RESEARCH ARTICLE

Model Based on Alkaline Phosphatase and Gamma-Glutamyltransferase for Gallbladder Cancer Prognosis

Xin-Sen Xu, Run-Chen Miao, Ling-Qiang Zhang, Rui-Tao Wang, Kai Qu, Qing Pang, Chang Liu*

Abstract

Purpose: To evaluate the prognostic value of alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) in gallbladder cancer (GBC). Materials and Methods: Serum ALP and GGT levels and clinicopathological parameters were retrospectively evaluated in 199 GBC patients. Receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off values of ALP and GGT. Then, associations with overall survival were assessed by multivariate analysis. Based on the significant factors, a prognostic score model was established. Results: By ROC curve analysis, ALP ≥ 210 U/L and GGT ≥ 43 U/L were considered elevated. Overall survival for patients with elevated ALP and GGT was significantly worse than for patients within the normal range. Multivariate analysis showed that the elevated ALP, GGT and tumor stage were independent prognostic factors. Giving each positive factor a score of 1, we established a preoperative prognostic score model. Varied outcomes would be significantly distinguished by the different score groups. By further ROC curve analysis, the simple score showed great superiority compared with the widely used TNM staging, each of the ALP or GGT alone, or traditional tumor markers such as CEA, AFP, CA125 and CA199. Conclusions: Elevated ALP and GGT levels were risk predictors in GBC patients. Our prognostic model provides infomration on varied outcomes of patients from different score groups.

Keywords: gamma-glutamyltransferase - alkaline phosphatase - gallbladder cancer - prognosis

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Introduction

Gallbladder cancer (GBC), the most common malignant neoplasm of the biliary tract, is usually associated with gallstone disease, late diagnosis, unsatisfactory treatment, and poor prognosis (Siegel et al., 2015). As none specific chemotherapy or radiotherapy for the disease has emerged with satisfying effects, resection of gallbladder remains the priority treatment for patients with resectable tumors (Dutta, 2012). Currently, the prognosis of GBC is very poor, with a 32% five-year survival rate for lesions confined to the gallbladder mucosa and a 10% one-year survival rate for more advanced stages (Lazcano-Ponce et al., 2001). Thus, identifying patients with higher risks of poor prognosis is useful for guiding us to choose the best treatment.

Many trials have explored the prognostic markers of GBC in patients undergoing gallbladder resection, such as tissue gene expression and somatic mutation (Li et al., 2014; Shu et al., 2015). However, these markers are either far from satisfactory regarding sensitivity and specificity, or are only confined to laboratory research, which is not feasible clinically.

Alkaline phosphatase (ALP) is a hydrolase enzyme that removes phosphate groups from many types of molecules. Gamma-glutamyltransferase (GGT) is an enzyme responsible for transferring gamma-glutamyl functional groups. They are particularly concentrated in liver and bile duct, and are routinely tested in patients with hepatobiliary diseases to evaluate liver function (Pratt and Kaplan, 2000). On the other hand, ALP and GGT have long been supposed to play potential roles in the diagnosis of malignant tumor. Several studies have confirmed the dignostic roles of ALP and GGT in liver cancer (Lopez et al., 1996; Hann et al., 2012). In addition, we also demonstrated that higher levels of ALP and GGT might predict poor prognosis of liver cancer, indicating the prognostic roles of ALP and GGT in predicting cancer survival (Xu et al., 2014).

However, in GBC, the values of ALP and GGT have not been explored yet. In the current study, we sought to evaluate the effects of ALP and GGT on the long-term prognosis of GBC patients underwent gallbladder resection. Furthermore, we tried to combine these serum markers to construct a new scoring model, to differentiate varied outcomes of GBC patients more accurately.

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Materials and Methods

Patient selection

In this study, eligible patients were identified using the clinical database in our unit (First Affiliated Hospital, Xi'an Jiaotong University, Xi'an, China) and the study period was from June 2008 to November 2013. There were 199 patients identified as suitable. All patients underwent gallbladder resection and had biopsy proven gallbaldder cancer. The selection criteria included: *(i)* patients were diagnosed with only biopsy proven GBC, with no concomitant other malignancies; *(ii)* patients had serum ALP and GGT measured at study entry; and *(iii)* patients had a minimum follow-up time of 1 year.

