RESEARCH ARTICLE

Risk Factors for Early Recurrence of HBV-related Hepatocellular Carcinoma Meeting Milan Criteria after Curative Resection

Wen-Jiang Zhu[1], Chu-Ying Huang[2], Chuan Li[1], Wei Peng[1], Tian-Fu Wen[1]*, Lv-Nan Yan[1], Bo Li[1], Wen-Tao Wang[1], Ming-Qing Xu[1], Jia-Yin Yang[1], Li Jiang[1]

Abstract

Background: The prognosis of patients with hepatocellular carcinoma (HCC) after curative resection varies greatly. Few studies had investigated the risk factors for early recurrence (recurrence-free time ≤ 1 year) of hepatitis B virus (HBV)-related HCCs meeting Milan criteria. Methods: A retrospective analysis was performed on the 224 patients with HCC meeting Milan criteria who underwent curative liver resection in our center between February 2007 and March 2012. The overall survival (OS) rate, recurrence-free survival (RFS) rate and risk factors for early recurrence were analyzed. Results: After a median follow-up of 33.3 months, HCC reoccurred in 105 of 224 patients and 32 died during the period. The 1-, 3- and 5-year OS rates were 97.3%, 81.6% and 75.6% respectively, and the 1-, 3- and 5-year RFS rates were 73.2%, 53.7% and 41.6%. Cox regression showed alpha-fetoprotein (AFP) > 800 ng/ml (HR 2.538, 95% CI 1.464-4.401, *P*=0.001), multiple tumors (HR 2.286, 95% CI 1.123-4.246, *P*=0.009) and microvascular invasion (HR 2.518, 95% CI 1.475-4.298, *P*=0.001) to be associated with early recurrence (recurrence-free time ≤ 1-year) of HCC meeting Milan criteria. Conclusions: AFP > 800 ng/ml, multiple tumors and microvascular invasion are independent risk factors affecting early postoperative recurrence of HCC. In addition resection appears capable of replacing liver transplantation in some situations with safety and a better outcome.

Keywords: Hepatocellular carcinoma - Milan criteria - early recurrence - factors - curative resection

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Introduction

Milan criteria (single tumor ≤ 5 cm, or showing up to 3 nodulars and each nodular ≤ 3 cm, without neither major vascular invasion nor distant metastasis), proposed in 1996 (Mazzaferro et al., 1996), was widely accepted as a standard for liver transplantation (LT) because of its satisfactory outcome. However, due to the shortage of liver donation and the limited waiting time with tumor progression, most of the patients were compromised to undergo resection. For those patients with preserved liver function, hepatectomy can be performed with relatively less technically difficult and timely convenient. But debate still exists on which should be the optimal therapy to small hepatocellular carcinoma (HCC). A recent study suggested that resection could achieve the similar 5-year OS rate as LT did but HCC recurred more frequently after resection than it did after LT (Lee et al., 2010). Small HCC with Child-Pugh A stage can achieve a favorable 5-year OS rate and a considerable proportion of it can even achieve a 5-year or even 10-year recurrence-free survival (RFS) by resection (Poon et al., 2001).

Recurrence is a tough issue of affecting the prognosis of patients. Plenty of studies investigated the factors of recurrence after liver resection. It has been generally confirmed that microvascular invasion, multi-nodular, high serum levels of alpha-fetoprotein, poor differentiation, tumor size, etc. were risk factors (Poon et al., 2000; Poon et al., 2002; Sumie et al., 2008; Kim et al., 2009; Park et al., 2009; Fan et al., 2011). The postoperative recurrence within 1 year was detected in a considerable proportion of patients who underwent hepatectomy in our center. Yet rare studies focused on postoperative early recurrence (recurrence within 1 year) of HCC meeting Milan Criteria. In this study, we sought to retrospectively analyze the clinical outcome of 224 patients with HBV-related HCC fulfilling Milan Criteria, who had been treated by primary curative resection, and to identify what kind of factors would lead to the early recurrence. So that we can confirm which group of patients would get more benefit from resection than LT.

