference in the distribution of other clinicopathological and hematological indexes between patients with high or low AFU levels (all P>0.05, *Table 2*).

Survival outcomes and prognostic analysis

In our cohort, 42 patients (42/160, 26.3%) had occurred death at the last follow-up (1 June 2018), and 37 deaths of them were directly related with ESCC. Median follow-up time was 83.8 months (range, 64.0–107.5 months), and the 5-year and 10-year OS rate for all 106 consecutive patients with pathological stage T1N0 ESCC was 79.4% and 66.3%, respectively.

In univariate analysis, patients with a low AFU level (≤17.95 U/L) showed a significantly longer 10-year OS than patients with a high AFU level (>17.95 U/L) (83.4% and 46.7%, P=0.028, *Table 1* and *Figure 2*). Additionally, univariate analysis also indicated that age, alcohol consumption, AST level, and LDH level were prognostic factors (*Table 1*). After Cox multivariable regression analysis, young patients (<65 years), patients with no history of alcohol consumption, and patients with low AFU level (≤17.95 U/L) were still significantly associated with a longer OS (*Table 1* and *Figure 2*).

Discussion

This study is the first to demonstrate and report that AFU

Table 1 Baseline characteristics of all 160 patients with pathological stage T1N0 ESCC and analysis of the prognostic factors for OS

Characteristic	N (%)	Univariate analysis		Multivariate analysis	
		10-year OS rate (%)	P*	Hazard ratio (95% CI)	P*
Gender			0.823		
Male	105 (65.6)	54.3			
Female	55 (34.4)	71.0			
Age (years)			0.008	0.408 (0.210–0.792)	0.008
<65	119 (74.4)	63.9			
≥65	41 (25.6)	42.1			
Smoking history			0.462		
Yes	86 (53.8)	63.9			
No	74 (46.2)	42.1			
Alcohol consumption			0.040	0.306 (0.136–0.690)	0.004
Yes	22 (13.8)	59.1			
No	138 (86.2)	67.7			
Differentiation			0.939		
Well	24 (15.0)	59.3			
Moderate	84 (52.5)	54.4			
Poor	52 (32.5)	69.7			
Tumor location			0.191		
Upper	26 (16.3)	84.3			
Middle	104 (65.0)	55.2			
Lower	30 (18.7)	58.9			
Length of tumor, cm			0.105		
≤1.8	42 (26.3)	39.4			
<1.8	118 (73.7)	72.6			
Surgical approach			0.926		
Sweet	102 (63.8)	55.1			
McKeown	58 (36.2)	68.5			
Depth of tumor invasion			0.253		
T1a	38 (23.8)	80.7			
T1b	122 (76.2)	54.1			
Adjuvant therapy			0.168		
Yes	6 (3.8)	100			
No	154 (96.2)	57.0			

Table 1 (*continued*)

Table 1 (*continued*)

Characteristic	N (%)	Univariate analysis		Multivariate analysis	
		10-year OS rate (%)	P*	Hazard ratio (95% CI)	P*
Resected lymph nodes count			0.061	1.192 (0.625–2.272)	0.593
≤13	53 (33.1)	44.7			
>13	107 (66.9)	74.1			
ALT			0.066	1.994 (0.940–4.233)	0.072
≤18.55	88 (55.0)	59.0			
>18.55	72 (45.0)	57.6			
AST			<0.001	1.861 (0.897–3.860)	0.095
≤15.35	27 (16.9)	34.9			
>15.35	133 (83.1)	62.7			
ALT/AST			0.324		
≤0.75	49 (30.6)	61.4			
>0.75	111 (69.4)	57.3			
GGT			0.250		
≤21.85	82 (51.3)	54.8			
>21.85	78 (48.7)	61.0			
LDH			0.035	2.215 (0.924–5.308)	0.075
≤170.20	108 (67.5)	53.6			
>170.20	52 (32.5)	58.7			
AFU			0.028	0.371 (0.164–0.839)	0.017
≤17.95	52 (32.5)	83.4			
>17.95	108 (67.5)	46.7			