Data collection

Data collected included patient demographics, presence of complications, gallstone history, tumor characteristics, preoperative serum markers such as ALP, GGT, CEA, AFP, CA125 and CA199 levels. Surgery procedure records and tumor pathology were also recorded. The American Joint Committee on cancer (AJCC) staging system was used for pathological and/or clinical staging. Approval for the study was obtained from the Institutional Review Board, which conformed to the standards of the Declaration of Helsinki.

Surgery and follow-up

The surgical management of GBC included an en bloc cholecystectomy and partial hepatectomy, supraduodenal portal lymphadenectomy, and reconstruction of the bile duct, if necessary. Patients were followed every 6 months. Physical examination with cross-sectional imaging studies (computed tomography or magnetic resonance imaging) was obtained for each patient on every follow-up visit.

Statistical analysis

Chi-square and t tests were used for categorical and continuous variables, respectively, with a $P<0.05$ considered significant. Serum markers were compared using the Wilcoxon rank test. Survival was analyzed by the Kaplan-Meier Curves and compared by the log-rank test, stratified by ALP and GGT, with the cutoff value determined by the receiver operating characteristic (ROC) curve. A forward stepwise multiple logistic regression model was developed using preoperative variables with a significant univariate P value. All data were analyzed by the SPSS version 19.0 software (SPSS, Chicago, IL, United States).

Results

ROC curves showed the cut-off value for elevated ALP and GGT

Between 2008 and 2013, 199 patients with GBC were enrolled in our study. The mean age at diagnosis was 64 years old. There was a female preponderance with 138 women and 61 men. There were 186 patients underwent radical cholecystectomy, and 13 patients underwent palliative resection.

In order to determine the optimal of ALP and GGT to differentiate prognosis, we performed the ROC curve analysis. It revealed an optimal cutoff of 211 U/L for ALP and 43 U/L for GGT (Figure 1). As for ALP, the area under the ROC curve (AUC) was 0.706, with a 95%CI of 0.626-0.785, while for GGT, the AUC was 0.679, with a 95%CI of 0.589-0.768. On the condition of 211 U/L for ALP, the sensitivity and specificity were 0.527 and 0.830, respectiely. While on the condition of 43 U/L for GGT, the sensitivity and specificity were 0.753 and 0.623, respectiely. For the purpose of convenience, we chose the cutoff value of 210 U/L for ALP and 43 U/L for GGT, with no impairment of accuracy.

Parameters associated with overall survival

Based on the cut-off value for elevated ALP and GGT, we performed the univariate analysis and multivariate analysis to explore the potential parameters associated with the overall survial (Table 1). According to the univariate analysis, tumor differentiation (HR, 1.782, 95%CI, 1.281-2.479), tumor stage (HR, 4.950, 95%CI, 2.175-11.263), ALP (HR, 2.039, 95%CI, 1.471-2.828), GGT (HR, 2.381, 95%CI, 1.621-3.496) and CA125

Table 1. Predictive Variables for Overall Survival of Gallbladder Cancer Patients by Multivariate Analysis Using Forward Stepwise Multiple Logistic Regression Model

Clinical Variables	Univariate analysis HR (95% CI)	*P*	Multivariate analysis HR (95% CI)	*P*
Age (≥60 years versus <60 years)	1.047 (0.735-1.491)	0.799		
Sex (female versus male)	1.026 (0.719-1.465)	0.886		
Gallstone (yes versus no)	1.387 (0.997-1.929)	0.052		
Complication1 (yes versus no)	0.885 (0.633-1.237)	0.474		
Tumor size (≥5 cm versus <5 cm)	1.136 (0.792-1.630)	0.488		
Tumor number (multiple versus single)	2.074 (0.845-5.090)	0.111		
Tumor differentiation (>II versus I-II)	1.782 (1.281-2.479)	0.001	1.364 (0.975-1.908)	0.071
Tumor stage (III/IV versus I/II)	4.950 (2.175-11.263)	<0.001	4.372 (1.914-9.986)	<0.001
Serum markers				
ALP (≥210 U/L versus <210 U/L)	2.039 (1.471-2.828)	<0.001	1.559 (1.046-2.324)	0.029
GGT (≥43 U/L versus <43 U/L)	2.381 (1.621-3.496)	<0.001	1.609 (1.005-2.575)	0.048
CEA (≥5 mg/L versus <5 mg/L)	0.871 (0.384-1.972)	0.74		
AFP (≥10 U/L versus <10 U/L)	1.048 (0.726-1.512)	0.804		
CA125 (≥35 U/mL versus <35 U/mL)	1.463 (1.033-2.074)	0.032	1.222 (0.853-1.751)	0.286
CA199 (≥37 U/mL versus <37 U/mL)	1.364 (0.986-1.889)	0.061		

