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Research Article

Clinical Analysis of the Risk Factors of Early and Late Phase Recurrence after Surgical Excision of Hepatocellular Carcinoma: Experience of One Center with 345 Patients

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Abstract

Background: Resection excision operation is an important treatment alternative for respectable HCC (hepatocellular carcinoma); unfortunately, many patients undergo the experience of early or late phase recurrence after surgical excision of the tumor lesion. Our study here aims to investigate the risk factors associated with early and late phase recurrence after surgery as well as to establish a predictive model to facilitate improving our predictive ability about HCC recurrence and accumulating the comprehensive treatment experience.

Methods: We conducted a retrospective analysis of 345 patients who received surgical excision of the tumor lesions in our hospital. Patients were divided into three classes: no recurrence, early phase recurrence and late phase recurrence. Risk factors of early and late recurrence were analyzed statistically. On the basis of the risk factors associated with early and late phase recurrence, the mathematics models for early and late phase recurrence were established.

Results: The cumulative survival rates without recurrence at 1,2,3,4, and 5 years were 68.8%, 40.1%, 35.7%, 30.2%, and 27.5% respectively. According to the widely accepted definition: early phase recurrence occurs within 2 years after the surgery and late phase recurrence happens 2 years after the resection. 197 patients had early phase recurrence and 25 ones had late phase recurrence. Cox multivariate proportion hazard model suggested that multiplicity of tumor lesions, high preoperative serum fibrinogen level, high preoperative serum GGT level and too much blood transfused are independent risk factors significantly correlated with early phase recurrence. In contrast with the risk factors associated with early phase recurrence, multiplicity of tumor lesions, severe liver cirrhosis and portal vein hypertension, high preoperative serum CHE level and high preoperative serum GGT level were identified as risk factors associated with late phase recurrence. Patients with at least two of the four early phase recurrence risk factors were much more likely to have early phase recurrence and patients with three or more late recurrence risk factors were prone to experience late phase recurrence.

Conclusions: Different kinds of risk factors are associated with early and late phase recurrence. Early phase recurrence occurs due to metastases within the liver while late phase recurrence happens as a result of impaired liver function reserve and carcinogenesis de novo from the cirrhotic liver.

Keywords: Hepatocellular carcinoma; Early and late phase recurrence; Surgical excision; Risk factors; Prognosis; High-risk group and low-risk group; Cut-off value

Introduction

As a common kind of malignant tumor, HCC (hepatocellular carcinoma) causes about 1 million deaths due to its increasing incidence annually and poor 5-year survival rate of less than 5% without treatment [1-4]. Surgery, which includes keratectomy and liver transplantation, is still believed by most surgeons to be the most effective treatment alternative for the patients with respectable HCC lesions. Although liver transplantation is the most curative treatment, it also has its own shortcomings. The lack of donor livers, long waiting time, higher perioperative morbidity and long-term or even lifetime immunosuppression therapy still restrict the wide application of the liver transplantation in the treatment of HCC. Over the last several decades surgical techniques and perioperative management of patients undergoing keratectomy have become more and more sophisticated which makes the procedure of resection of HCC much safer than that of several decades ago [1,3,4,5]. The safety of the surgical procedure is indicated by much lower mortality and morbidity rates than those

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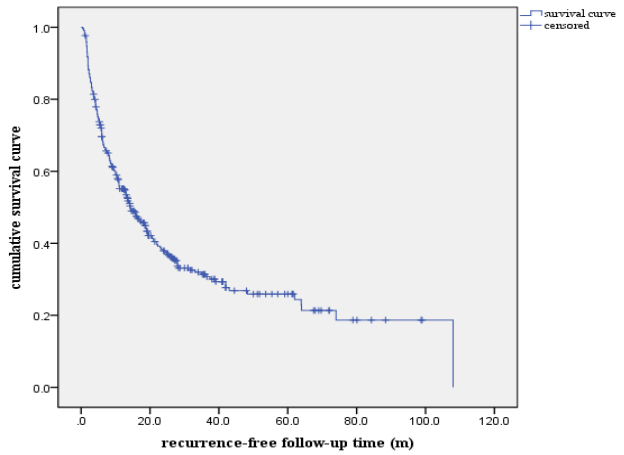

Figure 1: The cumulative recurrence-free survival curve of the 345 patients.

of several decades ago [1,3,4,5]. Even after a safe surgical resection of the HCC, the long-term prognosis of patients with HCC remains poor due to the high incidence of recurrence (68%-96%) [1,4,5,7,9]. Thus it is critical to develop effective therapeutic methods to control tumor recurrence with an ultimate goal of prolonging the life of HCC patients. By now, various kinds of risk factors associated with HCC recurrence have been reported. These factors include tumor-related ones, background liver status, the type of the surgery and even some molecular and immunological markers [1-8,12,14,17,20]. However, the exact causes and mechanism of recurrence still remain mysterious. In this study, we investigated the pattern of recurrence time and a few potential risk factors that may help us predict the early and late phase recurrence of HCC after a hepatectomy for HCC.

Methods

Patients

Between March 2005 and May 2013, 475 patients received surgical excision of HCC at the department of hepatobiliary surgery and liver transplantation of The Third Affiliated Hospital of Sun Yat Sen University. Of all these patients, 130 were excluded from the study. 57 patients received other treatment options prior to the resection surgery and 73 ones had simultaneous intraoperative microwave ablation because of the multiple lesions within the liver. 345 patients were included in the present study. Curative resection includes the complete removal of the tumor and the visible portal vein tumor thrombus with a negative microscopic margin. Patients receiving anti-HBV before and after the hepatectomy are recorded in the study and we analyzed the relationship between the anti-HBV therapy and late phase recurrence. The procedures and the related methods of this study and using human sample had received approval by the Ethics Committee of our hospital before the implementation and all the patients involved in this research had signed written informed consent.