*, P value less than 0.05 indicated a statistically significant difference. ESCC, esophageal squamous cell carcinoma; OS, overall survival; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; AFU, alpha-l-fucosidase.

significantly and independently predicts prognosis in patients with ESCC. Patients with pathological stage T1N0 ESCC who had low AFU levels (≤17.95 U/L) had a significantly longer 10-year OS than patients with high AFU levels (>17.95 U/L) (83.4% and 46.7%, P=0.028). Furthermore, a high AFU level was associated with a history of smoking, a high level of ALT, and a high level of GGT.

As a tumor biomarker, AFU was first introduced by Deugnier *et al.* in the diagnosis of HCC, and subsequent studies have also confirmed that serum AFU diagnosed HCC earlier than ultrasonography (9-11). Fawzy Montaser and colleagues found that AFU examination detected abnormalities earlier than abnormal ultrasonography

imaging observable at 6–9 months (12). In a meta-analysis from Gan *et al.*, the pooled sensitivity of AFU for the diagnosis of HCC was 0.72 while the pooled specificity was 0.78, and the AUC was 0.8125. They concluded that AFU could be used as a tumor marker for the diagnosis of HCC with great value in clinical application (16). Currently, the independent prognostic value of AFU levels for postoperative survival of patients with HCC, colorectal carcinoma and breast cancer has also been reported (11-16). HCC and colorectal carcinoma patients with an elevated AFU level are more likely to die from cancer, but an elevated AFU level predicts a better OS rate among triple-negative breast cancer patients. In our research, similar to
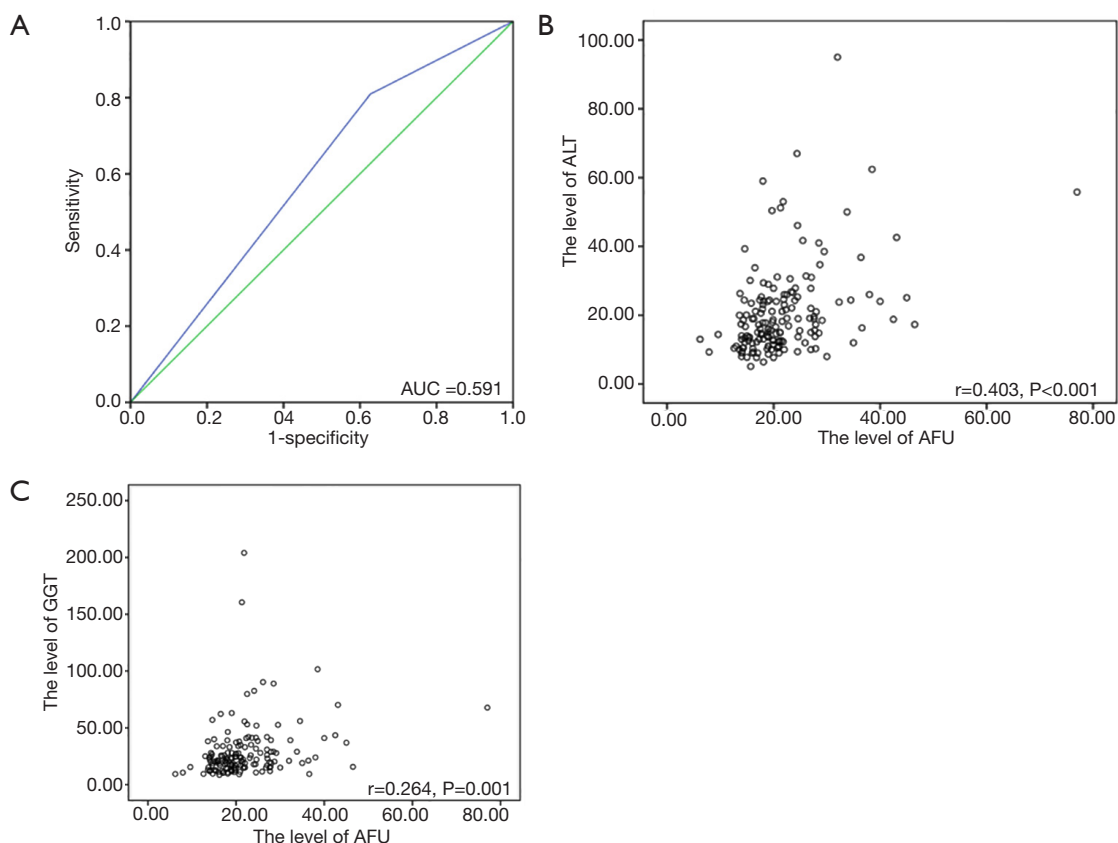
Figure 1 The predictive value of AFU in long-term survival and Pearson's linear correlation analysis. (A) The receiver operating characteristic curve of the AFU; (B) distribution of the levels of AFU and ALT; (C) distribution of the levels of AFU and GGT. AFU, alpha-l-fucosidase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