Materials and Methods

Inclusion Criteria

A total of 283 patients with HCC meeting Milan Criteria who underwent curative liver resection between February 2007 and March 2012 in the Department of Liver

*[1]Liver Transplantation Center, Department of Liver Surgery, [2]Department of Medical Oncology, West China Hospital of Sichuan University, Chengdu, China *For correspondence: ccwentianfu@sohu.com*

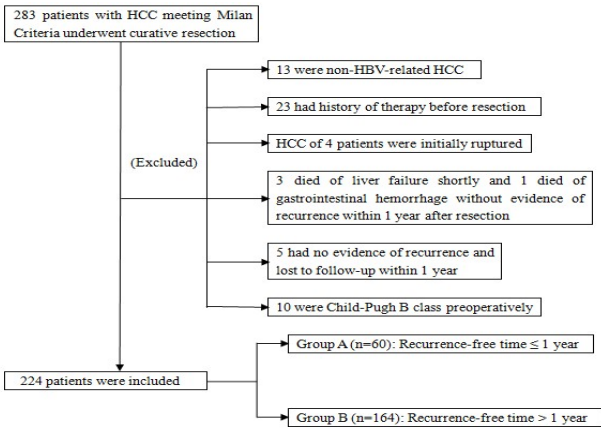

Figure 1. Flow Diagram Illustrating the Process of Selecting Target Patients

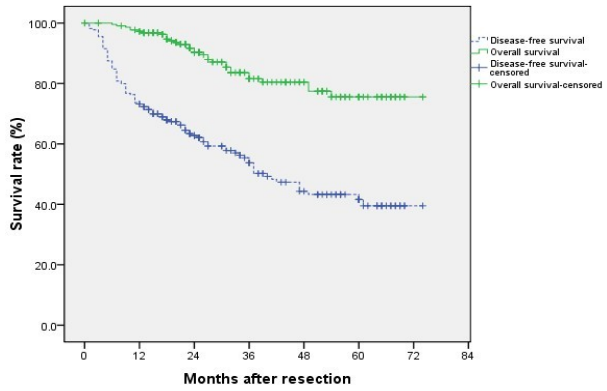

Figure 2. The Curve of RFS Rate and OS Rate of HCC Within Milan Criteria after Curative Resection

Surgery and Liver Transplantation Center of West China Hospital were included in this study. All of the operations were performed by 6 experienced surgeons. Due to Milan Criteria was a preoperative standard to screen patients for LT, the size of the tumors and the presence of vascular invasion were referred to the display of the preoperative contrast-enhanced Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). HCC were diagnosed before operation based on at least two kinds of radiology examination, serum AFP levels and history of hepatitis etc.. In this study, the diagnosis of HCC was definitely confirmed by postoperative pathological report. Patients of HCC but combined with other kind of malignant tumor were excluded finally. The differentiation of cancer cells was determined by Edmondson grade. Among the 283 patients, 13 were either HCV-related or non-viral-related HCC, 23 had a history of therapy including hepatectomy, radiofrequency ablation, transhepatic arterial chemotherapy and embolization (TACE), high intensity focused ultrasound (HIFU), percutaneous ethanol injection (PEI), chemotherapy and radiotherapy before, 3 were sent to emergency department for the rupture of HCC and transferred to our department, 1 was diagnosed as HCC and combined with primary lung cancer during the time of follow-up visit and it was not sure which kind of carcinoma he died of, 3 died of liver failure 3 days to 2 months after operation, 1 died of gastrointestinal hemorrhage 9 months after operation. All the cases above were excluded in this study. Among the 240 patients left, both 5 who had no evidence of HCC recurrence and lost to follow-up within one year after discharging the hospital, and 10 with preoperative Child-Pugh class B liver function were not included in this study, either. None of the 283 patients were with Child-Pugh class C liver function initially. In the end, 224 patients were included and retrospectively analyzed in this study. HCC recurrence within 1 year after operation was defined as early recurrence, and based on this basis they were divided into Group A (n=60, recurrence-free time ≤ 1 year) and Group B (n=164, recurrence-free time > 1 year) (Figure 1). 45 patients in group B had HCC recurrence during the period of follow-up visit.

Follow-up Visit

All of the 224 patients were regularly followed every 3 months in the first 3 years and every 6 months in the subsequent years. The status of disease was assessed by serum liver biochemistries, AFP levels, HBV-DNA levels and radiology examination. One or two kinds of radiology examination like ultrasound scan, ultrasound contrast, contrast-enhanced CT, and MRI was chosen based on the specific situation. All the patients would conventionally get anti-cancer therapy with Chinese patent drug like Huaier granule (Ren et al., 2009) and would get anti-virus therapy if their HBV-DNA levels was > 1.00E+03 copies/ml after discharging the hospital. Tumor recurrence was identified by new lesion in at least two radiology examinations and referred to the increase of AFP levels. It was not sufficient enough to diagnose recurrence of HCC only by the increasing level of AFP. The deadline of follow-up visit was the end of March 2013 or the patients' death. The overall median follow-up time was 33.3 months (3-74 months).

