Postoperative Transarterial Chemoembolization Benefits Patients with High γ -Glutamyl Transferase Levels after Curative Hepatectomy for Hepatocellular Carcinoma: A Survival Stratification Analysis

Hansheng Liu,^{1*} Aiqun Zhang,^{1*} Niansong Qian,^{1*} Lijie Gao,¹ Lining Xu,² Wenzhi Zhang,¹ Kai Jiang,¹ Shouwang Cai,¹ Zhiqiang Huang¹ and Jiahong Dong¹

¹The Hospital & Institute of Hepatobiliary Surgery, Chinese PLA General Hospital, Beijing, P.R. China

²The Department of General Surgery and Intensive Care Unit in Clinical Division of South Building, Chinese PLA General Hospital, Beijing, P.R. China

Hepatocellular carcinoma (HCC) is the most common liver tumor in Asian countries, and hepatectomy is currently regarded as the optimal curative treatment for HCC; however, the postoperative outcome remains unsatisfactory. Aiming at further clarification of prognostic factors after hepatectomy, we adopted a detailed stratification on survival periods. A total of 428 HCC patients undergoing curative hepatectomy were firstly divided into two groups using 2-year survival as cutoff point. Multivariate analysis showed that tumor-related factors, including vascular invasion ($P < 0.001$), high Edmondson grade ($P < 0.001$), large tumor size ($P < 0.001$) and high serum alpha-fetoprotein level ($P = 0.001$), were significant determinants for early death within 2 years, while postoperative transarterial chemoembolization (TACE) was demonstrated a protective factor ($P = 0.013$). Then the 281 patients with survival > 2 years were divided into two subgroups according to survival or death during follow-up to examine the late death related factors. We found that high serum γ -glutamyl transpeptidase (GGT), indicating severity of underlying liver disease, was significantly linked to death in this stage ($P = 0.006$). In further comparison of survival rates between subgroups stratified by early- and late-death indicators, we found the long-term outcomes of patients with high serum GGT were poor, regardless of the factors related with primary tumor. Furthermore, postoperative TACE decreased late death rate of patients with high GGT levels. In conclusion, despite the overwhelmed effects of primary tumor in the early stage after hepatectomy, postoperative TACE is beneficial for HCC patients with poor liver status.

Keywords: γ -glutamyl transpeptidase; hepatectomy; hepatocellular carcinoma; survival period; transarterial chemoembolization

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Hepatocellular carcinoma (HCC) is the most common liver tumor in Asian countries. Its prognosis remained unfavorable in spite of remarkable progress in diagnostic methods and treatment modalities. Among the various primary treatments for HCC, hepatic resection offers the best effectiveness, however the postoperative outcome remains unsatisfactory due to high incidence of recurrence. According to recent reports, the 5-year survival rate is about 50% (Makuuchi and Sano 2004; Bruix and Sherman 2005; Koniaris et al. 2011).

Many studies have been conducted in the past decades to reveal postoperative prognosis factors for HCC patients.

The patients' gender and age, the accompany disease, pathological status of the liver, original tumor, and treatment-related factors are related to postoperative survival in HCC patients (Qin and Tang 2002; Chok et al. 2009; Ting et al. 2012). However, controversy existed for which of these factors are the most important on different stage of the disease after hepatic resection. Recently, there have been a few studies in seeking of survival predictors for early death (death within 1 or 2 years in different reports) (Regimbeau et al. 2004; Kondo et al. 2005; Kaibori et al. 2008; Nanashima et al. 2010; Moriguchi et al. 2012). The major findings were original tumor related factors (ie. vascular

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*These authors contributed equally to this work.

Correspondence: Jiahong Dong, The Hospital & Institute of Hepatobiliary Surgery, Chinese PLA General Hospital, Fuxing Road 28, Beijing 100853, P.R. China.
e-mail: dongjh301@163.com

invasion, tumor size, higher mitoses, etc.) were associated with increased risk of early death due to recurrence. Kaibori et al. (2008) found that the hepatic functional reserve in the early postoperative period significantly influenced early recurrence and early death after resection of large HCC. There were also some reports clarifying early detection of recurrence site and suppression of co-existing hepatitis necessary for long postoperative survival (Takata et al. 2000; Kim et al. 2011).