the results in patients with HCC and colorectal carcinoma, the AFU levels were similarly able to predict the prognosis of pathological stage T1N0 ESCC patients (11,12,14-16).

A review on the mechanism of AFU revealed that an increased fucose or fucosylation content and aberrant fucosylation on the membrane surface of tumor cells may be associated with the metastatic capacity of tumors and could help abnormal cells escape immunological recognition (17-20). Thus, fucose expression or fucosylation might play a critical role in tumor progression and poor survival (17-19). However, these fucosylated glycoconjugates could be degraded by AFU, which would maintain a lower fucose content, prevent tumor metastasis, and induce immune responses (17-20). Accordingly, the serum AFU level, which would be consequently consumed, decreasing the fucosylated glycoconjugates synthesized by the neoplasm, could be used to predict the preoperative survival of patients with malignant tumors (17,19). Although previous studies have reported the level of AFU activity in malignant tumors

to be positively correlated with TNM staging or Duke's stage, low AFU serum content in early stage patients still precisely reflects tumor load and the degree of malignancy (14,15). In addition, AFU may be a potential therapeutic molecular target for clinical applications (13,17,18,20).

As specific, inexpensive and easily available biomarkers, ALT, AST, GGT and LDH are generally used in the preoperative assessment of liver function (21). In recent years, increasing studies have noted that these biomarkers are not only related to the risk of malignant tumor occurrence, but also associated with survival after anti-tumor treatment for cancers, such as gastric cancer, breast cancer, liver cancer, ESCC and other cancers (22-24). In a retrospective study, Huang *et al.* reported that ESCC cases with a lower ALT/AST ratio showed a worse prognosis than cases with a higher ratio (P<0.001); this was observed not only in the entire cohort but also in both the T1–2 and N0 subgroups (22). However, the predictive value of the ALT/AST ratio for long-term survival was not found in our

Table 2 The relationships between AFU levels and baseline characteristics in patients with stage T1N0 ESCC

Characteristic	AFU ≤17.95 (n=52)	AFU >17.95 (n=108)	P*
Gender			0.900
Male	39	80	
Female	13	28	
Age (years)			0.069
<65	29	76	
≥65	23	32	
Smoking history			0.019
Yes	21	65	
No	31	43	
Alcohol consumption			0.123
Yes	4	18	
No	48	90	
Performance status			0.115
0	45	99	
1	5	9	
2	2	0	
Differentiation			0.582
Well	10	14	
Moderate	26	58	
Poor	16	36	
Tumor location			0.932
Upper	9	17	
Middle	34	70	
Lower	9	21	
Length of tumor, cm			0.893
≤1.8	14	28	
<1.8	38	80	
Surgical approach			0.958
Sweet	33	69	
McKeown	19	39	
Reconstructed organ			
Gastric tube	52	107	–
Anastomosis			0.759
Hand-sewn	5	12	
Stapled	47	95	