Surgical modalities

If 3 or more segments (according to the Coined classification were resected, the procedure was called a major hepatectomy. 180 (52%) patients underwent major hepatectomies, of whom 27 (5%) were resected 3 or more discontinuous segments. The resection operation of 165 patients were minor ones that include non-anatomical wedge resections (no more than two segments) or enucleations (50, 14.3%) and left lateral segmentectomy (44, 12.8%). The average number of resected hepatic segments is 3.2 0.4 (range 0-6). Anatomical resection, defined as any type of systematic resection of the portal region based on the Coined classification system, was performed in 299 patients (75.1%), while 99 patients underwent non-anatomical resections (24.9%. In this study we classified incomplete removal of tumor-bearing portal region such as wedge resection or enucleation as non-anatomical resection while discontinuous segments resection was defined as anatomical resection as long as each resection of the patient was anatomical resection.

Diagnosis of HCC

Nowadays most HCC patients were detected and evaluated using contrast ultrasonography, contrast CT and MRI. If the lesion is larger than 2cm in diameter, a single imaging modality with arterial hypervascular and venous washout characteristic is suggestive of HCC; otherwise 2 or more kinds of imaging modalities with arterial hypervascular and venous washout characteristic are needed to confirm the diagnosis of HCC. The diagnosis of HCC patients were confirmed by histopathological investigation after the resection. Pathological grading was based on the Edmodson-Steiner criteria.

Follow-up

All the follow-up processes of the patients after resection were carried out in the outpatient or inpatient department of our hospital and the recurrence of HCC was closely monitored prospectively. The follow-up protocol was made up of monthly serum AFP (alpha-fetoprotein) monitoring and contrast ultrasonography, contrast CT or MRI once every 3 months after the resection of the HCC lesion. The changes of the serum tumor markers before and after the surgical operation as well as those at the confirmation of the recurrence were assessed. Tumor recurrence was confirmed according to the same criteria applied to the initial diagnosis of HCC and if hepatic re-resection was done, the recurrence was diagnosed by histopathological investigation. The number, size and location of recurrence (intrahepatic or extrahepatic) were then recorded. Recurrences outside of the liver (i.e metastases) were investigated by contrast ultrasonography, CT, MRI or PET-CT using 18F-FDG.

Statistical analysis

Descriptive statistics had several parameters including mean, range, standard deviation and proportion. For the continuous variables that have been previously widely used by the clinicians, the widely accepted cut-off values of these variables were directly used, otherwise the cut-off values were calculated by the ROC curve method. In univariate analysis, χ^2 test was adopted to determine the variables significantly associated with early and late phase recurrence. The multivariate analysis of prognostic factors for HCC early and late phase recurrence was done using the Cox's proportional hazards model. All the 23 variables were entered into a backward stepwise regression model. Step selections were based on the maximal likelihood ratio tests and only significant variables were reserved in the multivariate Cox's proportional hazards model analysis. The Kaplan-Meir method was used to evaluate survival rates and the log-rank test was applied to compare survival rates. SPSS18.0 for Windows (Chicago IL, USA) was used to to perform all the statistical evaluation.

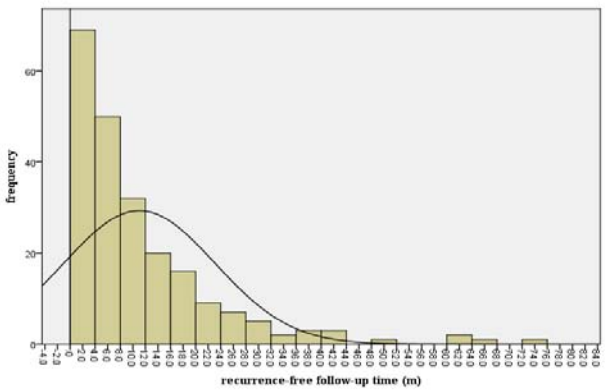

Figure 2: The distribution of the time from the hepatectomy to recurrence of the 237 patients.

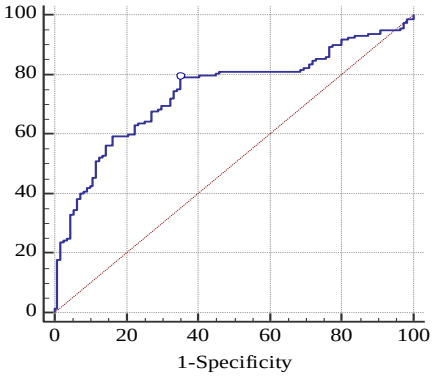

Figure 3: The ROC curve for the prediction of the combination of all the independent risk factors for early phase recurrence (AUC=0.739, P<0.001).

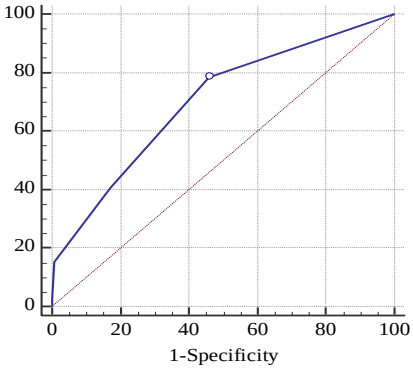

Figure 4: The ROC curve determining the best cut-off value predicting early phase recurrence (AUC=0.700, P<0.001).

For ROC (receiver operating characteristic) curve analysis, we used the Medcalc (version120 to calculate the sensitivity, specificity, area under the curve and to select the optimal cut-off value for predicting HCC recurrence. A variable was considered statistically significant when its P value was lower than 0.05.