The post-operational survival periods after early death point (1 or 2 years) might be a stage involving liver-related incidents such as intrahepatic or extrahepatic metastasis originated from primary tumor, multicentric carcinogenesis in the residual liver, fibrosis and hepatitis of the diseased liver, and also the following therapeutic strategies. Having survived through early postoperative stage, the influence from primary tumor might no longer be crucial, while the other factors mentioned above might prove more important. However, the predominant survival predictors for this important period were still unclear. Nowadays, 5-year survival with or without tumor recurrence has become the aim for the treatment of HCC (Koniaris et al. 2011; Fuks et al. 2012), rendering it is extremely critical to elucidate the cause of death during this late postoperative stage, which would further guide early intervention. The current study focused on further clarification of prognostic factors after hepatectomy. Having adopted a more detailed stratification on survival periods, we revealed some useful information.

Patients and Methods

Patients

Data collection and analysis in this study was approved by the Ethics Committee of PLA General Hospital. From July 1998 to March 2006, a total of 591 patients underwent their first hepatic resections of HCC at the Hospital & Institute of Hepatobiliary Surgery, Chinese PLA General Hospital. Patients with any of the following conditions were not included in this study: died within 30 days after operation (perioperative deaths; 4 patients); with sign of extrahepatic organ or lymph node metastasis before or during operation (46 patients); with residual tumors detected in the remnant liver after hepatectomy (15 patients); with positive macroscopic or microscopic surgical margin (9 patients); with unclassified Edmondson grade for tumor necrosis due to prior treatment of HCC (ie. preoperative transarterial chemoembolization (TACE), microwave ablation, radiofrequency ablation, etc.; 34 patients). Of the remaining 483 patients, 32 patients were lost to our follow-up; 7 patients were confirmed died in non-liver-related disease (ie. coronary heart disease, stroke, non-liver-tumor, accident, etc.) and 16 patients died in unknown causes. These patients were also excluded. The remaining 428 patients with curative hepatic resection were the subjects of this study.

Study design

All the surviving patients were followed up for at least 2 years after operation in order to clarify predictor for death beyond 2 years after operation (defined as late death in this study). Patients were firstly classified into two groups using 2 years as a cutoff point. One

group were those patients who die within 2 years after hepatic resection ($n = 147$), and the other were those with survival > 2 years after operation ($n = 281$). Twenty-eight clinicopathological factors were compared to clarify the risk factors for early death after hepatic resection. These selected clinicopathological data included the basic information of patients, parameters related to underlying liver disease, tumor-related factors as well as the treatment-related factors, which were listed in Table 1. Then the patients with survival > 2 years were further divided into two subgroups according to their survival during follow up (died, $n = 130$; survived, $n = 151$) to find out late-death associated predictors. Furthermore, survival rates were compared between subgroups stratified by early- and late-death predictors (Fig.

Table 1. The characteristics of HCC patients ($N = 428$).

Variables	Number
Days in hospital	13.77 ± 7.06
Age (years)	50.79 ± 10.61
Sex ratio (male/female)	374/54
Symptoms (with/without)	259/169
Serum creatinine level (umol/L)	81.64 ± 16.66
Underlying liver status factors	
Etiology (+/-)*	358/70
Child-Pugh class (A/B and C)	364/64
Liver cirrhosis (with/without)	365/63
ALT (U/L)	71.29 ± 135.64
AST (U/L)	63.86 ± 104.57
GGT (U/L)	106.80 ± 126.21
ALP (U/L)	103.58 ± 63.23
Albumin (g/L)	39.94 ± 5.17
Total bilirubin (umol/L)	26.57 ± 45.29
Platelet ($\times 10^9/L$)	140.47 ± 53.80
INR	1.24 ± 0.18
Tumor-related factors	
Maximum diameter (cm)	6.27 ± 3.69
Number of tumors (solitary/multiple)	351/77
Tumor distribution (unilobar/bilobar)	385/43
Serum AFP ($\leq 400/> 400$ ng/ml)	260/168
Edmondson grade (I-II/III-IV)	323/105
Vascular invasion ($n, \%$)	39, 9.1%
Bile duct invasion ($n, \%$)	18, 4.2%
Treatment-related factors	
Inflow occlusion (yes/no)	354/74
Hepatectomy (anatomy/nonanatomy)	144/284
Duration of operation (min)	197.91 ± 69.66
Blood transfusion (ml)	318.61 ± 484.23
Postoperative TACE (yes/no)	178/250

Edmondson grade I-II corresponding to well or moderately differentiated tumors; Edmondson grade III-IV corresponding to poorly differentiated tumors. Anatomy hepatectomy, the resection of a portion of liver parenchyma based on the distribution of the portal pedicles and the location of the hepatic veins; nonanatomy hepatectomy, was that performed not limited by those anatomical scissuras.

*HBs-Ag and/or HCV-Ab.

Fig. 1. The scheme of study design.

1).