Table 2 (*continued*)

Table 2 (*continued*)

Characteristic	AFU ≤17.95 (n=52)	AFU >17.95 (n=108)	P*
Depth of tumor invasion			0.065
T1a	17	21	
T1b	35	87	
Adjuvant therapy			0.965
Yes	2	4	
No	50	104	
Resected lymph nodes count (mean ± SD)	19.4±11.2	20.1±12.1	0.676
Resected lymph nodes stations (mean ± SD)	6.48±3.50	6.11±3.36	0.644
ALT			0.012
≤18.55	36	52	
>18.55	16	56	
AST			0.581
≤15.35	10	17	
>15.35	42	91	
ALT/AST			0.063
≤0.75	21	28	
>0.75	31	80	
GGT			0.005
≤21.85	35	47	
>21.85	17	61	
LDH			0.971
≤170.20	35	73	
>170.20	17	35	

*, P value less than 0.05 indicated a statistically significant difference. ESCC, esophageal squamous cell carcinoma; OS, overall survival; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; AFU, alpha-l-fucosidase.

patients with pathological stage T1N0 ESCC. In addition, unlike results in hepatitis B-induced HCC, the level of AST or ALT alone was not related to the clinical outcome of ESCC, and this was shown in not only a previous report but also our study (22,23). Recently, the prognostic capabilities of GGT and LDH in ESCC have also been widely demonstrated, and the cases enrolled in these related studies included all TNM stages (I–IV) (22-25). However, in adjusted survival analysis, these two biomarkers did not play significant prognostic roles in our study, which was consistent with a subgroup analysis from Huang *et al.* (22). Possible reasons for the differences described above include the following.

First, when used to assess liver function, these biomarkers could also indicate anti-inflammatory and anti-oxidant properties, exposure to certain carcinogens, and alcohol intake (26,27). Accordingly, higher levels of these biomarkers were positively associated with more severe systemic inflammatory response syndrome (SRS), a more advanced stage and distant metastasis, as indicated
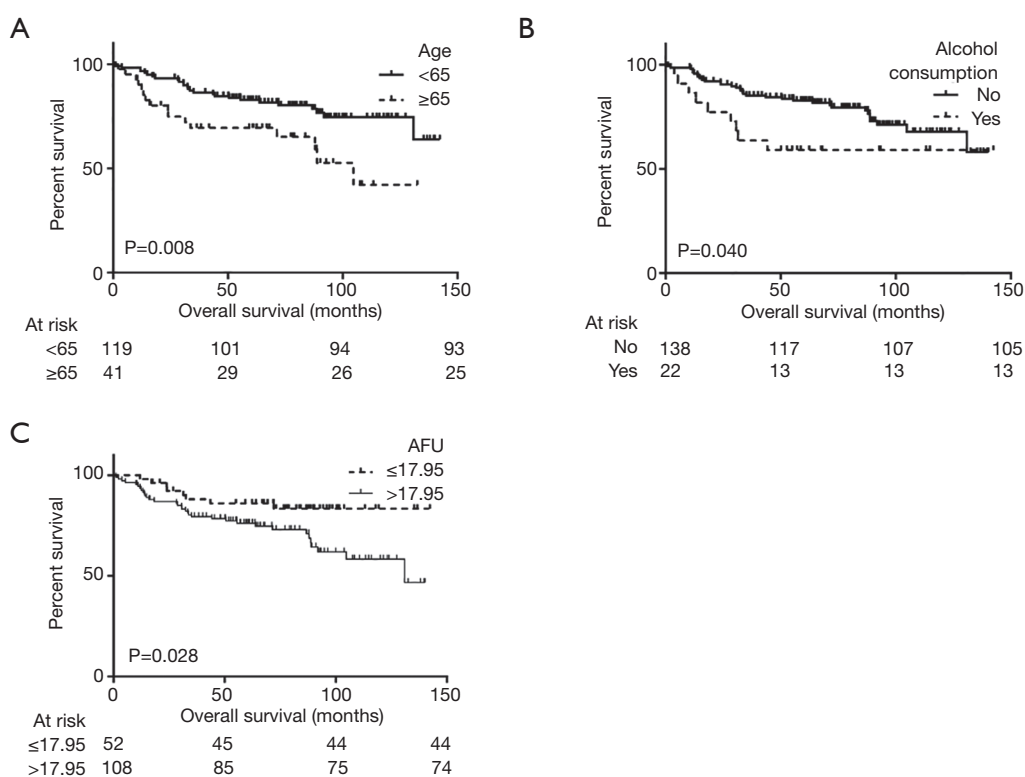
Figure 2 Kaplan-Meier survival curves for patients with pathological stage T1N0 ESCC according to (A) age at diagnosis, (B) alcohol consumption, (C) the level of serum AFU. ESCC, esophageal squamous cell carcinoma.