Results

Patient characteristics

306 men (88.7%) and 39 women (11.3%) were incorporated in the present study with a mean age of 50.01±11.62 years. Assessed by the Child-Pugh classification system, all the patients belonged to grade A or B. 317 patients (92.2%) belonged to Child-Pugh A and 28 patients (7.8%) belonged to Child-Pugh B. At the start of the hepatectomy, 237 patients (68.7%) had only one tumor lesion and 108 patients (31.3%) had multiple tumors. The median nodule diameter was 5.38 cm (range 1.0-18.2cm). Regarding the etiology of the HCC, 310 (77.9%) of all the patients were HBV-positive while only 3 were confirmed HCV-positive. All the patients had liver fibrosis of different grades and 272 patients (68.3%) had cirrhosis background. Table 1 shows the demographics of all the patients that contain preoperative, intraoperative and tumor-related parameters pertain to the initial hepatectomy.

Cumulative risk for recurrence

237 patients underwent the experience of recurrence during the follow-up time of 1 to 113 months after surgery. And the mean time from surgery to tumor recurrence was 18.02±19.22 months (range 0.5 to 108 months). Of these patients who had recurrence, 210 (89%) patients experienced intrahepatic recurrence while 21 (9%) had intrahepatic and extrahepatic recurrence at the same time or had extrahepatic recurrence subsequently. The remaining 6 (2.0%) patients had extrahepatic tumor recurrence without any intrahepatic recurrence lesions. Extrahepatic metastases were diagnosed through imaging techniques and (or) pathological analysis of the resected extrahepatic recurrence lesions. The sites where the extrahepatic tumor recurrence occurred included the lungs (18 patients), brain (5 patients), bone (3 patients) and extrahepatic bile duct (1 patients). There was no recurrence occurring near the cut surface. Almost all the recurrences were multiple in number and occurred in both liver lobes or opposite lobe. One hundred and eight patients did not have the experience of recurrence after a mean follow-up time of 31.5 months. The cumulative recurrence-free Kaplan-Meier curve of the 345 patients having received hepatectomy surgery was demonstrated in (Figure 1). The cumulative recurrence-free survival rate was 68.8%, 40.1%, 35.7%, 30.2% and 27.5% at 1,2,3,4 and 5 years respectively. The recurrence time distribution curve of the 237 patients with recurrence was shown in (Figure 2). Two distinctively different recurrence peaks were detected according to the recurrence time distribution curve. The first peak was at one year after the surgery and it was the most likely time when recurrences occurred. The second peak was at 4 years after the hepatectomy. We could also infer from the recurrence time distribution pattern that recurrence after hepatectomy should be classified into early phase (within 2 years) and late phase recurrence (after two years).

Univariate analysis of early and late phase tumor recurrence

197 patients experienced early phase recurrence, while late phase recurrence happened to forty patients. The results of risk factors associated with early phase recurrence and late phase recurrence by the means of univariate analysis were shown in (Table 2) and (Table 4). Fourteen factors significantly associated with early phase tumor recurrence were determined through univariate analysis, namely pathological differentiation, number of the tumor lesions, vascular invasion, neutrophil cell count, AST, ALB, PA, GGT, FIB, Intraoperative blood transfusion, ALT, CHE, ADA and maximal

Table 1: The descriptive analysis of the clinic pathological factors of the 345 patients (N = 345).

Variables		Mean±SD (range)/(n,%)
Gender	Male	306(88.7)
	Female	39(11.3)
Age(years)		50.01±11.62(16-79)
Smoking	No	229(66.4)
	Yes	116(33.6)
Type II Diabetes Mellitus	No	311(90.1)
	Yes	34(9.9)
Preoperative antiviral therapy	No	180(52.2)
	Yes	165(47.8)
Portal hypertension and cirrhosis	No	108(31.3)
	Mild to moderate	226(65.5)
	Severe	11(3.2)
Maximal diameter of the tumor		5.80±3.58(1.00-18.20)
Number of the tumor lesions	Single	237(68.7)
	Multiple	108(31.3)
Vascular invasion	No	200(58.0)
	Yes	145(42.0)
Extrahepatic metastasis	No	336(97.4)
	Yes	9(2.6)
Cancerous thrombus of the IVC or portal vein	No	307(89.0)
	Yes	38(11.0)
Rupture of the tumor	No	323(93.6)
	Yes	22(6.4)
Margin	Negative	339(98.3)
	Positive	6(1.7)
AFP	<100	172(49.9)
	101-200	34(9.9)
	201-400	23(6.7)
	>400	116(33.6)
Neutrophil cell count		28.34±21.58(1.0-113.0)
Platelet cell count		178.49±78.63(2.41-465.00)
Lymphocyte count		1.67±0.66(0.23-4.21)
Mononuclear cell count		0.52±0.46(0.04-6.25)
ALP		89.47±40.88(32-305)
ALT		49.76±55.22(6-562)
AST		49.84±48.35(12-477)
TC		4.46±1.10(1.37-8.15)
CHE		6742.32±3055.07(1711-49262)
ALB		39.77±4.21(22.4-52.5)
PA		176.63±58.19(24-357)
GGT		99.65±136.12(14-1549)
ADA		13.93±6.05(1-43)
FIB		3.27±1.19(1.17-10.27)
Child-Pugh Grading	A	317(92.2)
	B	27(7.8)
Intraoperative blood transfusion		415.22±651.04(0-4200)
AST/ALT		1.20±0.67(0.34-4.70)
Pathological differentiation	Well	59(17.1)
	Moderate	261(75.7)
	Poor	25(7.2)
Recurrence	No	123(35.7)
	Yes	222(64.3)
Follow-up time (months)		28.34±21.58(1-113)
Follow-up time without recurrence (months)		18.02±19.22(0.5-108)

AFP: Alpha Fetoprotein; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotranferase; TC: Total Cholesterol; CHE: Cholinesterase; ALB: Albumin; PA: Pre-Albumin; GGT: Glutamyl Transpeptidase; ADA: 3.5.4.4 Adenosine Deaminase; FIB: Fibrinogen.

diameter of the tumor. It was also through univariate analysis that eleven parameters significantly associated with late phase tumor recurrence were identified, i.e portal hypertension and cirrhosis, maximal diameter of the tumor, platelet cell count, lymphocyte count, TC, ALB, CHE, GGT, ADA, FIB and Child-Pugh grading.