The following factors were assessed by postoperative pathological examination of resected specimens: liver cirrhosis, tumor differentiation, tumor size, vascular invasion, bile duct invasion and the number of tumor nodules. Tumor size was measured as the longest diameter of the tumor. If multiple lesions were visible, the largest was measured. The histological grade of tumor differentiation was assigned, based on the Edmondson and Steiner's classification system. Vascular invasion was confirmed by the presence of macroscopic portal vein tumor thrombosis (ie. invasion of second-order or more proximal branches of the portal vein) or hepatic vein tumor thrombosis. Bile duct invasion was verified by postoperative pathological examination as presence of macroscopic bile duct wall tumor infiltration or bile duct tumor thrombi. In our study, tumors with more than 2 nodules (including satellite nodules surrounding a larger main tumor) were defined as multiple tumors. Days in hospital were the length of hospital stay after operation. All serum test results were collected prior to operations.

The postoperative adjuvant TACE was performed on the HCC patients according to their personal intention, but not recommended for those with notorious hepatic function after hepatic resection. The first TACE was performed 4-12 weeks after hepatic resection. In principle, the hepatic arterial catheter was placed via the femoral artery using the Seldinger technique. Through this catheter, a micro-catheter was introduced and the tip of which was advanced to the feeding arteries of target foci under fluoroscopy guidance. Then emulsion of the anticancer agent and lipiodol was injected, followed by selective embolization of the feeding artery using gelatin sponge particles (Peng et al. 2009). The course was repeated for 2 to 5 cycles according to the status of the patients.

Follow-up

All patients were followed up in the outpatient clinic after operation with regular surveillance for recurrence by abdominal ultrasonography every 2 to 6 months. The end-point of follow up was the time of death. Every surviving patient was followed up until April 2011 or later. Recurrences were diagnosed using ultrasonography, computed tomography or hepatic arteriography. In this study, for the patients who died of intrahepatic recurrence or extrahepatic metastasis, the cause for death was defined as cancer recurrence. For the patients who died of ascites, gastrointestinal hemorrhage, acute hepatitis, or coma but without tumor recurrence, the cause for death was

defined as liver failure. Survival time was defined as the period between the date of initial hepatic resection and the date of death for the dead patients, or between the date of initial hepatic resection and the date of last follow-up for the surviving patients. The data were censored at the last follow-up for surviving patients.

Statistical analysis

All statistical analysis were performed using SPSS statistical software (version 17.0). Continuous data were expressed as mean $\pm$ s.d. and were compared between groups using *t* test or the Mann-Whitney *U* test. Categorical variables were compared using the chi-squared test (or Fisher's exact test where appropriate). Logistic regression analysis was used for multivariate analysis of risk factors for different survival times and to estimate hazard ratios (HR). Cumulative survival was calculated according to the Kaplan-Meier method and checked for significance with the log rank tests. *P* value of < 0.050 was considered statistical significant.

Result

Patient characteristic

Clinical and pathologic features of patients are listed in Table 1. The characteristics of patients with or without postoperative TACE were also compared via univariate analysis, and no significant difference was found. In this study cohort, 338 patients had hepatitis B infection, 13 patients had hepatitis C, and 7 patients had both. The median follow-up was 51 months (range 1.5-151). The postoperative 2-, 5-, 8- and 10-year survival rates were 65.7%, 46.3%, 33.2% and 26.7%, respectively. Upon the time of the last follow-up, 277 patients had died; the mean postoperative survival time was 31.9 ± 26.8 (range 2-119) months. There were 251 males and 26 females, with mean age of 50.9 ± 10.4 (range 10-89) years old. Fig. 2 shows the distribution of survival time of those 277 patients after hepatectomy, which suggesting the first 24 months the peak period of death (147 deaths, accounting for 53.07% of total). Based on this time cutoff point, patients died within 2 years postoperatively were defined as early death.

Predictors for postoperative early death

To identify predictors for early death after hepatic resection of HCC, all selected clinicopathologic factors were included for univariate analysis. The early death predictors were identified as follows: male; days in hospital; hepatitis B and/or C infection (as underlying liver disease-related factors); high serum alpha-fetoprotein (AFP) level, bilobar involvement of tumor, histological vascular invasion, large tumor size and high Edmondson grade (as tumor-related factors); postoperative TACE (as treatment-related factors) (Table 2). These selected indicators were then enrolled for logistic regression analysis. Vascular invasion ($P < 0.001$), large tumor size ($P < 0.001$), high serum AFP level ($P = 0.001$) and high Edmondson grade ($P < 0.001$) were demonstrated significant determinants for early death. Also, we found postoperative TACE was negatively associated with early death, which might be a protective factor (HR, 0.558; 95% CI (confidence interval), 0.351-

Fig. 2. Distribution of survival time after hepatectomy among 277 dead patients.