[1]complication includes diabetes, hypertension, liver cyst or renal cyst

(HR, 1.463, 95%CI, 1.033-2.074) were identified as the risk factors of prognosis. Putting these variables with a significant P value into a forward stepwise multiple logistic regression model, only tumor stage (HR, 4.372, 95%CI, 1.914-9.986), ALP (HR, 1.559, 95%CI, 1.046-2.324) and GGT (HR, 1.609, 95%CI, 1.005-2.575) were identified as indendpent prognostic factors.

Construction of the preoperative prognostic scoring model

Inspired by the preoperative prognostic score published by Wang et al. in liver cancer patients who underwent liver transplantation, we tried to establish a scoring model using the three preoperative factors, namely, ALP, GGT, and tumor stage, which were found to be independently significant by multivariate regression analysis (Wang et al., 2011).

With respect to each factor, we devided the patients into two different groups and performed the Kaplan-Meier curve and log-rank test (Table 2). Between the high ALP and low ALP group, the patients with higher ALP level also got a higher GGT level and higher tumor markers such as CA125 and CA 199. Similarly, between the high GGT and low GGT group, the patients with higher GGT level also got a higher ALP level and higher tumor markers such as CA125 and CA 199. It revealed some connections between ALP and GGT in determining the prognosis of GBC patients. When deviding the patients by the tumor stage, only tumor differentiation was shown to be significant different.

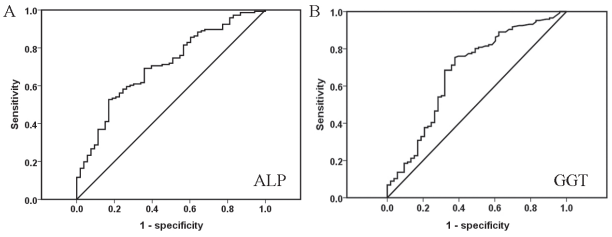

Figure 1. Receiver Operating Characteristic Curves to Discriminate 199 Patients with Different Prognosis by the Appropriate Cutoff Values of Alkaline Phosphatase (A) and Gamma-Glutamyltransferase (B). ALP: Alkaline phosphatase; GGT: gamma-glutamyltransferase

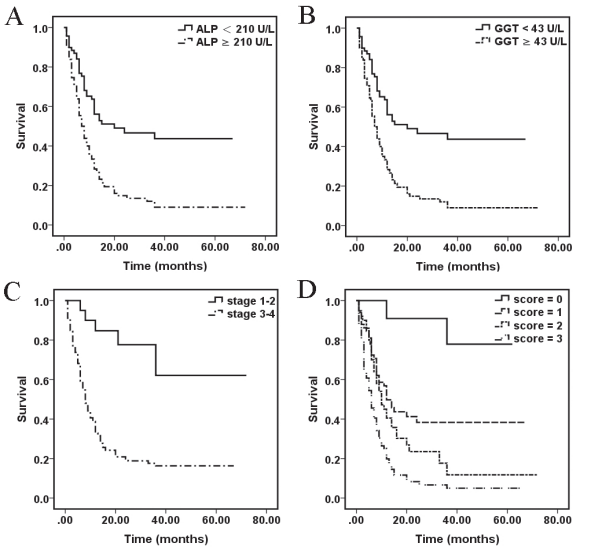

Figure 2. Impact of Alkaline Phosphatase (A), Gamma-Glutamyltransferase (B) and Tumor Stage (C) on the Overall Following Surgical Resection, as Classified by the Cutoff Value of Alkaline Phosphatase, Gamma-Glutamyltransferase and Tumor Stage, Respectively. (D) Varied outcomes of gallbladder cancer patients as classified by different prognostic scores