Statistical Analysis

Categorical data were compared by the chi-square or Fisher's exact probabilities test. Continuous variables were compared by t test or Mann-Whitney U test for variables with an abnormal distribution. The OS rate and RSF rate were analyzed by the Kaplan-Meier method and the differences were analyzed by the log-rank test. And the Cox proportional hazards model was used for the multivariate analysis of prognostic risk factors. It was considered statistically significant when the P value was less than 0.05. All analyses were performed by the SPSS software program ver. 16.0 (SPSS Inc, Chicago, IL).

Results

After the median follow-up of 33.3 months, 105 patients (47%) had HCC recurrence and 32 (14%) died during the period. 60 patients (27%) had HCC recurrence within 1 year after resection. The OS rate of 1, 3 and 5 years of the oncological patients fulfilling the Milan Criteria who underwent curative hepatic resection were 97.3%, 81.6% and 75.6% respectively, and the RSF rate of 1, 3 and 5 years of them were 73.2%, 53.7% and 41.6% (Figure 2). For those patients in group A and group B, the OS rate at 1, 2 and 3 years were 89.1%, 64.4%, 41.1% and 100%, 98.3%, 94.0% respectively ,with significant

Table 1. Demographic and Clinical Data of 224 Patients with HBV-related Hepatocellular Carcinoma Meeting Milan Criteria Who Underwent Curative Hepatic Resection

Factors	Group A n=60	Group B n=164	*P* value
Sex			0.780
Male	53(88%)	147(90%)	
Female	7(12%)	17(10%)	
Age (years)	47.68±11.21	50.24±11.04	0.127
Total bilirubin (mmol/ml)	16.64±7.14	15.92±6.56	0.482
Albumin (g/L)	42.93±4.02	42.03±3.92	0.130
INR*	1.06±0.11	1.06±0.11	0.767
Platelet (×10^9/L)	102.30±36.82	109.26±46.28	0.246
≤ 80×10^9/L	21(35%)	50 (30%)	0.520
> 80×10^9/L	39(65%)	114(70%)	
White blood cell (×10^9/L)	4.85±1.39	5.45±2.82	0.035
Neutrophil (×10^9/L)	2.86±0.96	3.40±2.19	0.011
Lymphocyte (×10^9/L)	1.56±0.61	1.55±0.70	0.877
NLR**	2.04±0.84	2.36±1.22	0.061
Ratio of Neutrophil (%)	59.00±8.92	60.76±9.19	0.201
Ratio of Lymphocyte (%)	32.08±8.07	29.54±8.24	0.042
HBeAg			0.222
Positive	15(25%)	29(18%)	
negative	45(75%)	135(82%)	
HBV-DNA (copies/ml)			< 0.001
< 1.00E+03	21(35%)	73(45%)	
> 1.00E+04	14(23%)	34(21%)	
< 1.00E+05	13(22%)	17(10%)	
> 1.00E+05	12(20%)	40(24%)	
Alpha-fetoprotein			0.003
< 800 ng/ml	38(63%)	135(82%)	
> 800 ng/ml	22(37%)	29(18%)	
Preoperative size of tumor (mm)	34.60±10.84	33.20±10.17	0.370
Number of tumors (based on preoperative radiology images)			0.018
Single nodular	54(90%)	161(98%)	
Multi-nodular	6 (10%)	3(2%)	
Number of tumors (based on pathological report)			0.008
Single nodular	46(77%)	148(90%)	
Multi-nodular	14(23%)	16(10%)	
Edmondson-Steiner grade			0.019
I, II	30 (50%)	110 (67%)	
III, VI	30 (50%)	54(33%)	
Microvascular invasion			< 0.001
Positive	22(37%)	22(13%)	
Negative	38(63%)	142(87%)	
Laparoscopic resection			0.075
Yes	6(10%)	5(3%)	
No	54(90%)	159(97%)	
Combined with splenectomy			0.295
Yes	2(3%)	14(9%)	
No	58(97%)	150(91%)	
Transfusion in the Operation			0.745
Yes	1(2%)	6(4%)	
No	59(98%)	158(96%)	
Hospital stays (days)	9.22±3.72	8.98±3.35	
Ishak scores***	(n=26)	(n=65)	0.255
2-4 scores	6(23%)	23(35%)	
5-6 scores	20(77%)	42(65%)	