Multivariate analysis of the independent adverse risk factors significantly correlated with early phase and late phase recurrence were done by Cox's multivariate proportional hazard model analysis. Contrary to univariate analysis, only 4 factors were proven to be associated with early phase tumor recurrence: Number of tumor lesions [OR=2.139 P <0.001 95% CI: 1.535-2.982] FIB [OR=1.604, P =0.01, 95% CI: 1.121-2.294], GGT [OR=2.091, P <0.001, 95% CI: 1.478-2.958] and Intraoperative blood transfusion [OR=1.611, P =0.005, 95% CI: 1.158-2.241]. Otherwise, 4 factors were detected to induce late phase tumor recurrence, namely Number of tumor lesions [OR=3.895, P =0.003, 95% CI: 1.580-9.604], Portal hypertension and cirrhosis [OR=3.762, P =0.003, 95% CI: 1.677-8.485], CHE [OR=1.000, P =0.007, 95% CI: 0.999-1.004] and GGT [OR=1.004, P =0.030, 95% CI: 1.000-1.008]. The results of Cox's multivariate proportional hazard model analysis of early and late phase recurrence are shown in (Table 3) and (Table 5).

Predicting early phase tumor recurrence

By the means of ROC analysis, the cut-off value that best predicts the risk of early phase recurrence is 2. The patients in our research were then stratified into high-risk (patients with at least 2 adverse independent risk factors) and low-risk (absence of any or only 1 adverse independent risk factors) groups. Figure 3 shows the cumulative recurrence rates for the high-risk and low-risk groups. High-risk patients and low-risk patients were proven to have significantly different early phase recurrence rates (P<0.001, log-rank test).

Establishing and validation of the mathematics model for early phase tumor recurrence

Cox's multivariate proportional hazard model analysis identified that serum GGT level, tumor lesion number, preoperative fibrinogen level and intraoperative blood transfusion are the independent risk factors for early phase tumor recurrence. On the basis of the results

Table 2: Univariate analysis of the risk factors associated with early phase recurrence.

Variables	No recurrence	Recurrence	Total	X^2 value	P value
Gender					
Male	94	142	236	2.053	0.152
Female	17	15	32		
Smoking					
Yes	34	57	91	0.934	0.334
No	77	100	177		
Type II Diabetes Mellitus					
Yes	7	19	26	2.493	0.114
No	104	138	242		
Preoperative antiviral therapy					
Yes	51	83	134	1.246	0.264
No	60	74	134		
Portal hypertension and cirrhosis					
No	41	47	88	2.987	0.07
Mild to moderate	70	104	174		
Severe	0	6	6		
Vascular invasion					
Yes	32	59	91	2.221	0.01*
No	79	98	177		
Extrahepatic metastasis					
Yes	2	2	4	0.123	0.726
No	109	155	264		
AFP					
1-100	57	83	140	1.126	0.771
101-200	13	17	30		
201-400	9	8	17		
>400	32	49	81		
Child-Pugh grading					
A	107	43	250	2.930	0.0087
B	4	14	18		
Pathological differentiation					
Well	28	18	46	9.422	0.01*
Moderate	79	128	207		
Poor	4	11	15		
Maximal diameter of the tumor					
≥3cm	68	59	127	14.627	0.001*
<3cm	43	98	141		
Neutrophil cell count					
≤3.72	78	94	192	3.058	0.08
>3.72	33	63	96		
PA					
≤195	77	90	167	21.16	<0.001*
>195	30	71	101		
GGT					
≤96	99	107	206	16.182	<0.001*
>96	12	50	62		
Age					
≤60	78	121	199	1.573	0.21
>60	33	36	69		
Number of tumor Lesions					
>3	95	111	206	19.951	<0.001*
≤3	16	46	62		
Platelet cell count					
≤150	36	70	106	4.109	0.045
>150	75	87	162		
Lymphocyte count					
≤1.72	53	106	158	11.480	0.006*
>1.72	59	51	110		
Mononuclear cell count					
≤0.57	89	111	200	3.086	0.079
>0.57	22	46	68		
ALP					
≥83	74	81	161	3.433	0.064
<83	37	70	107		
ALT					
≤52	27	17	44	8.631	0.003*
>52	84	140	224		
AST					
≤38	76	78	154	9.390	0.002*
<38	35	79	114		
TC					
≤4.1	50	59	109	1.465	0.326
>4.1	58	88	146		
CHE					
≤7556	60	115	175	10.573	0.001*
>7556	51	42	93		
ALB					
≤39.2	71	77	148	5.854	0.016*
>39.2	40	80	120		
GGT					
≤96	99	127	206	16.182	<0.001
>96	12	50	62		
ADA					
≤19	99	127	206	4.388	0.048*
>19	12	30	42		
FIB					
≥2.48	93	109	202	7.221	0.007*
<2.48	18	48	66		
AST/ALT					
≤0.9	52	65	117	0.765	0.382
>0.9	53	78	131		
Intraoperative blood transfusion					
≤400	86	96	182	7.985	0.005*
>400	25	61	86		

Table 3: Cox's multivariate proportional hazard model analysis of early phase recurrence.

