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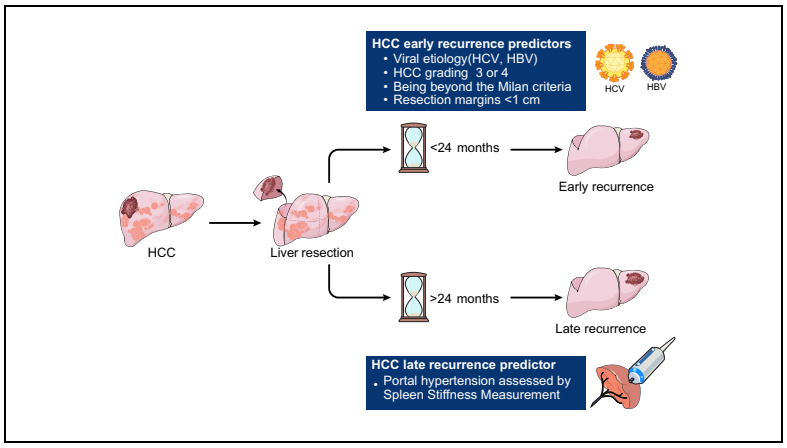
Role of liver and spleen stiffness in predicting the recurrence of hepatocellular carcinoma after resection

Graphical abstract



Highlights

- Predictive factors for early and late recurrence of HCC are different.
- Early recurrence of HCC is associated with underlying primary HCC and surgical techniques and strategies.
- Late recurrence is associated with the degree of portal hypertension assessed by spleen stiffness measurement.

Authors

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Lay summary

The main result of this study is that spleen stiffness measurement, evaluated by transient elastography, seems to be the only predictor of the late recurrence of hepatocellular carcinoma, defined as recurrence after 24 months from liver resection. Indeed, spleen stiffness measurement is directly correlated with the degree of liver disease and portal hypertension, which are both involved in carcinogenesis.

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Role of liver and spleen stiffness in predicting the recurrence of hepatocellular carcinoma after resection

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Background & Aims: Hepatocellular carcinoma (HCC) is a frequent complication of liver disease. When feasible, hepatic resection is the first-choice therapy. However, tumor recurrence complicates at least 2/3 hepatic resections at 5 years. Early recurrences are mainly tumor or treatment-related, but predictors of late recurrences are undefined. We aimed to evaluate the factors related to HCC recurrence after curative resection, with liver and spleen stiffness measurement (LSM and SSM) as markers of severity and duration of the underlying liver disease.

Methods: We enrolled patients with chronic liver disease and primary HCC suitable for hepatic resection. We followed up patients for at least 30 months or until HCC recurrence. We performed uni- and multivariate analyses to evaluate the predictive role of tumor characteristics, laboratory data, LSM and SSM for both early and late recurrence of HCC.

Results: We prospectively enrolled 175 patients. Early HCC recurrence at multivariate analysis was associated with viral etiology, HCC grading (3 or 4), resection margins <1 cm and being beyond the Milan criteria. HCC late recurrence at univariate analysis was associated with esophageal varices (hazard ratio [HR] 3.321, 95% CI 1.564–7.053), spleen length (HR 3.123, 95% CI 1.377–7.081), platelet/spleen length ratio if <909 (HR 2.170, 95% CI 1.026–4.587), LSM (HR 1.036, 95% CI 1.005–1.067), SSM (HR 1.046, 95% CI 1.020–1.073). HCC late recurrence at multivariate analysis was independently associated only with SSM (HR 1.046, CI 1.020–1.073). Late HCC recurrence-free survival was significantly different according to the SSM cut-off of 70 kPa ($p = 0.0002$).

Conclusions: SSM seems to be the only predictor of late HCC recurrence, since it is directly correlated with the degree of liver disease and portal hypertension, both of which are involved in carcinogenesis.

Lay summary: The main result of this study is that spleen stiffness measurement, evaluated by transient elastography, seems to be the only predictor of the late recurrence of hepatocellular carcinoma, defined as recurrence after 24 months from liver resection. Indeed, spleen stiffness measurement is directly correlated with the degree of liver disease and portal hypertension, which are both involved in carcinogenesis.