0.886; $P = 0.013$) (Table 3).

Predictors for postoperative late death

By univariate analysis, five underlying liver disease-related factors (high levels of serum γ -glutamyl transpeptidase (GGT), serum alkaline phosphatase (ALP), serum total bilirubin, high international normalized ratio (INR), and liver cirrhosis) and only two tumor-related factors (high Edmondson grade and serum AFP > 400 ng/ml) were associated with late death (Table 4). Logistic regression analysis revealed that high serum GGT level and high Edmondson grade were the independent factors for late death ($P = 0.006$ and 0.010 , respectively; Table 5). In this multivariate analysis model, continuously elevated GGT level was associated with late death. When serum GGT levels were dichotomized at cut-off point of 80 U/L, GGT > 80 U/L was also tightly correlated with survival, with HR more significant (HR, 3.249; 95% CI, 1.946-5.425; $P < 0.001$). Serum GGT level of 80 U/L was chosen as the cut-off point, because the most significant P value can be generated in survival analysis by log rank tests ($P < 0.001$, Table 6).

The patients with survival > 2 years were further subgrouped by the selected late death factors and compared for survival rates. The survival rates of patients with both low serum GGT level and low Edmondson grade were significantly higher than those with high serum GGT level, high Edmondson grade and both high subgroups, after the 2-year mark ($P = 0.001$, 0.010 and < 0.001 , respectively; Fig. 3). The patients with low Edmondson grade but high serum GGT level were also at continued risk of dying postoperatively. These revealed that not only tumor-related factors, the status of diseased liver was as well a key factor that influenced the survival rate in the later years after hepatectomy.

Effect of tumor-related factors and serum GGT level on different survival periods

To further evaluate the prognostic significance of these screened predictors on different stages of disease after hepatectomy, the patients were further subgrouped by combining each tumor-related factor with their serum GGT levels (as parameters related to underlying liver disease), respectively. For each combination, patients were divided into four subgroups for pairwise comparison of survival using the Kaplan-Meier method.

For the patients with vascular invasion, the survival curves stratified by serum GGT level almost overlapped with each other, and no significant difference was recognized in survival rates (Fig. 4A). This phenomenon was also observed in the patients with poorly differentiation (Edmondson grade III-IV) (Fig. 4C). All these patients had poor prognosis (Table 7). These show that serum GGT level has limited effect on the survival of tumor-dominated patients regardless of early or late death, because tumor-related factors might overshadow underlying liver disease-related factors as the primary factors affecting prognosis in these patients.

However, the prognostic value of serum GGT level is significantly elevated when it comes to patients with less advanced HCC (Table 7). In the comparison of the patients with less invasive HCC, such as those with highly differentiated tumor (Edmondson grade I-II), serum GGT > 80 U/L was associated with a decrease in the 2-year survival rate from 78.7% to 63.0%, 5-year survival rate from 63.8% to 37.0% and 8-year from 49.8% to 21.6%, respectively ($P < 0.001$, Fig. 4D). Similar results were also observed in the patients with tumor size ≤ 5 cm ($P = 0.001$, Fig. 4F), without vascular invasion ($P < 0.001$, Fig. 4B) and AFP ≤ 400 ng/ml ($P = 0.005$, Fig. 3H), respectively. Even for the patients with tumor size > 5 cm (Fig. 4E) or AFP > 400 ng/

Table 2. Univariate analysis of factors associated with early death.