Variables	β	SE	Wald	P	95% CI	OR
Number of tumor Lesions	0.760	0.169	20.131	0.000*	1.535-2.982	2.139
FIB	0.473	0.183	6.696	0.010*	1.121-2.294	1.604
GGT	0.738	0.177	17.365	0.000*	1.478-2.958	2.091
Intraoperative blood transfusion	0.477	0.168	8.027	0.005*	1.158-2.241	1.611

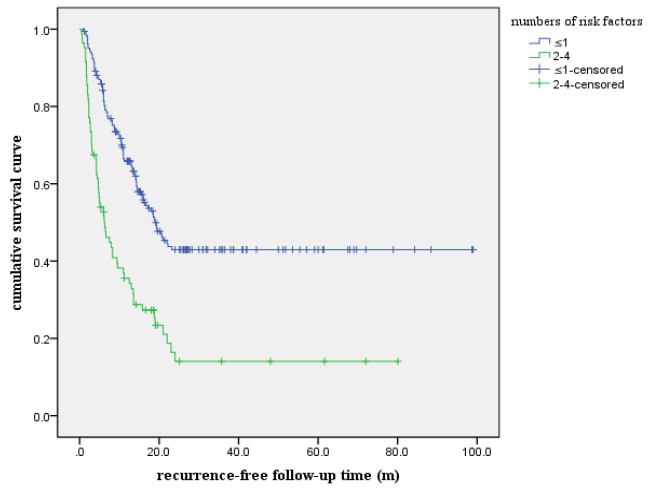

Figure 5: Cumulative recurrence-free survival curve for early phase recurrence after hepatectomy (patients were stratified into high-risk and low-risk group) (log-rank test, χ^2=32.86, P<0.001).

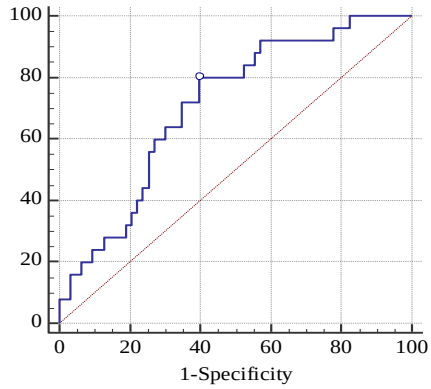

Figure 6: The ROC curve for the prediction of the combination of all the independent risk factors for late phase recurrence (AUC=0.709, P<0.001).

of Cox's multivariate proportional hazard model analysis, the mathematics model for early phase tumor recurrence wash (t)=h0(t) exp(0.738*GGT+0.760*tumor lesion number+0.473*preoperative fibrinogen level+0.477*intraoperative blood transfusion).

Predicting late phase tumor recurrence

By the means of ROC analysis, the cut-off value that best predicts the risk of late phase recurrence is 3. The patients in our research were then stratified into high-risk (patients with at least 3 adverse independent risk factors) and low-risk (less than 3 adverse independent risk factors) groups. Figure 3 and Figure 5 shows the cumulative recurrence rates for the high-risk and low-risk groups.

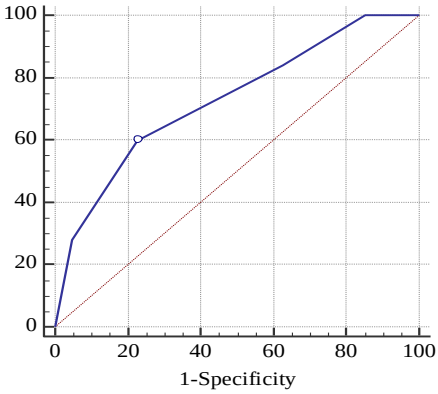

Figure 7: The ROC curve determining the best cut-off value predicting late phase recurrence (AUC=0.728, P<0.001).

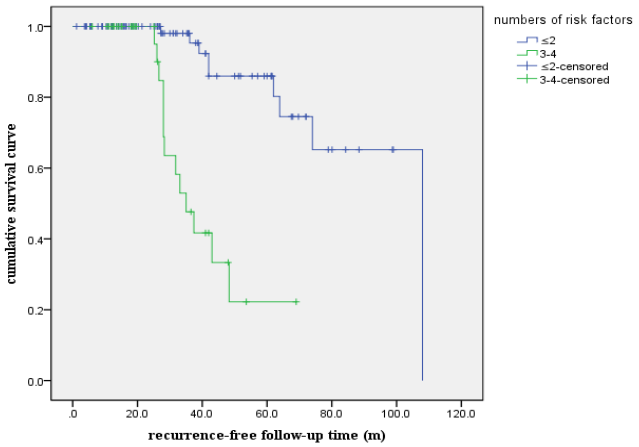

Figure 8: Cumulative recurrence-free survival curve for early phase recurrence (Patients were divided into high-risk and low-risk group) (log-rank test, χ2 value = 33.57, P<0.001).

High-risk patients and low-risk patients were proven to have significantly different late phase recurrence rates (P<0.001, log-rank test) (Figure 6).

Establishing the mathematics model for late phase tumor recurrence

Cox's multivariate proportional hazard model analysis identified that serum GGT level, tumor lesion number, preoperative CHE level and background liver cirrhosis status were the independent risk factors for late phase recurrence. On the basis of the results of Cox's multivariate proportional hazard model analysis, the mathematics model for late phase tumor recurrence wash (t)=h0(t)exp(1.360 *tumor lesion number-2.869*background liver cirrhosis status +0.049*CHE+0.08*GGT).

Discussion

Hepatectomy can simultaneously remove the main tumor lesion and the surrounding tissue that may contain microscopically lesions that cannot be detected by conventional imaging techniques. And hepatectomy can be carried out almost without any waiting time. The two advantages mentioned above make hepatectomy the first choice treatment for most respectable HCC. Although the prognosis for

Table 4: Univariate analysis of the risk factors associated with late phase recurrence.