Introduction

Hepatocellular carcinoma (HCC) is a frequent complication in patients with chronic liver diseases, and one of the most common malignancies worldwide.[1,2] Liver resection is the first option for the treatment of patients with small solitary tumors and preserved liver function.[1,2] Tumor recurrence complicates 70% of cases of hepatic resection at 5 years, and is the expression of both intrahepatic metastasis (mainly stated as early recurrence) and the development of *de novo* tumors (late recurrence).[3–8]

Some studies[9–12] recently explored the differences between early and late recurrence and investigated the risk factors for each type of recurrence. Predictive factors for early recurrence, *i.e.* recurrence within 24 months of surgery, are well established and are mainly tumor- or treatment-related (*i.e.* tumor size, tumor number, presence of microsatellites and vascular invasion).[13] By contrast, only poor data are available for the prediction of late recurrence, *i.e.* recurrence 24 months post-surgery, which is probably related to the evolution of the underlying chronic liver disease. Among the possible predictive factors for late HCC recurrence, the presence and the degree of portal hypertension (PH) could play an important role. In fact clinically significant PH influences the natural history of advanced liver disease, as the degree of PH is directly correlated with the risk of developing complications,[14] including HCC.[15]

The measurement of hepatic venous pressure gradient (HVPG) is the gold standard method used to assess PH, which stratifies the severity and prognosis of patients with chronic liver diseases. HVPG >10 mmHg has been identified as an independent predictive factor for HCC development.[15] However HVPG is invasive, thus in the last decade, several authors[16,17] have tried to assess PH with non-invasive methods. In particular, the role of liver[16] and spleen stiffness[18–20] (LS and SS) have been investigated as non-invasive markers of PH and its complications. In addition, our research group also identified spleen

Keywords: Hepatocellular carcinoma recurrence; Liver resection; Liver stiffness measurement; Spleen stiffness measurement; Portal hypertension.
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stiffness measurement (SSM) as a predictor of clinical complications in patients with compensated cirrhosis, including HCC.[19]

The investigation of the degree of both liver fibrosis and PH with non-invasive tests could thus identify patients at risk of recurrence after resection. In fact, 2 recent studies correlated the degree of pre-resection LS, a marker of liver fibrosis and PH, with the late recurrence of HCC.[21,22]

To the best of our knowledge, the role of SS as a predictor of HCC recurrence has not yet been investigated. The aim of this study was to evaluate the role of liver stiffness measurement (LSM) and SSM in the prediction of HCC recurrence after curative resection.

Patients and methods

Between October 2008 and January 2014, patients with a first HCC diagnosis who were suitable for curative hepatic resection according to American Association for the Study of Liver Disease (AASLD) guidelines 2005[23] were prospectively and consecutively enrolled before surgery and followed up for at least 30 months after curative resection in order to identify HCC recurrence. The study was carried out at the Department of Medical and Surgical Sciences of the S. Orsola Hospital, in Bologna, Italy and approved by the local Ethics Committee; informed consent was obtained from all patients.

The inclusion criteria for the study were: patients with chronic liver disease and primary HCC suitable for hepatic resection. Exclusion criteria were patients with HCC recurrence and patients who refused surgical treatment.

HCC diagnosis was performed on the basis of clinical and/or histological and/or biochemical and/or radiological parameters, according to AASLD guidelines.[23] HCC staging (mainly size and number) was performed according to imaging evaluations, including computed tomography (CT) and magnetic resonance imaging (MRI).[23]

In brief, we judged patients suitable for surgery according to the most recent guidelines available when the study started,[23] thus, we included patients with normal bilirubin concentration, and the absence of decompensated liver cirrhosis. Neither LSM nor SSM were used for the selection of surgical candidates. Ultrasound (US) was performed on all patients during surgery in order to detect any additional nodules that had not been revealed pre-operatively and to ascertain a tumor-free margin of at least 1 cm. During parenchymal transection, clamping was always adopted to control bleeding; central venous pressure was maintained under 5–6 mmHg to prevent bleeding from hepatic veins.

Liver and spleen stiffness measurements

LSM and SSM were assessed by transient elastography within 1 week before surgery using a FibroScan® (Echosens, Paris, France) after an overnight fasting and a complete abdominal US examination before surgery. LS values were obtained as previously reported[18] and according to the Liver Stiffness Study Group "Elastica" of the Italian Association for the Study of the Liver[24] and the recommendations of the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB).[25,26] For each patient, LS values were considered adequate if the success rate was >60%, and the interquartile range (IQR) was <30% of the median value.

SSM was performed as previously described[18,19] with the same probe used to perform LSM. SS values were obtained, after overnight fasting and under US assistance. The same guidelines for LS measurement were applied (*i.e.*, success rate >60%, IQR <30%) to SS to consider the examination adequate.[24] An LSM was always performed in the cirrhotic liver parenchyma, far from the HCC, using US as a guide.