*INR, international normalized ratio; **NLR, neutrophil to lymphocyte ratio; ***Only 91 patients had Ishak scores assessed in their pathological reports

Table 3. Multivariate Analysis of Independent Risk Factors For Early Recurrence after Curative Resection from Cox Regression Analysis

Factors	HR	95% CI	p-Value
AFP > 800 ng/ml	2.538	1.464-4.401	0.001
Multiple tumors	2.286	1.231-4.246	0.009
MVI	2.518	1.475-4.298	0.001

difference between the two groups ($P < 0.001$) (Figure 3).

Compared the demographic and clinical data of group A and group B, there were no significant difference in the sex, age, total bilirubin, Albumin, INR, Platelet, lymphocyte, NLR, the ratio of neutrophile granulocyte, HBeAg, preoperative size of tumor, hepatectomy combined with splenectomy, transfusion in the operation and hospital stays after resection (Table 1).

Group A had lower level of White blood cell than group B (4.85±1.39 vs. 5.45±2.82, *P*=0.035), and lower level of neutrophile granulocyte (2.86±0.96 vs. 3.40±2.19, *P*=0.011). But the proportion of lymphocyte in group A was higher than that in group B (32.08±8.07% vs. 29.54±8.24%, *P*=0.042). There was higher proportion of patients with AFP > 800 ng/ml (37% vs.18%, *P*=0.003) and with Edmondson-Steiner grade III and IV (50% vs. 33%, *P*=0.019) in group A. There was statistic difference in the level of HBV-DNA between the two groups ($P < 0.001$). However, we further analyzed the data of the two groups based on the cutoff level of HBV-DNA was 1.00E+03 copies/ml, 1.00E+04 copies/ml and 1.00E+05 copies/ml respectively, no statistic significance could be observed (35% vs. 45%, 58% vs. 66%, 80% vs. 66%, *P*=0.201, 0.342, 0.491 respectively). Only 91 patients had Ishak scores assessed in the pathological reports, as is shown in Table 1, 26 were in group A and 65 were in group B. Generally, Ishak scores 5 and 6 was considered cirrhosis, but no difference could be observed between the two groups (23% vs. 35%, *P*=0.255).

According to the result of Cox regression, AFP > 800 ng/ml, laparoscopic resection, MVI, Edmondson-Steiner grade, multiple tumors and ratio of lymphocyte were statistically significant in univariate analysis (Table 2). Only AFP > 800 ng/ml (HR 2.538, 95% CI 1.464-4.401, *P*=0.001), multiple tumors (HR 2.286, 95% CI 1.123-4.246, *P*=0.009) and MVI (HR 2.518, 95% CI 1.475-4.298, *P*=0.001) remained to be significant risk factors in the multivariate analysis (Table 3). In order to reflect the objective reality more reasonably, the number of tumors was referred to pathological report instead of preoperative radiology findings.

Table 2. Univariate Analysis for Early Recurrence after Curative Resection from Cox Regression Analysis

Factors	HR*	95% CI**	p-Value
AFP > 800 ng/ml	2.191	1.295-3.707	0.003
Laparoscopic resection	2.698	1.160-6.278	0.021
MVI	2.820	1.667-4.770	< 0.001
Edmondson-Steiner grade((I, II/III, IV)	1.866	1.125-3.096	0.016
Multiple tumors	2.298	1.262-4.184	0.007
NLR	0.770	0.583-1.018	0.067
Neutrophil	0.844	0.697-1.023	0.084
Ratio of Lymphocyte	1.031	1.001-1.063	0.045

*HR, hazard ratio, **CI, confidence interval

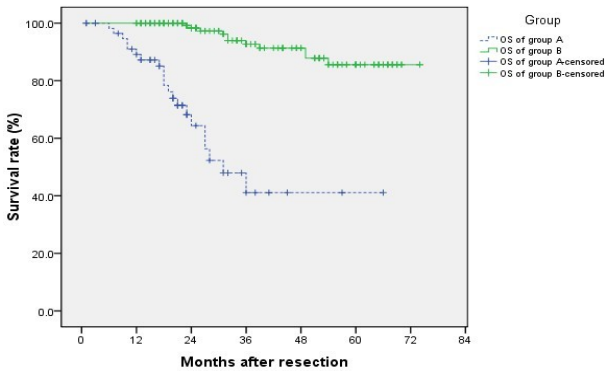

Figure 3. The Overall Survival of Group A and Group B. Patients in group A had a significantly lower overall survival than patients in group B ($P < 0.001$)