Variables	No recurrence	Recurrence	Total	χ^2 value	P value
Gender					
Male	104	23	127	0.946	0.530
Female	19	2	21		
Age					
≥60	35	6	41	0.206	0.650
<60	88	19	107		
Smoking					
No	89	18	107	0.001	0.971
Yes	34	7	41		
Type II Diabetes Mellitus					
No	112	24	136	0.681	0.691
Yes	11	1	12		
Preoperative antiviral therapy					
No	64	15	79	0.530	0.467
Yes	59	10	69		
Portal hypertension and cirrhosis					
No	43	6	49	6.094	0.048*
Mild to moderate	79	17	96		
severe	1	2	3		
Maximal diameter of the tumor					
≤9.5cm	119	21	140	6.603	0.028*
>9.5cm	4	4	8		
Number of tumor lesions					
≤3	118	22	40	2.558	0.120
>3	5	3	8		
Vascular invasion					
No	81	18	99	0.354	0.552
Yes	42	7	49		
Extrahepatic metastasis					
No	121	25	146	0.412	0.521
Yes	2	0	2		
Cancerous embolism					
No	118	22	140	1.719	0.352
Yes	5	3	8		
Margin					
Negative	121	23	144	3.210	0.073
Positive	2	2	4		
AFP					
1-100	62	14	76	0.965	0.810
101-200	14	2	16		
201-400	10	3	13		
>400	37	6	43		
Neutrophil cell count					
≤2.59	36	12	48	3.327	0.068
>2.59	87	13	100		
Platelet cell count					
≤180	61	20	81	7.753	0.005*
>180	62	5	67		
Lymphocyte count					
≤1.51	42	15	57	5.865	0.015*
>1.51	81	10	91		
Mononuclear cell count					
≤0.28	20	8	28	3.356	0.067
>0.28	103	17	20		
ALP					
≤54	23	2	25	1.694	0.193
>54	100	23	123		
ALT					
≤45	92	15	107	1.271	0.132
>45	31	10	41		
AST					
≤32	60	8	68	2.356	0.125
>32	63	17	80		
TC					
≤4.3	66	7	73	4.833	0.028*
>4.3	53	16	69		
ALB					
≤41.9	78	21	99	3.976	0.046*
>41.9	45	4	49		
CHE					
≤7403	66	21	87	7.895	0.007*
>7403	57	4	61		
PA					
≤195	62	16	78	1.918	0.186
>195	59	8	67		
GGT					
≤58	72	7	79	7.785	0.008*
>58	51	18	69		
ADA					
≤22	115	20	135	4.723	0.03*
>22	8	5	13		
FIB					
≥2.19	107	17	124	5.516	0.019*
<2.19	16	8	24		
Intraoperative blood transfusion					
≥1550ml	7	1	8	1.493	0.222
<1550ml	116	25	140		
AST/ALT					
≤0.77	32	10	42	1.999	0.151
>0.77	91	15	106		
Child-Pugh grading					
A	117	20	137	8.525	0.014*
B	5	6	11		
Pathological differentiation					
Well	28	11	39	4.829	0.089
Moderate	88	13	101		
Poor	7	1	8		

Table 5: Cox's multivariate proportional hazard model analysis of late phase recurrence.

Variables	β	SE	Wald	P	OR	95% CI
Number of tumor Lesions	1.360	0.460	8.723	0.003	3.895	1.580-9.604
Portal hypertension and cirrhosis	-2.869	1.005	10.475	0.003	3.762	1.677-8.485
CHE	0.049	0.000	7.368	0.007	1.000	.999-1.004
GGT	0.08	0.002	4.717	0.030	1.004	1.000-1.008

most HCC patients has improved a lot over the last several decades, tumor recurrence remains the most common cause of treatment failure and even the deaths of HCC patients [1,2,6,9,17,20,22]. It has been reported in several studies that early phase recurrences are mainly due to intrahepatic dissemination and therefore are associated with aggressive pathological tumor factors such as vascular invasion, tumor diameter, multiplicity of tumor lesions, incomplete tumor capsule and tumor cell dissemination during the surgery [6,9,10,21,27,30,38]. It has been postulated that the factors mentioned above are very much likely to lead to intrahepatic metastasis and subsequently to early phase tumor recurrences [2,5,9,12,19,24,46]. Many experts believe early phase recurrences are associated with invasive nature of the tumor cells [1-5,15,26,34,42,46]. However, some other researchers found that liver status plays a much more important role in the tumor recurrence [5,12,26,34,45]. Thus, the precise mechanism of tumor recurrence still remains controversial.

In our study, we divided the tumor recurrences into two categories according to the recurrence time distribution pattern. Recurrences within two years after the surgery were defined as early phase recurrences and recurrences beyond two years after the surgery were defined as late phase recurrences. Moreover, it has been suggested by many experts in HCC treatment that different phases of recurrences are associated with different kinds of risk factors [1,7,9,18,25,39,42,46]. These findings may in part explain why many previous studies came to conflicting conclusions, since these studies did not distinguish early phase recurrences from late phase recurrences. Poon et al, were the first to distinguish early recurrences from late phase recurrences and find different phases of recurrences were correlated with different kinds of risk factors [2,9,14,20,27,36,42,46]. So they furthermore suggested that early phase recurrences mainly originated from intrahepatic metastases and late phase recurrences actually represented new tumor formation. However, the exact time that separates early phase recurrences from late phase recurrences is still controversial. Poon et al, suggested one year, which is different from our the two years we concluded from the recurrence time distribution pattern [2,9,14,20,27,36,42,46]. This difference might be due to different patient features. Imaura et al, also proposed that two years should be the demarcation time, although their research determined different risk factors from ours [5,14,17,18,23,46]. (The HCC patients in their research are HCV-positive, whereas most patients in our research are HBV-positive).

Our research found 4 risk factors associated with early phase recurrence, namely tumor lesion number, serum GGT level, high preoperative fibrinogen level and intraoperative blood transfusion. The identification of portal vein and IVC (inferior vena cava) cancerous thrombus as an adverse independent risk factors indicates that the main route by which early phase recurrence occurs is spreading through the portal vein. Several previous researches have pointed out that preoperative vascular invasion or even portal vein and IVC cancerous thrombus often heralds fulminant recurrence and extremely terrible prognosis [1-4,7,14,27,33,46].