HCC recurrence diagnosis

HCC recurrence was defined according to previous studies[27,28] and the recent AASLD guidelines as early (if occurring <24 months) or late (if occurring >24 months).[1] The follow-up protocol included a clinical assessment by physical examination, US and laboratory exams every 3 months. HCC recurrence was diagnosed according to modifications of alpha-fetoprotein levels and US appearance, confirmed either by multiphasic CT or multiphasic MRI.[23]

Statistical analysis

Continuous variables were reported as median (interquartile range [IQR] or 25th–75th percentiles), and categorical variables were reported as counts (percentage). To identify factors associated with HCC recurrence, we considered the following variables: gender (male/female), age, body mass index (BMI), etiology (hepatitis C virus [HCV], hepatitis B virus [HBV], HCV-HBV *vs.* other causes such as alcohol or non-alcoholic steatohepatitis [NASH]), HCC grade tumor, HCC maximum diameter, HCC number of nodules (≤1 *vs.* >1), macrovascular invasion, microvascular invasion, histologic margins of the resection <1 cm, HCC satellitosis, localization of HCC on the right lobe/left lobe/both lobes, esophageal varices (EV), spleen length (<12 cm *vs.* ≥12 cm), platelet to spleen length ratio (<909 *vs.* ≥909), LSM and SSM assessed by FibroScan®. As most of the patients would have been in Child-Pugh class A with a low model for end-stage liver disease (MELD) score, these 2 variables were not considered in univariate and multivariate analyses.

We prospectively and consecutively enrolled all patients suitable for curative surgery. Patients were followed for 24 months in order to detect early recurrences and then for at least further 6 months starting from the 24th month in order to detect late recurrences. We performed 2 separate analyses: the first considering the first 24 months and the second a follow-up of at least a further 6 months. We assumed that if a patient had an early recurrence, he/she could not have a late recurrence. In order to identify the factors associated with early and late HCC recurrence, 2 separate analyses were performed. First, considering all the participants enrolled, a Cox regression analysis was conducted to assess the ability of the above variables in predicting the risk of HCC recurrence in the first 24 months after the resection. Second, considering only patients with at least 24 months of follow-up without early HCC recurrence, a similar Cox regression analysis was performed to assess the ability of the same variables to predict late HCC recurrence. In order to define the meaning and possible use of our results in clinical practice, we selected a cut-off value that would optimize the prediction of late HCC recurrence. According to this cut-off value, the included patients were divided into 2 sub-groups; then using the Kaplan-Meier approach the risk of late HCC recurrence was estimated and compared between the 2 sub-groups. The log-rank test was used for these comparisons. We planned to enroll about 25 patients/years and expected about a 60% recurrence rate in these patients.

We performed 2 non-planned *post hoc* analyses: first, for conflicting results[29,30] we decided to not include alpha-

fetoprotein (AFP) as a predictor of early HCC recurrence in the initial study model; however, subsequently studies demonstrated a possible role for AFP.[31,32] Thus, we tried to assess the role of AFP in predicting early HCC recurrence. The available AFP values were rescaled (divided by 100) and introduced into the model. Second, we noted that HCC late recurrence occurred mostly in patients with Metavir score F4; thus, we performed an analysis introducing Metavir score (F4 compared to score 1, 2, 3) for the prediction of late HCC recurrence.

For both early and late recurrence analyses, the same statistical approach was used. Firstly, several univariate Cox regression analyses were performed considering all the variables. Subsequently, only the variables significantly associated with the recurrence in univariate analyses were entered into a multivariate model. Finally, the best multivariate model was identified, adopting a backward elimination procedure. Data were analyzed considering deaths and liver transplantations as competing events. The estimated hazard ratios (HR) with their 95% CIs were calculated. *P* values less than 0.05, (2-tailed), were considered statistically significant. SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA.) was used for the statistical analyses.

Results

Patient characteristics

Of the 175 enrolled patients, 18 were lost to follow-up. The characteristics of the remaining 157 patients, followed up for at least 24 months from the inclusion or until HCC recurrence, is shown (Table 1). Among the overall population, 64 out of 157 (40.8%) patients did not develop HCC recurrence, 66 out of 157 (42%) patients developed an early HCC recurrence within 24 months (16 after 12 months); 27 out of 157 (17.2%) patients developed late recurrence (after 24 months) (Fig. 1).

Of the 157 patients evaluated, 1 patient who did not develop HCC recurrence underwent liver transplantation within 24 months from liver resection, 6 patients who did not develop HCC recurrence died (4 out of 6 for liver-related causes and 3 out of 6 died within 24 months of surgery) and finally 1 patient who developed late recurrence died due to liver-related causes.

The majority of the enrolled population was male (87.9%) with a median age at diagnosis of 62 years. The prevalent etiology at diagnosis was an HCV-related liver disease. Of the enrolled population, 94.9% had a Child-Pugh score A. Of our population, 3.8% had stage F1 liver fibrosis on the biopsy specimen sampled during surgery, according to the Metavir classification,[33] 8.3% had stage F2, 31.2% had stage F3, and 56.7% had stage F4. Seventy-two (45.9%) patients underwent atypical liver resection and 85 patients (54.1%) underwent anatomical resection. All patients underwent an R0 resection with absence of tumor at the resection margin.