Discussion

Since Milan Criteria was proposed in 1996, it has been a classical criteria to select patients to undergo LT. However, some surgeons considered that it was too strict to exclude some patients beyond Milan Criteria which might to benefit from LT (Yao et al., 2001; Zheng et al., 2008). Due to the shortage of organ donor and the risk of disease progress on the waiting list, resection was suggested as the first-line treatment to the patients with small HCC and preserved liver function, and could achieve the favorable 5-year OS rate. The 5-year OS rate of resected HCC meeting Milan Criteria, according to the recent reports, ranged from 27% to 80% (Baccarani et al., 2008; Ueno et al., 2009; Lim et al., 2012), even 10-year OS rate in some centers could reach to 56% (Park et al., 2009), and the 5-year RFS rate ranged from 21% to 57% (Ochiai et al., 2004; Lee et al., 2010; Lim et al., 2012). In our study, we observed that the 1-, 3- and 5-year OS rate were 96%, 81% and 76% respectively, and the 1-, 3- and 5-year RFS rate were 68%, 51% and 39% respectively. Lei et al. (2013) concluded that the 1-, 3-, and 5-year OS rate of HCC meeting Milan Criteria after LT in our center were 92.0%, 85.7% and 74.1% respectively. Lee et al. (2010) in their recent study suggested that resection could achieve the similar 5-year OS rate as LT but HCC recurred more frequently after resection than it did after LT. Koniaris et al. (2011) even concluded in their studies that resection was associated with a more favorable patient survival compared with LT performed on patients with HCC within Milan Criteria. Among the total of 283 HCC patients who underwent resection within Milan Criteria and with Child-Pugh class A and B between February 2007 and March 2012, only 3 patients (1.1%) died of liver failure shortly after resection. Thus, for HCC with preserved liver function, resection can be a safe method and can achieve an ideal outcome. What is more, LT is associated with higher cost-effectiveness, higher operation risk and higher possibility of opportunistic infection because of immunosuppressor. Therefore, we agree that resection, instead of LT, ought to act as the first-line treatment, so that the limited organ donation can be applied more reasonably to benefit more patients. However, due to assuming higher risk of recurrence after resection, many surgeons suggested that salvage LT could compensate for this disadvantage (Majno et al., 2000; Belghiti et al., 2003; Cherqui et al., 2009). Perhaps risk factors of early recurrence ought to be pointed out when patients waiting the liver donor.

According to the Figure 3, Group A had a poorer OS rate than Group B ($P < 0.001$). The 1-, 2- and 3-year OS rate of patients with HCC recurrence within 1 year were 87%, 53% and 40% respectively. 21 of the 60 patients of group A died during the period of follow-up visit, the median time from recurrence to death was 12.2±7.4 months. Similarly, 11 patients died in group B and the median time from recurrence to death was 9.9±8.0 months. There was no statistic significance (P=0.442). Therefore, the RFS rate might be associated with the OS rate. Minagawa et al. (2003) and Roayaie et al. (2011) claimed in their studies that the recurrence-free time less than 1 year was an independent and adverse prognostic factor after repeat resection, but Huang et al. (2012) reported the recurrence-free time was less than 18 months. The patients who have a long RFS rate may achieve a better OS rate. Therefore, for those patients who have risk factor of early recurrence, perhaps some intervene like TACE or sorafenib should be taken aiming at those risk factors preventively would improve the prognosis after resection.

AFP is generally considered as a marker of screening and diagnosing HCC. We found that AFP > 800 ng/ml is an independent risk factor of early recurrence in our study (HR 2.538, 95% CI 1.464-4.401, P=0.001). It is not surprising that high level of AFP was an adverse factor of prognosis of HCC after hepatectomy. But the cutoff value of AFP varies in different studies. Zhou et al. (2010) analyzed the data of 158 patients with HCC ≤ 3 cm and found that AFP > 100 ng/ml was related to postoperative recurrence within 1 year. Wang et al. (2009) found that AFP > 400 ng/ml was a statistically significant univariate factor affecting RFS rate of all the HCC patients. However, no studies, as far as we know, had discussed about what cutoff value of AFP was related to the early postoperative recurrence of HCC within Milan Criteria after resection. We also analyzed 400ng/ml as the cutoff value of AFP, but no statistical significance was seen in univariate analysis (HR 1.476, 95% CI 0.886-2.460, P=0.135). Perhaps for HCC meeting Milan Criteria, patients with AFP > 800 ng/ml should be prior on the waiting list of LT.