Variables	Die within 2 years (n = 147)	Survival > 2 years (n = 281)	P
Days in hospital	14.83 ± 8.06	13.21 ± 6.41	0.036
Age (years)	49.60 ± 10.84	51.41 ± 10.45	0.094
Sex ratio (male/female)	137/10	237/44	0.009
Symptoms (with/without)	97/50	162/119	0.094
Serum creatinine level (umol/L)	80.80 ± 16.55	82.08 ± 16.73	0.449
Underlying liver status factors			
Etiology (+/-)*	132/15	226/55	0.013
Child-Pugh class (A/B and C)	121/26	243/38	0.251
Liver cirrhosis (yes/no)	127/20	238/43	0.638
ALT (U/L)	69.27 ± 72.96	72.35 ± 158.99	0.784
AST (U/L)	69.68 ± 85.90	60.81 ± 113.14	0.366
GGT (U/L)	120.53 ± 133.23	99.61 ± 122.01	0.104
ALP (U/L)	107.50 ± 70.70	101.28 ± 57.78	0.329
Albumin (g/L)	39.74 ± 5.09	40.05 ± 5.21	0.553
Total bilirubin (umol/L)	28.13 ± 51.54	25.75 ± 41.72	0.605
Platelet (× 10 ⁹ /L)	144.42 ± 62.17	138.41 ± 48.85	0.272
INR	1.25 ± 0.22	1.23 ± 0.16	0.239
Tumor-related factors			
Maximum diameter (cm)	7.34 ± 3.55	5.70 ± 3.64	< 0.001
Number of tumors (solitary/multiple)	116/31	235/46	0.228
Tumor distribution (unilobar/bilobar)	123/24	262/19	0.002
Serum AFP (≤ 400/> 400 ng/ml)	67/80	193/88	< 0.001
Edmondson grade (I-II/III-IV)	90/57	233/48	< 0.001
Vascular invasion (yes/no)	28/119	11/270	< 0.001
Bile duct invasion (yes/no)	6/141	12/269	0.926
Treatment-related factors			
Inflow occlusion (yes/no)	122/25	232/49	0.911
Hepatectomy (anatomy/nonanatomy)	56/91	88/193	0.159
Duration of operation (min)	204.97 ± 67.85	194.22 ± 70.43	0.130
Blood transfusion (ml)	343.63 ± 338.85	305.60 ± 544.78	0.442
Postoperative TACE (yes/no)	51/96	127/154	0.036

Die within 2 years, die within 2 years after hepatectomy; Survival > 2 years, survived for more than 2 years after hepatectomy; Edmondson grade I-II corresponding to well or moderately differentiated tumors; Edmondson grade III-IV corresponding to poorly differentiated tumors; Anatomy hepatectomy, the resection of a portion of liver parenchyma based on the distribution of the portal pedicles and the location of the hepatic veins; Nonanatomy hepatectomy, was that performed not limited by those anatomical scissuras.

*HBs-Ag and/or HCV-Ab.

Table 3. Multivariate analysis of factors associated with early death.

Variables	Hazard ratio (95% CI)	P*
Maximum tumor size (cm) [†]	1.115 (1.051-1.182)	< 0.001
Serum AFP (> 400 ng/ml)	2.151 (1.371-3.375)	0.001
Vascular invasion (yes)	5.310 (2.409-11.706)	< 0.001
Edmondson grade (III-IV)	3.244 (1.989-5.293)	< 0.001
Postoperative TACE (yes)	0.558 (0.351-0.886)	0.013

CI, confidence interval; Edmondson grade III-IV corresponding to poorly differentiated tumors.

[†]Were modeled at continuous variables of maximum tumor size; *Logistic regression analysis.

Table 4. Univariate analysis of factors associated with late death.

Variables	Die beyond 2 years (<i>n</i> = 130)	Sur. beyond 2 years (<i>n</i> = 151)	<i>P</i>
Day in hospital	14.00 ± 6.93	12.54 ± 5.88	0.059
Age (years)	52.48 ± 9.76	50.48 ± 10.96	0.111
Sex (male/female)	114/16	123/28	0.152
Symptom (with/without)	77/53	85/66	0.619
Serum creatinine level	81.47 ± 15.57	82.61 ± 17.70	0.567
Underlying liver status factors			
Etiology (+/-)*	109/21	117/34	0.180
Child-Pugh class (A/B and C)	110/20	133/18	0.397
Liver cirrhosis (yes/no)	117/13	121/30	0.022
ALT (U/L)	88.81 ± 184.40	58.18 ± 132.32	0.116
AST (U/L)	72.71 ± 125.12	50.57 ± 101.01	0.102
GGT (U/L)	121.92 ± 137.76	80.40 ± 103.26	0.005
ALP (U/L)	111.36 ± 65.42	93.08 ± 51.56	0.011
Albumin (g/L)	39.57 ± 4.63	40.46 ± 5.65	0.144
Total bilirubin (umol/L)	31.56 ± 55.34	20.74 ± 23.68	0.040
Platelet (× 10 ⁹ /L)	132.83 ± 47.19	143.21 ± 49.89	0.076
INR	1.25 ± 0.16	1.20 ± 0.16	0.022
Tumor-related factors			
Maximum diameter (cm)	6.02 ± 3.65	5.43 ± 3.62	0.167
Number of tumors (solitary/multiple)	107/23	128/23	0.578
Tumor distribution (unilobar/bilobar)	122/8	140/11	0.707
Serum AFP (≤ 400/> 400 ng/ml)	80/50	113/38	0.017
Edmondson grade (I-II/III-IV)	100/30	133/18	0.013
Vascular invasion (yes/no)	7/123	4/147	0.238
Bile duct invasion (yes/no)	8/122	4/147	0.147
Treatment-related factors			
Inflow occlusion (yes/no)	108/22	124/27	0.833
Hepatectomy (anatomy/nonanatomy)	43/87	45/106	0.555
Duration of operation (min)	202.19 ± 63.46	187.36 ± 75.45	0.078
Blood transfusion (ml)	337.88 ± 439.95	277.81 ± 621.17	0.358
Postoperative TACE (yes/no)	56/74	71/80	0.508