Of the 157 enrolled patients, SSM was invalid in 15 patients (9.6%) and in 6 of them LSM was also invalid (3.8%). In addition, among these 15 patients with invalid SSM, 9 patients developed early HCC recurrence and only 1 patient developed late HCC recurrence. Of the 6 patients with invalid LSM, 4 patients developed early HCC recurrence and none of these 6 patients developed late HCC recurrence.

The median length of follow-up in the patients without late recurrence was 1,871 days, and in patients with recurrences, it was 951 days. The post-operative and 90-day mortality rate from surgery were zero.

Risk factors for early (<24 months) HCC recurrence

Among all variables evaluated in the univariate logistic Cox regression analysis (Table 2), considering 157 patients at risk of early recurrence, early recurrence was associated with etiology, HCC diameter, HCC grading, resection margins <1 cm, satellitosis and being beyond the Milan criteria. Multivariate Cox analysis showed that only viral (HCV, HBV) etiology, HCC grading (3 or 4), resection margins <1 cm and being beyond the Milan criteria were independently associated with early recurrence. Early HCC recurrence-free survival is shown (Fig. 2).

Risk factors for late (>24 months) HCC recurrence

For the Cox univariate analysis, we considered 87 patients followed for at least 24 months (4 patients excluded due to incomplete follow-up, in particular 1 patient underwent liver transplantation within 24 months of surgery, and 3 patients died within 24 months of surgery) without early recurrence. HCC late recurrence was associated with the presence of esophageal varices, spleen length, platelet/spleen length ratio <909, LSM and SSM. At Cox multivariate analysis, only an increase in SSM values was independently associated with a major risk of the late recurrence of HCC (Table 3). We defined SSM 70 kPa as the optimal cut-off value, which allowed us to obtain a positive predictive value of 75% and a negative predictive value of 75%. Late HCC recurrence-free survival is shown in the subgroup of patients with SSM >70 kPa compared to those with SSM ≤70 kPa, with a statistically significant difference ($p = 0.0002$) (Fig. 3).

Post hoc analyses

We performed 2 non-planned *post hoc* analyses regarding the role of AFP in predicting early HCC recurrence and Metavir F4 fibrosis score in predicting late HCC recurrence.

When we planned the study, AFP was not included in the model and thus, it was available for a subset of 80 patients. We included AFP in the model for early HCC recurrence, obtaining a statistically significant result at univariate analysis: HR 1.039 (CI 1.013–1.066) for each 100 ng/ml AFP increase, $p = 0.0036$. However, at multivariate analysis AFP was not statistically significant.

Including Metavir score in a *post hoc* analysis as a predictor of late HCC recurrence, we obtained a statistically significant result at univariate analysis: HR 5.891 (CI 2.028–17.114) for Metavir F4 compared to Metavir F1,2,3, $p = 0.0011$. In the multivariate analysis no variable remained statistically significant: Metavir score HR 3.72 (CI 0.946–14.481) $p = 0.060$; SSM HR 1.028 (CI 0.995–1.062) $p = 0.0966$.

Discussion

The aim of our study was to investigate the potential role of LSM and SSM in predicting early and late HCC recurrence after surgery in cirrhotic patients. The most significant result was that an increase in SSM was identified as the only predictor of late HCC recurrence. In addition, we found that viral etiology and tumor characteristics (HCC grade, resection margins <1 cm, being beyond the Milan criteria) were independently associated with early recurrence of HCC.

HCC is a frequent complication in patients with chronic liver diseases, and 1 of the most common malignancies worldwide.[1,2] Tumor recurrence complicates 70% of cases of hepatic resection at 5 years, and is the expression of both intrahepatic metastasis

Table 1. Demographics and clinical data of study population.