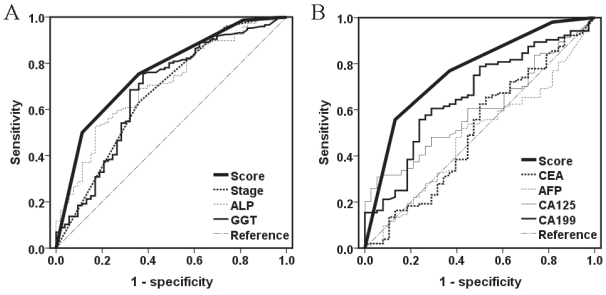

Figure 3. Comparison of Different Receiver Operating Characteristic Curves (ROC) Based on Specific Prognostic Tumor Markers. (A) Comparison of area under the ROC curve between the score model and tumor stage, alkaline phosphatase and gamma-glutamyltransferase. **(B)** Comparison of area under the ROC curve between the score model and traditional tumor markers such as CEA, AFP, CA125 and CA199

Table 2. Comparison of Baseline Characteristics of Patients with Different Alkaline Phosphatase, Gamma-Glutamyltransferase and Tumor Stage

Factors	ALP ≥210 (n=86)	ALP < 210 (n=113)	*P*	GGT ≥43 (n=130)	GGT <43 (n=69)	*P*	Stage (I-II) (n=20)	Stage (III-IV) (n=179)	*P*
Age (mean±sd)	63.9±11.3	63.4±11.0	0.753	63.5±11.0	63.8±11.3	0.817	66.6±12.1	63.3±11.0	0.599
Sex (male/female)	62/24	76/37	0.535	88/42	50/19	0.522	7/13/15	125/54	0.62
Complication	28	54	0.042	52	30	0.653	6	76	0.343
Gallstone	51	56	0.197	76	31	0.075	9	98	0.481
Tumor size (mean±sd)	3.62±0.34	3.81±2.14	0.549	3.77±2.26	3.65±2.12	0.721	4.09±2.55	3.70±2.17	0.452
Tumor number (mean±sd)	1.07±0.34	1.01±0.09	0.061	1.05±0.27	1.03±0.12	0.363	1.00±0	1.04±0.25	0.478
Differentiation (poor/high)	47/39	51/62	0.2	70/60	28/41	0.101	1/19/15	97/82	<0.001
Stage (I/II/III/IV)	1/6/24/55	2/11/44/56	0.25	1/7/39/83	2/10/29/28	0.007	3/17/0/0	0/0/68/111	<0.001
Serum markers									
ALP (U/L) (median)	610	102	<0.001	345	85.3	<0.001	136	168	0.218
GGT (U/L) (median)	502	31	<0.001	371	20.2	<0.001	25	156	0.056
CEA (mg/L) (median)	4.06	3.52	0.331	3.82	3.57	0.823	2.12	3.91	0.325
AFP (U/L) (median)	2.82	2.99	0.612	2.83	3.02	0.621	2.41	2.88	0.494
CA125 (U/ml) (median)	34	24.9	0.002	32.8	24.2	0.014	18.1	30.7	0.101
CA199 (U/ml) (median)	332	35.6	<0.001	236	27.1	0.002	35.4	132	0.286

According to the Kaplan-Meier curves, for both ALP and GGT, a significant difference was observed in the overall survival between patients with normal and elevated levels (P<0.05) (Figure 2A-B). Unexceptionally, patients in the advanced tumor stage also got a poorer prognosis than patients in the early stage (P<0.05) (Figure 2C). To give each of the positive factors a score of 1, we built a scoring model consisting of 4 different risk groups, namely, the score of 0, 1, 2 and 3. Varied outcomes in overall survival stratified by different scores were shown in Figure 2D. Based on this scoring model, we could easily judge the different risks of overall survival in GBC patients.

Predictive value of the prognostic scoring model

The discriminatory performance of the scoring model, as measured by area under the ROC curve, resulted in AUC of 0.768 (95%CI, 0.680-0.855), which was superior to each of the factors alone when predicting the overall survival (Figure 3A). Meanwhile, we also explored the values of traditional tumor markers in differentiating the varied outcomes of GBC patients, such as the CEA (AUC, 0.510, 95%CI, 0.398-0.623), AFP (AUC, 0.484, 95%CI, 0.379-0.590), CA125 (AUC, 0.600, 95%CI, 0.503-0.696) and CA199 (AUC, 0.662, 95%CI, 0.562-0.761). Compared with the traditional tumor markers in predicting the prognosis, the scoring model also showed a superiority (Figure 3B).

Discussion

ALP and GGT have long been supposed to play potential roles in the diagnosis of malignant tumor. Previously, we also demonstrated the prognostic values of ALP and GGT in liver cancer. While in this study, we revealed for the first time the potential values of ALP and GGT in predicting the overall survival in gallbladder cancer. In addition, we also successfully established a scoring model based on ALP and GGT levels, which was superior than most previous prognostic markers in gallbladder cancer.