Die beyond 2 years, died beyond 2 years during follow-up periods; Sur. beyond 2 years, survived beyond 2 years during follow-up periods; Edmondson grade I-II corresponding to well or moderately differentiated tumors; Edmondson grade III-IV corresponding to poorly differentiated tumors; Anatomy hepatectomy, the resection of a portion of liver parenchyma based on the distribution of the portal pedicles and the location of the hepatic veins; Nonanatomy hepatectomy, was that performed not limited by those anatomical scissuras.

*HBs-Ag and/or HCV-Ab.

Table 5. Multivariate analysis of factors associated with late death.

Variables	Hazard ratio (95% CI)	<i>P</i> *
GGT (U/L) [†]	1.003 (1.001-1.006)	0.006
Edmondson grade (III-IV)	2.323 (1.219-4.428)	0.010

CI, confidence interval; Edmondson grade III-IV corresponding to poorly differentiated tumors.

[†]Were modeled at continuous variables of GGT; *Logistic regression analysis.

Table 6. Overall survival rates of patients with different serum GGT levels.

Variables	1-year	2-year	3-year	5-year	8-year	10-year	P *
GGT $\leq$ 80 U/L	85.6%	71.2%	64.0%	55.2%	42.9%	37.0%	< 0.001
GGT > 80 U/L	72.5%	57.9%	48.3%	37.7%	19.8%	13.9%	

*Log rank test.

Fig. 3. Kaplan-Meier survival curves of the 281 patients with survival > 2 years stratified by late death related indicators. a, Edmondson grade I-II, serum GGT $\leq$ 80 U/L ($n = 148$); b, Edmondson grade III-IV, serum GGT $\leq$ 80 U/L ($n = 30$); c, Edmondson grade I-II, serum GGT > 80 U/L ($n = 85$); d, Edmondson grade III-IV, serum GGT > 80 U/L ($n = 18$). The survival rates of patients with both low Edmondson grade and low serum GGT level (a) were significantly higher than those with high Edmondson grade (b), high serum GGT level (c) and both high (d) subgroups, after the 2-year mark ($P = 0.010$, 0.001 and < 0.001 , respectively).

ml (Fig. 4G), the survival curve for patients with low serum GGT level became very gentle in the later follow-up years, while in high serum GGT level subgroup still steep. These indicate that high serum GGT level was also a predictor on survival throughout the disease course after hepatic resection for less advanced HCC. Furthermore, as effect of the primary tumor diminish with longer postoperative survival, serum GGT level turned out a survival predictor for patients with advanced HCC.

Role of postoperative TACE on patients with high GGT levels

As the long-term outcomes of patients with high serum GGT levels were poor, we further evaluated the role of postoperative TACE on these patients. We found postoperative TACE had limited effect on the survival of HCC within 2 years after operation, especially in the subgroups with serum GGT $\leq$ 80 U/L (Fig. 5A). However, in the patients with serum GGT > 80 U/L, the 5-year and 8-year survival rates of postoperative TACE subgroup were much

higher than those without TACE, although the survival rates were almost the same at the end of the postoperative follow-up years (Fig. 5B). It suggested that postoperative TACE would lower the late death rate of patients, especially protective for the subgroup with high serum GGT level (Table 8).

Discussion

Among the studies concerning the postoperative outcomes of HCC patients, some focused on associated risk factors and compared survival rates (Ker et al. 2003; Chok et al. 2009; Ju et al. 2009; Ting et al. 2012). While some others used log rank test for univariate analysis, followed by Cox proportional hazards regression models for multivariate analysis in consecutive series of patients (Poon et al. 2000; Sasaki et al. 2006). However, these analysis methods cannot directly indicate the impact of different risk factors on different survival periods. Therefore, some scholars performed post-operational prognosis analysis according to different survival times (Regimbeau et al. 2004; Kondo et

Fig. 4. Kaplan-Meier survival curves of patients with different serum GGT levels.

(A) Patients with vascular invasion; (B) Patients without vascular invasion; (C) Patients with poorly differentiated tumor (Edmondson grade III-IV); (D) Patients with highly differentiated tumor (Edmondson grade I-II); (E) Patients of tumor size > 5 cm; (F) Patients of tumor size ≤ 5 cm; (G) Patients of serum AFP > 400 ng/ml; (H) Patients of serum AFP ≤ 400 ng/ml.