by numerous studies (21-25). In other words, few of these enzymes were released by active inflammatory cells or tumor cells in early stage carcinoma; hence, their levels were not sufficient to distinguish prognostic differences in the population with a lower tumor load (15,22). Second, alcohol intake was positively related to the serum content of these liver function indexes, especially the ALT/AST ratio and GGT, which was also validated in our study (P=0.048 and 0.031, respectively; compared by independent t test) (22,25). However, current or former alcohol consumption was an uncommon phenomenon in our cohort (22/160, 13.8%); therefore, the serum contents of these liver function indexes remained low, and no significant individual differences were found (26). Third, our research was carried out at a single center with a small number of patients from a retrospective database, this likely leading to selection bias, which was also one of the limitations of this study.

To date, little data was available regarding the correlation between serum AFU level and liver dysfunction. Reported by Cheng *et al.* in a large-scale study in HCC following hepatectomy, the positive correlation between

the level of AFU and AFP was found (9). Recently, in a similar prognosis study, Li and his coworkers tried to explore the correlation between the level of AFU and transaminase (including ALT and AST) in intrahepatic cholangiocarcinoma, but no significant correlation was detected. In this study, we first found that a high AFU level was associated with a high level of ALT and GGT in ESCC, and the possible explanations for the relationships remained unclear. However, after multivariable regression analysis, only the AFU was proved to be independent prognostic index with OS. This was exactly the argument that supports our conclusion: serum AFU showed a better prognostic value than other liver function index for long-team survival in patients with early stage ESCC.

This is the first study to explore the association between AFU levels and long-term survival in patients with ESCC, and the prognostic value of serum AFU content in early stage malignant tumors was also demonstrated and reported (9-15). However, several limitations of this study should be noted when interpreting the results. First, the study spanned a long period of time (almost 10 years),

and much progress in testing, surgical procedures, and perioperative management occurred during that time (9,11,17,19,20). Second, the results might be unreliable given current attitudes. In addition, chronic hepatitis viral infection and alcoholic hepatitis are common phenomena in China, likely leading to abnormal non-neoplasm-related liver function (27,28). Another obvious limitation of this study was our selection of the best cutoff value, because a different definition of high AFU content might lead to a different result, which might weaken the clinical application of this novel biomarker (9-15) . Even so, the potential prognostic value of serum AFU content in ESCC has been demonstrated, and future research directions regarding molecular targets for ESCC treatment have also been proposed.

Conclusions

In conclusion, compared with other serum biomarkers, serum AFU levels showed a better prognostic value for long-team survival in patients with early stage ESCC. This enzyme may be a potential therapeutic molecular target for ESCC.

Acknowledgments

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This retrospective study was approved by the board-certified Research Ethics Committee at our center (No. B2014-110) and written informed consent was obtained from all patients.

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Table S1 World Health Organization (WHO) level of drinking risk (in 2014)

WHO risk level	Grams (g) of pure alcohol per day	
	Female	Male
Abstinence	0	0
Low risk	1–20	1–40
Medium risk	21–40	41–60
High risk	41–60	61–100
Very high risk	61+	101+