Clinically, serum ALP and GGT levels are usually tested to evaluate liver function. ALP and GGT are indicative of liver disease, hepatitis, biliary obstruction, and so on. Interestingly, emerging evidence indicates that increasing levels of ALP and GGT may be linked to high cancer risk. Du et al. (2014) indicated the serum bone-specific ALP as a biomarker in the diagnosis of osseous metastases in cancer patients. It was suggested that ALP indicated proliferation in nucleolar localization in an electron microscopic cytochemistry study (Yamamoto et al., 2003). Cancer cells showed higher ALP activity in the nucleolus and changes in localization during the cell cycle. Kunutsor et al. also summarised a positive log-linear association of GGT levels with overall cancer risk, with a hazard ratio of 1.32 (Kunutsor et al., 2015). It assumed that GGT played a major role in glutathione metabolism, the major thiol antioxidant in the body, and was thus involved in cellular defence and protection of cells against further oxidative stress. However, the specific pathways still remain unclear. On the other hand, with respect to hepatobiliary tumors specifically, the probable biliary obstruction might also partially account for the increased levels of ALP and GGT in the diagnosis of malignant tumors.

Considering the prognostic roles of ALP and GGT, a previous national health survey indicated that elevated GGT was associated with mortality from all causes, liver disease, cancer, and diabetes, while ALT was associated with only liver disease mortality, suggesting the prognostic values of ALP and GGT in predicting overall survival (Ruhl and Everhart, 2009). With respect to cancer, ALT was recently supposed to be a poor survival indicator in prostate cancer, nasopharyngeal cancer and colorectal cancer (Saif et al., 2005; Sonpavde et al., 2012; Xie et al., 2014). In addition, ALP has also been included in the Chinese University Prognostic Index, an liver cancer staging system that assigns a score of 3 when ALP is >200 IU/L (Leung et al., 2002). On the other hand, GGT was also recognized as a novel adverse prognostic marker in renal cancer, colorectal cancer, esophageal cancer, liver cancer and cholangiocarcinoma (He et al., 2013; Yin et al., 2013; Hofbauer et al., 2014; Ma et al., 2014; Yang et al., 2014). These previous studies strongly indicated the valuable prognostic roles of ALP and GGT in malignant tumors.

In this study, we systematically explored the cut-off value of ALP and GGT by using ROC curve analysis in predicting prognosis in GBC patients. We found that the cut-off value of ALP and GGT was 210 U/L and 43 U/L, respectively. The ALP level was a littler higher than the average value previously reported (120 U/L), while the GGT level was similar (Yin et al., 2013). We speculate that the probable biliary obstruction in gallbladder cancer might partially contribute to this bias, however, the specific mechanisms need further exploration.

By multivariate analysis, it turned out that ALP, GGT and tumor stage were independent prognostic predictors of overall survival. Kaplan-Meier curves demonstrated poor prognosis in patients with higher levels of ALP and GGT, and with advanced tumor stage. Based on the outcomes of multivariate analysis, we further established a simple prognostic model consisting of ALP, GGT and tumor stage, which could differentiate the varied outcomes of patients clearly. By ROC curve analysis, the simple score showed great superiority compared with the widely used TNM staging, or compared with each of the ALP or GGT alone. Further analysis showed that, even in the presence of traditional tumor markers such as CEA, AFP, CA125 and CA199, the scoring model still manifested great superiority. The high mortality and poor prognosis of gallbladder cancer are in great need of prognostic models to predict the overall survival of these patients (Wang et al., 2008). Here we presented the simple scoring model consisting of ALP, GGT and tumor stage, which was superior than most previous prognostic markers in gallbladder cancer.

However, it is worth noting that there were some conditions the elevated ALP and GGT levels were not due to the tumor biology. For instance, during pregnancy, hyperparathyroidism, Paget's disease, sarcoidosis and some bone conditions, the ALP level might also be

upregulated. Slightly elevated serum GGT was also found to correlate cardiovascular diseases, metabolic syndrome, alcohol addiction and chronic liver disease. Thus, we should not be solely dependent on these markers in the prediction of GBC survival. Further studies are still needed to confirm and update our preoperative scoring model to predict the prognosis of gallbladder cancer.

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