Table 7. Overall survival rates of patients with different serum GGT levels in subgroups stratified by each tumor-related risk factor.

Variables	GGT $\leq$ 80 U/L			GGT $>$ 80 U/L			P*
	2-year	5-year	8-year	2-year	5-year	8-year	
Vascular invasion							
yes	30%	30%	0	27.6%	13.8%	0	0.336
no	72.9%	56.3%	44.4%	63.8%	37.6%	22.5%	< 0.001
Edmondson grade							
I-II	78.7%	63.8%	49.8%	63.0%	37.0%	21.6%	< 0.001
III-IV	48.4%	29.0%	21.8%	41.9%	23.3%	13.6%	0.152
Tumor size							
≤ 5 cm	78.9%	64.0%	48.5%	65.5%	39.7%	25.2%	0.001
> 5 cm	57.3%	39.3%	33.0%	54.2%	30.8%	17.7%	0.019
Serum AFP levels							
≤ 400 ng/ml	77.1%	58.8%	46.6%	68.9%	44.4%	27.8%	0.005
> 400 ng/ml	58.8%	47.5%	35.2%	46.6%	22.7%	11.9%	< 0.001

Edmondson grade I-II corresponding to well or moderately differentiated tumors; Edmondson grade III-IV corresponding to poorly differentiated tumors.

*Log rank test.

Fig. 5. Kaplan-Meier survival curves of patients with/without postoperative TACE. (A) Patients of serum GGT $\leq$ 80 U/L; (B) Patients of serum GGT $>$ 80 U/L.

Table 8. Overall survival rates of patients with/without postoperative TACE in different serum GGT level subgroups.

Variables	With PO TACE			Without PO TACE			P*
	2-year	5-year	8-year	2-year	5-year	8-year	
GGT $\leq$ 80 U/L	74.1%	57.1%	43.4%	68.8%	53.6%	42.4%	0.683
GGT $>$ 80 U/L	66.7%	43.9%	31.9%	52.7%	27.7%	13.5%	0.040

PO TACE, postoperative TACE.

*Log rank test.

al. 2005; Kaibori et al. 2008; Nanashima et al. 2010; Moriguchi et al. 2012). In our study, longer follow up was conducted, and more detailed stratification on survival periods was performed to elucidate the effect of each risk factor on survival.

Early death within 2 years after hepatectomy might result mainly from malignant behavior of the primary tumor

(Sasaki et al. 2006; Nanashima et al. 2010). In the present study, we found that vascular invasion, high Edmondson grade, large tumor size and high serum AFP levels were associated with early death. These data was mostly consistent with previous reports (Regimbeau et al. 2004; Park et al. 2006; Moriguchi et al. 2012). Generally, there was a correlation between tumor differentiation and tumor size,

and they both were predictors of microvascular invasion (Ker et al. 2003). Tumor thrombus tends to form in the portal vein, as it is the main outflow vessel of the tumor. It is generally accepted that HCC with portal vein tumor thrombi is associated with early intrahepatic recurrence and poor prognosis (Park et al. 2006).

Among the tumor-related factors that are typically associated with early death after hepatectomy, only poor tumor differentiation predicted an adverse outcome beyond 2 years after hepatectomy in logistic multivariate analysis. Recently, Eguchi et al. (2011) also demonstrated tumor differentiation as the strongest predictor of death from recurrent HCC within 5 years. High pre-operative serum GGT level was screened out as another independent risk factor associated with late death, whether continuously or dichotomously. Lise et al. (1998) and Ju et al. (2009) both had reported preoperative serum GGT level with prognostic value on HCC patients undergoing hepatic resection. Zhang et al. (2011) demonstrated that GGT level was an important prognostic factor to predict prognosis of patients with intermediate HCC treated with TACE, even in subgroup with normal AFP level. Guiu et al. (2011) validated GGT as an independent predictor of outcome after TACE for HCC in a European population.