Small lesion, especially the size of nodular smaller than 1cm, can hardly be detected by preoperative radiology imagines. Therefore, the number of tumor was based on postoperative pathological report can be more objective. As is presented in our study, the RFS rate of single tumor and multiple tumors were significantly different. Milan Criteria contains two kinds of presence of tumor, single tumor ≤ 5 cm and up to 3 nodulars and each ≤ 3 cm. Nevertheless, the two kinds of presence had different outcomes. The former presence had better RFS rate than the latter. It may indicate that the two presences of HCC should be administrated differently. And we suggest that single tumor ≤ 5 cm may be treated by resection primarily, and multiple tumors would be treated by LT if possible, or registered in the waiting list of salvage LT preferentially after hepatectomy. Fan et al. (2011) also demonstrated in their study that patients with multi-nodular tumors had a worse survival and might benefit from LT. The similar idea

was echoed by another previous study (Poon et al., 2002).

A number of studies showed that MVI was related with early recurrence after hepatectomy (Poon et al., 2000; Poon et al., 2002; Sumie et al., 2008; Kim et al., 2009), and this point was also proved in our study. Intrahepatic metastases via the portal venous system are widely accepted as a main mechanism for intrahepatic recurrence (Toyosaka et al., 1996). Hasegawa et al. (2005) demonstrated that anatomic resection can help to reduce the possibility of early recurrence resulting from vascular invasion and had a better outcome than nonanatomic resection. Though some authors found factors like AFP, tumor size, tumor number and Edmondson-Steiner grade et al. were able to predict the presence of MVI (Kim et al., 2008; Sakata et al., 2008; Zhao et al., 2013), MVI as a pathological finding cannot be able to guide preoperative strategy. Hence, due to postoperative recurrence easily, some authors proposed in their studies that HCC with MVI after resection should be recommended to salvage LT (Poon et al., 2002; Sala et al., 2004; Fuks et al., 2012).

In our study, the level of Neutrophil, the ratio of lymphocyte, Edmondson-Steiner grade, laparoscopic resection showed statistically significant in univariate analysis. But they were not sufficient enough to remain statistical significance in multivariate analysis. Mano et al. (2013) retrospectively analyzed 958 patients and found NLR was an independent predictor of survival after hepatectomy, and the best cutoff of NLR was 2.81. Kuang et al. (2011) demonstrated that peritumoral neutrophils played an crucial role in tumor progression. However, no impact of NLR (HR 0.770, 95% CI 0.583-1.018, P=0.067) and neutrophils (HR 0.844, 95% CI 0.697-1.023, P=0.084) were seen on the early recurrence in our study. For the impact of Edmondson-Steiner grade on early recurrence, similar result was observed in the cohort of HCC ≤ 3 cm (Zhou et al., 2010). Given that it was more easily to detect MVI in high grade tumors (Kim et al., 2008), the independence of Edmondson-Steiner grade might be extincted by MVI. It was deemed that laparoscopic liver resection was safe and could gain more benefit on selective HCC (Cheung et al., 2013). Our study was for this view.

New surgery strategy should be proposed for HCC within Milan Criteria considering those risk factors. Patients with AFP > 800 ng/ml and/or multiple tumor preoperatively, should be recommended to undergo LT firstly if possible, or undergo resection temporarily and be registered to wait for the salvage LT. Patients with MVI and/or new lesion detected in pathological findings has the superiority in the waiting list of salvage LT.

This study is limited by its retrospective analysis, limited samples and relatively short follow-up visit time. Hence, further studies are needed to reinforce our result. In conclusion, we found in our study that AFP > 800 ng/ml, multiple tumors and microvascular invasion were three independent risk factors affecting early recurrence of HCC within Milan Criteria after primary curative resection. It was necessary to propose the patients with those risk factors ought to be enrolled into the waiting list of salvage LT after primary resection. And resection was capable of replacing liver transplantation in some situation for its safety and ideal outcome.

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