Tumor lesion multiplicity is another adverse risk factor associated with early phase tumor recurrence and the possible explanation is that tumor lesion multiplicity reflects microscopic vascular invasion. Some other studies reported that serum AFP level, pTNM score, histological differentiation and tumor diameter are useful prognostic factors to predict early phase tumor recurrence [1-6,10,19,21,37,44,46]. However, in our research, none of the factors mentioned above were proven to be significantly associated with early phase tumor recurrence. Contrary to the results of other studies, none of the surgery-related factors were identified to contribute to early phase recurrence. To sum up, the vascular invasion behavior of tumor cells is believed to be the most important mechanism how the early phase tumor recurrence occurs.

Besides the factors that reflect tumor cell vascular invasion nature, some other factors which include serum GGT level, preoperative fibrinogen level and intraoperative blood transfusion are also identified to be independent adverse risk factors of early phase tumor recurrence. The following three passages will be dedicated to discussing how these three factors are related to early phase tumor recurrence.

GGT, also known as gama glutamyl transpeptidase, is widely distributed through the human body tissues. The kidney tissue has the most abundant GGT content, followed by pancreas, liver and heart tissue [5,11,22,36,44]. Elevated serum GGT level is most often seen in hepatobiliary diseases. In HCC patients, serum GGT level is often several times above the upper limit. In our study, GGT is proven to be negatively related to early phase tumor recurrence. The GGT gene hypomethylation status of the HCC malignancy is the possible reason why GGT level is significantly elevated in most HCC patients [24,30,44]. Both the malignant cells in the tumor and adjacent inflamed liver tissue are responsible for the significantly elevated serum GGT level [24,30,41,44]. So significantly elevated serum GGT level can in part reflect the invasive nature of the tumor cells of HCC patients. So we can also relate elevated serum GGT level to the early phase tumor recurrence after surgical removal of all the visible HCC lesions. GGT has different isoforms which include F(fetus) type, P (placenta) type and H (HCC malignant cell) type [44]. Of all these three types of GGT, HS-GGT is most valuable in the diagnosis of HCC [44]. Apart from aiding in the diagnosis of HCC, GGT especially HS-GGT can be used to predicting tumor recurrence [44].

As one of the most important technologies promoting the development of surgical treatment, blood transfusion plays an irreplaceable role in the treatment of HCC. Opelz et al, found that blood transfusion before kidney transplantation could prolong the graft survival time through suppression of the immune system of the recipients [21,32,35]. Subsequent researches concluded that patients receiving blood transfusion especially massive blood transfusion were much more likely to experience tumor recurrence and to have a worse prognosis (breast cancer lung cancer and renal cell carcinoma and so on) [21,32,35]. Despite the discoveries mentioned above, the precise mechanism of this phenomenon still remains unknown. The

following studies revealed that blood transfusion inhibits the immune system of the patients, such as the reactivity of the lymphocytes, NK cells (natural killer) and macrophages [21,32,35]. Blood transfusion makes the system produce all kinds of inhibitory factors enabling the tumor cells to escape from the attacks of the immune system and therefore to maintain able to grow and spread to other sites of the body. The fact that massive blood transfusion promotes tumor growth and recurrence also applies to HCC. Many retrospective studies of independent risk factors associated with HCC tumor growth and recurrence found that massive blood transfusion were significantly adversely correlated with recurrence and worse prognosis of HCC [21,32,35]. The development of modern surgical instruments, the perioperative management and the comprehensive treatment of HCC patients make the prognosis of the HCC patients much better than that of several decades ago [1-6,14,21,37,46]. However, the prognosis of HCC patients remains relatively poorer than other malignant diseases. And tumor recurrence remains the main factor affecting the long-term survival rates of HCC patients [1-9,15,26,33,46]. Intraoperative massive blood transfusion increases the recurrence rate and adversely affected the long-term prognosis of HCC patients. Hepatobiliary surgeons must do their best to improve their surgical skills and make use of the modern surgical instruments to reduce the unnecessary or uncalled-for blood transfusion to improve the prognosis of HCC patients to the maximum extend.

Many kinds of malignant tumors are with elevated serum fibrinogen and elevated level of fibrinogen is associated with tumor recurrence, vascular invasion and metastasis [6,14,25,37,44]. HCC is not exceptional. The liver plays a vital role in maintaining the normal function of the blood coagulation system. First, the liver syntheses many kinds of coagulation, anticoagulation and fibrinolytic factors. Second, the liver is responsible for eliminating the activated coagulation factors, plasminogen activator and fibrinogen degradation products. So, the liver is vital in keeping the balance between the coagulation system and the anticoagulation system. The coagulation state of the HCC patients is varied among the patients while most patients who have malignant tumors are usually in hypercoagulative state. The reason for the phenomenon mentioned above is that HCC patients usually have cirrhosis background. Some patients are in hypercoagulative state because they have multiple tumor lesions or lesions invading the vascular system which produce too much procoagulation factors while the background cirrhosis is not so severe. Some other patients are in hypo coagulation state because of the too severe cirrhosis background. As one of the factors involved in the coagulation system, fibrinogen level shows the same pattern as the hypercaogulation state. Normally, patients with liver diseases usually has low levels of fibrinogen, since the ability to produce fibrinogen of the patients with liver diseases is compromised. So, the tumor stage and the liver function are two main factors affecting the levels of fibrinogen. Fan et al found that HCC HepG2 produced more fibrinogen than normal cells [4,8,13,22,47]. They also concluded that the levels of fibrinogen of the patients with vascular invasion or cancerous thrombus were much higher than those of the patients without vascular or cancerous thrombus [4,8,13,22,47]. That the level of fibrinogen is significantly positively associated with tumor stage was also confirmed [4,8,13,22,47].` The fibrinogen level of stage III and IV patients is significantly higher than that of stage I and II patients when the patients have the same liver function status [4,8,13,22,47]. Pajumbo et al, found mice without fibrinogen expression are immune to tumor recurrence and metastasis, which was consistent with many clinical researches [4,8,13,22,47]. Possible reasons why fibrinogen is associated with tumor recurrence and metastasis are as follows: (1) fibriogen molecule serves as a bridge of tumor cells, platelet cells and endothelial cells to facilitate the recurrence and metastasis of tumor cells, (2) fibrinogen molecule helps the positioning and attachment of the malignant microembolism in the targeted organ, which is vital to tumor recurrence and metastasis, (3) fibriogen molecules serve as the temporary mesenchymal tissue that provide the tumor cells with nutrients and oxygen, (4) the microcomplex of fibrinogen molecules, tumor cells and platelets protect the tumor cells from attacks of the immune system [4,8,13,22,47]. In summary, serum fibrinogen level can be used as one of the markers predicting the tumor development, local invasion and metastasis.