As we know, GGT is a plasma membrane-bound heterodimeric enzyme secreted by hepatocytes and bile duct epithelial cells. Clinically, as a stable serum molecule released from liver, high levels of serum GGT are indicative of liver or bile tract-associated disease. Hepatic inflammation and fibrosis may damage hepatocytes, and then causes the obstruction of capillary bile duct, which ultimately lead to over excretion of GGT into the blood. There is a positive correlation between serum GGT level and the degree of liver fibrosis especially in chronic liver disease (Poynard et al. 1984). Comparing to serum ALT and AST with shorter half life period and easy fluctuation, serum GGT better reflects the liver state in the long disease progression of hepatitis. Ruhl and Everhart (2009) reported that elevated GGT was more likely to be related to mortality from all causes (including liver disease) than ALT. Persistent inflammation in hepatitis activity and maintained necrosis-induced hepatocyte regeneration may lead to chromosomal damage and increased the risk for malignant transformation after hepatic resection (Poon et al. 2000). In addition, HS-GGT (hepatoma specific GGT bands), a component of serum GGT by HCC tissue, is useful in diagnose of subclinical HCC (Xu et al. 1992; Tsutsumi et al. 1996; Wang et al. 2009). The subclinical HCC cannot be easily discovered before and during operation, and it may reoccur after operation. However, HS-GGT only accounts for 2.2% to 9.7% of total serum GGT, most of which is secreted by liver (Yao et al. 2000; Wang et al. 2009). Therefore, high GGT level might indicate increased inflammation, fibrosis and liver lobular structural disorder. These patients not only would likely suffer from high incidence of tumor recurrence, but also were restricted for further treatment

due to uncompensated liver function. GGT has critical clinical indication for evaluating and pursuing further treatments. Indeed, five underlying liver disease-related factors were associated with late death by univariate analysis in our study. Hence, we speculated, the severity of underlying liver disease was a key risk factor for poor long-term outcomes in a consecutive HCC patient cohort after hepatic resection. For more powerful conclusion, other liver status related factors, such as ICGR 15 (15-min indocyanine green retention rate), ^{99m}Tc -GSA (Technetium-99m-diethylenetriamine pentaacetic acid galactosyl human serum albumin) liver dynamic SPECT, HVP (hepatic venous pressure gradient measurement), and more histological or biochemical indexes should be included in prospective clinical trials.

Postoperative TACE was screened as a treatment-related factor associated with early death. But in further analysis, we found postoperative TACE benefited those with high GGT levels by lowering the rate of late death. We speculated that postoperative TACE might not be so effective on those with high remnant liver tumor burden in the short time after operation, because many patients with highly invasive cancer tend to die in the early follow-up years. In a few patients free from recurrence during the first 2 years after operation, some suffered from liver failure due to curative major liver section of advanced HCC nodule. In this way, the effect of primary tumor diminished in the later follow-up years, and postoperative TACE appeared to reduce the mortality of patients henceforth. Statistically, they were manifested as the $\text{HR} < 1$. Postoperative TACE did not alter the prognosis of those with low serum GGT levels, because these patients had more treatment choice after tumor recurrence due to their sufficient liver function reserve.

Here we should pay attention to those patients with high serum GGT levels, whether in advanced or less advanced HCC. Doubtlessly, patients with advanced HCC, regardless of GGT levels, should undergo close long-term surveillance postoperatively and adjuvant anticancer therapy was recommended if intrahepatic metastasis appeared. We also found the patients with less advanced HCC but high serum GGT level were at continued risk of dying postoperatively. So it raises a question on the further treatment of these patients after liver section. If tumor recurred, repeated hepatectomy may provide additional 5-year survival rate up to 50%, which seems be an ideal therapeutic option (Itamoto et al. 2007). However, this method may only be suitable for part of the patients, depending on their liver function reservation and distribution of recurrence foci. At this time, systemic postoperative adjuvant therapies (ie. microwave ablation, radiofrequency ablation, etc.) can also be choices to avert recurrence. In this study, postoperative TACE can improve the survival rate of these patients. The purpose of postoperative adjuvant TACE were to eliminate the shed tumor cells potentially released by surgical manipulation of the liver, to destroy small intrahepatic metastases that may not have been detected preop-

eratively and intraoperatively, and also to eradicate the de novo HCC in the residual liver. However, repeated postoperative TACE can cause progressive liver atrophy or hepatic insufficiency. Fuks et al. (2012) have described the effectiveness of hepatectomy while awaiting liver transplantation. Salvage liver transplantation might serve as a feasible and rational strategy for selected patients with recurrence restricted to the liver after previous resection. Furthermore, liver transplantation can be considered as a primary treatment, which might be more reliable than hepatectomy or salvage liver transplantation. Patients with HCC within Milan Criteria displayed 5-year survival rate more than 70% after liver transplantation (Mazzaferro et al. 1996). The optimal management for those patients with a poor prognosis remains to be clarified by further clinical trials.

In conclusion, the detailed stratification on survival periods could better elucidate the different impact of prognostic factor on survival after curative hepatectomy for HCC, which would help optimize treatment strategies for different HCC patients. Although overwhelmed by effect of primary tumor in the early stage after hepatectomy, postoperative TACE is beneficial for HCC patients with poor liver status.

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Conflict of Interest

None declared.

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