	Whole cohort (n = 157)	No HCC recurrence (n = 64)	Early HCC recurrence (n = 66)	Late HCC recurrence (n = 27)
Patient demographics				
Male, n (%)	138 (87.9)	54 (84.4)	58 (87.9)	26 (96.3)
Female, n (%)	19 (12.1)	10(15.6)	8 (12.1)	1 (3.7)
Age at HCC diagnosis, yr (median, IQR)	62 (37–85)	64 (42–85)	59 (35–87)	61 (48–79)
BMI (median, IQR)	25.8 (23.8–28)	26 (23.8–29.3)	26 (24–28)	25 (22.5–26.3)
ALT (median, IQR)	47 (31–74)	49 (25.5–72.5)	45.5 (33–76.3)	50 (28–108.5)
Esophageal varices, n (%)	39 (24.8)	9 (14.1)	18 (27.3)	12 (44.4)
Spleen length ≥12 cm, n (%)	83 (52.9)	23 (35.9)	41 (62.1)	19 (70.4)
Etiology, n (%)				
HCV	88 (56)	32 (50)	44 (66.7)	12 (44.4)
HBV	31 (19.8)	14 (21.9)	13 (19.7)	4 (14.8)
HCV-HBV	5 (3.2)	2 (3.1)	1 (1.5)	2 (7.4)
Alcohol/NASH	33 (21)	16 (25)	8 (12.1)	9 (33.3)
Child-Pugh score, n (%)				
A (%)	149 (94.9)	60 (93.8)	63 (95.4)	26 (96.3)
B (%)	8 (5.1)	4 (6.2)	3 (4.6)	1 (3.7)
MELD Score, n (%)				
<9	134 (95.3)	56 (87.5)	53 (80.3)	25 (92.6)
10–19	22 (4)	8 (12.5)	12 (18.2)	2 (7.4)
>20	1 (0.7)	0	1 (1.5)	0
HCC recurrence, n (%)	93 (59.2)	0	66 (42)	27 (17.2)
HCC primitive degree tumor, n (%)				
1	9 (5.8)	6 (9.4)	1 (1.5)	2 (7.4)
2	42 (26.9)	22 (34.4)	11 (16.9)	9 (33.3)
3	94 (60.2)	33 (51.6)	47 (72.3)	14 (51.9)
4	11 (7.1)	3 (4.7)	6 (9.2)	2 (7.4)
Alpha-fetoprotein (ng/ml), median (IQR) (n = 80)	10.5 (45.5)	10 (38.5)	11 (38)	14 (88)
HCC max diameter (mm), median (IQR)	35 (25)	34.5 (15.5)	40 (30)	28 (24)
HCC Nodules, n (%)				
1	104 (66.3)	45 (70.3)	40 (60.6)	19 (70.4)
2	32 (20.4)	13 (20.3)	13 (19.7)	6 (22.2)
3	12 (7.6)	2 (3.1)	8 (12.1)	2 (7.4)
>3	9 (5.7)	4 (6.3)	5 (7.6)	0
HCC microvascular invasion, n (%)				
No	70 (44.9)	33 (51.6)	24 (36.9)	13 (48.1)
Yes	86 (55.1)	31 (48.4)	41 (63.1)	14 (51.9)
HCC macrovascular invasion, n (%)				
No	135 (86)	57 (89.1)	53 (81.8)	24 (88.9)
Yes	22 (14)	7 (10.9)	12 (18.2)	3 (11.1)
Histologic Margins <1 cm, n (%)				
No	141 (89.8)	63 (98.4)	52 (78.8)	26 (96.3)
Yes	16 (10.2)	1 (1.6)	14 (21.2)	1 (3.7)
HCC satellitosis, n (%)				
No	134 (85.4)	59 (92.2)	52 (78.8)	23 (85.2)
Yes	23 (14.3)	5 (7.8)	14 (21.2)	4 (14.8)
HCC Liver lobe, n (%)				
Right	93 (59.3)	41 (64.1)	37 (56.1)	15 (55.6)
Left	47 (29.9)	16 (25)	21 (31.8)	10 (37)
Right + Left	17 (10.8)	7 (10.9)	8 (12.1)	2 (7.4)
Histologic METAVIR, n (%)				
F1	6 (3.8)	4 (6.3)	2 (3)	0
F2	13 (8.3)	10 (15.6)	3 (4.6)	0
F3	49 (31.2)	25 (39.1)	20 (30.3)	4 (14.8)
F4	89 (56.7)	25 (39.1)	41 (62.1)	23 (85.2)
Liver Stiffness (kPa), median (IQR) (n = 151)	13.6 (15.2)	11.8 (13.2)	12.4 (17.3)	18.2 (12.9)
Spleen Stiffness (kPa), median (IQR) (n = 142)	39.5 (29)	35 (24)	40 (30)	54.2 (31)

ALT, alanine aminotransferase; BMI, body mass index; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IQR, interquartile range; NASH, non-alcoholic steatohepatitis; MELD, model for end-stage liver disease; kPa, kilopascals.

(known as early recurrence) or the development of *de novo* tumors (late recurrence).[3–8,34]

The recognition of predictive factors for both early and late HCC recurrence could improve the management of these patients. While for early recurrence there is an agreement indicating that the most important predictors are the biological characteristics of the tumor,[35,36] few data are available for late recurrence.[21,22]

The results of the present study show that the late recurrence of HCC seems to be predicted by the severity of the liver disease, mainly expressed by LSM and SSM as non-invasive markers of PH, and not by the tumor characteristics. In fact, late