Contrary to early phase tumor recurrence, late phase tumor recurrence is associated with different risk factors. Tumor lesion number, background liver cirrhosis status, serum CHE level and GGT were found to be independent risk factors for HCC late phase recurrence. As discussed above, tumor lesion multiplicity is one risk factor for early phase recurrence, but we can also explain that tumor multiplicity implies that the liver is severely damaged and is prone to form new tumor lesions in the diseased tissue. It has been confirmed by many previous studies that late phase HCC recurrence after surgery is mainly due to intrahepatic carcinogenesis de novo and the microenvironment is the basis of tumor late phase recurrence [1-5,19,25,37,45,46]. Although liver cirrhosis status was found to be adversely affect tumor late phase recurrence, Li Bo et al, did not confirm liver cirrhosis as an independent risk factor for late phase tumor recurrence [46]. But their research concluded that continuous variables such as albumin level and ICGR15 (indocyanine green retention after 15 minutes) could more accurately reflect the damage degree of remnant liver tissue than categorical variables such as liver cirrhosis status [46]. ICGR15 test have been adopted by many surgeons and the hospital we work with has just initiated ICGR15 test. So we could have analyzed tumor late phase recurrence more accurately and objectively if we had adopted ICGR15 test to reflect liver function reserve instead of liver cirrhosis status. Although many surgeons believed antiviral therapy preoperatively and postoperatively may reduce the recurrence risk, we did not confirm this conclusion, however. It may be due to the fact that many patients lack the data of HBV infection and antiviral therapy. Li Bo et al, as well as many other surgeons concluded that perioperative antiviral therapy, no matter preoperatively or postoperatively, might reduce tumor late phase recurrence to a rather great extend [5,17,27,45,46]. We adopted HBVDNA to reflect HBV infection status and analyzed the relationship between preoperative antiviral therapy and tumor late phase recurrence. However, the result of our research was not so hopeful as other studies. Although we have not confirmed that perioperative antiviral therapy reduces the tumor recurrence risk, we still recommend that antiviral therapy be initiated perioperatively given many other experts confirmed the finding. Serum GGT level significantly increases either when tumor vascular invasion occurs or remnant liver damage is severe. We can explain why serum GGT level is significantly associated with tumor late recurrence since elevated GGT level could be related to cirrhotic liver background.

CHE, also known as cholinesterase, has two types, namely true CHE and false CHE. True CHE is mainly located within the neuromuscular junction and responsible for the breakdown of acetylcholine while false CHE is widely distributed all over the whole body (mainly the brain, serum, liver and intestine and kidney). The CHE in the serum is mainly produced by the liver. Most of the HCC patients have decreased CHE level, since most patients have the cirrhosis background (the liver produces most of the CHE in the serum). Our study found decreased CHE level was significantly associated with late phase tumor recurrence and the possible mechanisms are as follows. The liver produces most of the CHE in the serum and thus serum CHE level is one marker of cirrhosis and liver function reserve. Low serum concentration of CHE is indicative of liver microenvironment damage and thus is associated with late phase recurrence. Zhao et al found in their study that most of the molecules of the cholinergic system were expressed in HCC tumor tissue and the expression of CHE was significantly lower than the adjacent normal liver tissue or even the cirrhotic tissue [43]. It was also discovered in the experiment that CHE molecules could inhibit the growth and cloning of SMMC-7221 and HepG2 cells and Ache is the direct acting molecule of the inhibiting procedure [43]. They also confirmed that the possible molecular signal transduction pathway involved was inhibition of the MAPK-PI3K/Akt [43]. So the low serum CHE level is indicative of low expression of CHE in the tumor cells and cirrhotic tissue and thus is significantly associated with tumor late phase recurrence [43].

In summary, our study finds that intrahepatic metastasis from the primary tumor contributes to the early phase tumor recurrence after the surgery and the carcinogenesis de novo from the cirrhotic liver is the main reason why late phase tumor recurrence occurs. So it is understandable that early phase tumor is mainly associated with tumor-related factors and late phase tumor recurrence is chiefly associated with factors reflecting the severity of the damage of the remnant liver tissue. The recurrence and metastasis of the tumor can be avoided or delayed at least through the following means: to limit the extend of the excising and adopt sophisticated surgical skills to reduce the blood loss and the blood transfusion as far as possible, to adopt comprehensive treatment protocols for the patients with vascular invasion or cancerous thrombus, to control the long-standing infection of the HCC patients to preserve the liver function reserve of the remnant liver lobes. And furthermore, patients who are at high risk of recurrence, no matter early phase or late phase, should be closely monitored and receive corresponding treatment. Through all the efforts mentioned above, we can hope to increase the recurrence-free survival time of the patients as far as possible.

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