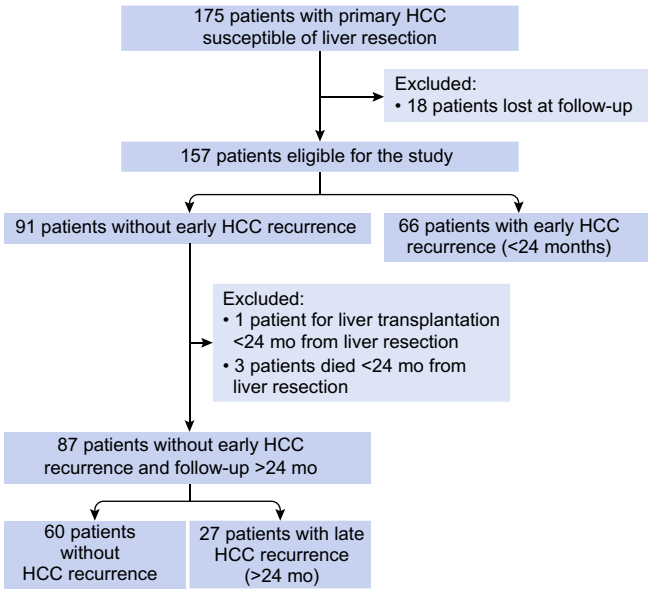

Fig. 1. Study flow-chart. HCC, hepatocellular carcinoma.

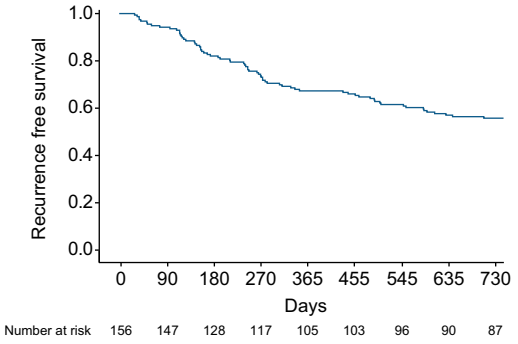

Fig. 2. Early HCC recurrence-free survival. Kaplan-Meier curve for early HCC recurrence-free survival. HCC, Hepatocellular carcinoma.

recurrence of HCC was associated with signs of PH such as the presence of EV, splenomegaly and its correlation with platelet count, LSM and SSM. Meanwhile, early HCC recurrence was associated with viral etiology, HCC diameter, HCC grade, resection margins <1 cm, satellitosis and being beyond the Milan criteria. These results suggest that late recurrence could be regarded as *de novo* tumor,[37,38] with a different tumor biology compared to early HCC recurrence,[35–36] which are distant in terms of time and not related to the primary HCC,[10] but to an increasingly severe liver disease.[10]

We observed a statistically significant difference in the curves for late HCC recurrence-free survival: patients with SSM >70 kPa had a higher recurrence rate (Fig. 3). Moreover, using a cut-off of 70 kPa for SSM either the positive and the negative predictive values were 75%.

To the best of our knowledge this is the first study documenting that SSM is an independent predictor of late HCC recurrence. SSM is an accurate non-invasive method for the assessment of the degree of PH, as previously demonstrated by our group[18] and others.[39] It is widely accepted that the degree of PH negatively influences the natural history of chronic liver disease,[14] including HCC occurrence.[15] We also found that SSM can predict liver disease complications, including HCC development.[19]

The predictive role of SSM in late HCC recurrence that we observed in the present study confirms that SSM represents an accurate non-invasive surrogate marker of PH, since PH is one of the pathogenetic factors involved in HCC development.[14] In fact cirrhosis scars may be associated with vascular proliferation due to an impaired oxygen delivery caused by intrahepatic shunts, veno-occlusive thrombotic lesions, a reduction in the

Table 2. Univariate and multivariate analyses for independent variables associated with HCC early recurrence.

Early HCC recurrence n = 157	Univariate		Multivariate	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Sex Male	1.012 (0.488–2.100)	0.9740		
Age*	0.988 (0.964–1.012)	0.3277		
BMI*	0.977 (0.922–1.035)	0.4271		
ALT*	0.998 (0.994–1.003)	0.4685		
Viral etiology	2.303 (1.104–4.803)	0.0261	2.337 (1.088–5.021)	0.0296
Esophageal varices	1.280 (0.733–2.233)	0.3856		
Spleen length ≥12 cm	1.581 (0.966–2.589)	0.0686		
Platelet/spleen length >909	1.089 (0.660–1.796)	0.7389		
Liver stiffness measurement*	1.005 (0.984–1.027)	0.6191		
Spleen stiffness measurement*	0.998 (0.985–1.011)	0.7849		
HCC liver lobe		0.7348		
Right	1			
Left	0.774 (0.355–1.686)			
Bilateral	0.908 (0.395–2.085)			
HCC max diameter (mm)*	1.010 (1.001–1.020)	0.0348		
HCC number of nodules >1	1.456 (0.885–2.394)	0.1393		
HCC grading 3–4	2.442 (1.282–4.653)	0.0066	2.077 (1.046–4.124)	0.0368
Histologic margins <1 cm	3.751 (2.213–6.360)	<0.0001	1.987 (1.060–3.727)	0.0322
HCC satellitosis	1.834 (1.037–3.244)	0.0372		
HCC macrovascular invasion	1.566 (0.838–2.924)	0.1596		
HCC microvascular invasion	1.401 (0.840–2.337)	0.1959		
HCC beyond Milan criteria	2.312 (1.428–3.742)	0.0006	2.132 (1.272–3.575)	0.0041

ALT, alanine aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma; HR, hazard ratio.
* HR for one-unit increase; Cox regression analysis.

Table 3. Univariate, multivariate and *post hoc* multivariate analyses for independent variables associated with HCC late recurrence.

Late HCC recurrence n = 87	Univariate		Multivariate		*Post hoc* multivariate	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Sex Male	0.976 (0.941–1.012)	0.1888				
Age*	0.964 (0.932–1.020)	0.1875				
BMI*	0.922 (0.860–0.989)	0.0224				
ALT*	1.001 (0.995–1.007)	0.7274				
Viral etiology	1.560 (0.707–3.441)	0.2711				
Esophageal varices	3.321 (1.564–7.053)	0.0018				
Metavir score	5.891 (2.028–17.114)	0.0011			3.720 (0.946–14.481)	0.0600
Spleen length ≥12 cm	3.123 (1.377–7.081)	0.0064				
Platelet/spleen length <909	2.170 (1.026–4.587)	0.0425				
Liver stiffness measurement*	1.036 (1.005–1.067)	0.0210				
Spleen stiffness measurement*	1.046 (1.020–1.073)	0.0005	1.046 (1.020–1.073)	0.0005	1.028 (0.995–1.062)	0.0966
HCC liver lobe		0.4709				
Right	1					
Left	1.352 (0.331–5.514)					
Bilateral	2.055 (0.483–8.743)					
HCC max diameter (mm)*	0.994 (0.977–1.011)	0.4867				
HCC number of nodules >1	1.035 (0.460–2.327)	0.9344				
HCC grading 3–4	1.131 (0.532–2.406)	0.7489				
Histologic margins <1 cm	1.527 (0.272–8.575)	0.6305				
HCC satellitosis	1.642 (0.556–4.853)	0.3697				
HCC macrovascular invasion	0.989 (0.291–3.356)	0.9856				
HCC microvascular invasion	1.105 (0.525–2.326)	0.7929				
HCC beyond Milan criteria	0.953 (0.405–2.246)	0.9129				

ALT, alanine aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma; HR, hazard ratio.
* HR for one-unit increase; Cox regression analysis.

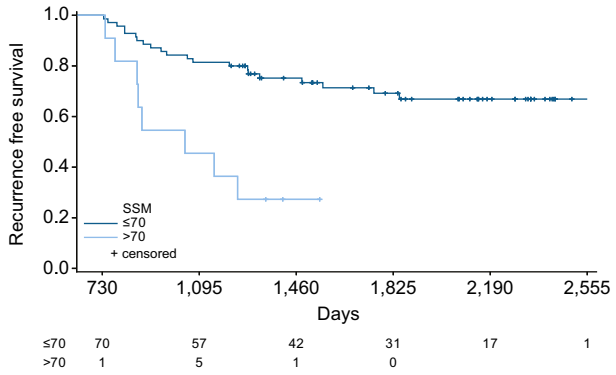

Fig. 3. Late HCC recurrence-free survival. Survival free of HCC late recurrence according to SSM ≤70 kPa or SSM >70 kPa. HCC, hepatocellular carcinoma; SSM, spleen stiffness measurement.

sinusoidal area, sinusoidal capillarization and increased resistance to blood flow.[40] Thus, angiogenic factors are more highly expressed by hepatocytes in cirrhotic nodules, above all by the production of hypoxia-inducible factor-1 and other cytokines, which induce both fibrogenesis and angiogenesis, finally leading to PH and carcinogenesis.[41]

As far as LSM is concerned, Jung *et al.*[21,22] found a correlation between late HCC recurrence and LSM. Conversely, we found a positive correlation between LSM and late HCC recurrence only at univariate analysis, while at multivariate analysis only SSM, which was not measured by Jung *et al.*,[21,22] correlated with late recurrence. A possible explanation for these different results is the known better accuracy[42] of SSM than LSM, as surrogate markers in evaluating PH, which plays an important role in HCC development and recurrence.[15]

It is worth noting that in our series most of the HCC late recurrences occurred in patients with Metavir score F4 (Table 1), confirming that the late HCC recurrences develop mostly in advanced chronic liver disease. However, the fibrosis staging according to the Metavir grade was not included in the model. Indeed, we expected that most of the included patients would have Metavir F4 and this scoring system would not be able to describe a further progression of chronic advanced liver disease. Otherwise, we included in the model other variables considered accurate in defining a disease progression in patients with Metavir F4, such as LSM and SSM, platelet count, spleen length and platelet count to spleen length ratio. Anyway, we performed a *post hoc* analysis to assess the possible predictive role of Metavir F4 compared to Metavir F1-2–3. At univariate analysis the result was statistically significant, but in the multivariate analysis no variable remained statistically associated to HCC late recurrences, underlining the critical problem of inflating the number of variables included in the model in the presence of suboptimal sample size.

Taking into account early recurrence, our results confirm findings of other studies[43–45] that this recurrence is predicted by intrinsic HCC characteristics and by the radicality of the surgical resection. In fact, early recurrence was associated at multivariate analysis with the viral etiology of the underlying liver disease, tumor differentiation (grading), resection margins <1 cm, and HCC staging beyond Milan criteria. When we planned the study, on the basis of conflicting results[29,30] we decided to not include AFP as a predictor of early recurrence. However, subsequently further studies become available and demonstrated a possible predictor role of this biomarker.[31,32] Thus, we performed a *post hoc* analysis in the subset of 80 patients with available AFP values. We obtained a statistically significant result at univariate analysis that did not remain significant in the multivariate analysis; with these limitations we cannot support the role of alpha-fetoprotein in our series for the prediction of early HCC recurrence.

Most of the patients enrolled had an HCV-related liver disease. HCV liver disease etiology has already been investigated and associated with a higher risk of HCC recurrence after hepatectomy.[46,47] Sasaki *et al.*[47] showed that patients with HCV had a 2–5% higher incidence of HCC recurrence than those with HBV after 20 months post-resection, and HCV was found to be an independent predictor of HCC recurrence. In addition, another recent study[48] highlighted that HCV association with HCC recurrence was stronger in the first year after resection and subsequently the trend decreased.

Regarding the Milan criteria, the relationship between tumor size, number, resection margins <1 cm and recurrence is clear.[49] HCC nodules ≥5 cm in diameter are associated with an increased recurrence rate[50,51] due to the higher risk of intrahepatic metastases, invasion of the portal vein,[50] and microvascular invasion,[51] which are all observed in the presence of larger tumors.

Our data confirm that tumor grade is another strong predictor of early recurrence,[27,49,52] although it is well known that its predictive value is related to microvascular invasion:[27,53,54] a poorly differentiated tumor brings a 2-fold increased risk of early recurrence compared to well-moderately differentiated tumors.[27]

By identifying completely different predictors among the early and late recurrences, our results may indirectly confirm previous studies which showed that 2 years (24 months) is the correct time interval to discriminate between early and late HCC recurrence.[2,37]

A limitation of the present study is that we observed only 27 late recurrences of HCC and, assessing many variables, we cannot rule out that there may have been a data bias and some overfitting. We aimed to assess the predictors of early and late recurrences after HCC curative resection, above all LSM and SSM, and we also had to test other plausible predictors. However, there is little data available on HCC recurrence predictors and we probably introduced too many variables compared to the realistically expected number of events. However, we performed the study in a single center to minimize other factors related to different surgical techniques.

Another limitation was that we were unable to evaluate, in patients with HCV-related liver disease, the possible effect of the new direct antiviral agents (DAAs) on HCC recurrence. In fact, all our patients were enrolled in a pre-era DAAs era and only 39 out of 93 (41.9%) patients with HCV reached the DAA era without HCC recurrence; 38 out of 39 patients (97.4%) reached sustained virological response and 5 of them developed late HCC recurrence. Due to the small sample size it is not possible to evaluate a potential effect of HCV eradication with DAAs on HCC recurrence. However, this association is still under debate.[55–57] The strength of our study is that in a single tertiary center we were able to explore both early and late recurrence in a cohort of candidates for HCC surgical resection, thus variability in surgical approach and technique were minimized. Inclusion in the study was based on accepted and validated criteria,[2] and consequently our cohort can be considered representative of patients with HCC and the results can be generalized or transferred to similar contexts. In addition, only around 10% (18/175) of the enrolled patients were lost at follow-up. Regarding the LSM and SSM feasibility, spleen stiffness measurement produced invalid results in 15 patients out of 157 (10%) (Fig. 1) and LSM was invalid in 6 of these 15 patients (3.8% out of 157 patients). These data are in accordance with the literature,[18,19,58] which reports percentages ranging from 10% to about 15%. Using patients with early recurrence-free survival as an inception cohort enabled us to demonstrate a statistically significant association of late HCC recurrence with SSM.

However other factors not explored in this study could play a role, and the actual predictive accuracy seems at least moderate, thus preventing the creation of an accurate predictive model. Additional studies are thus needed to validate the present results and to explore the possible role of other predictors.

It is known that a follow-up after resection is recommended due to high rates of treatable recurrences;[59] in fact, the European Association for the Study of the Liver recently published HCC recommendations advising a 3–4 month interval of follow-up in the first year after resection for primary HCC. For late HCC recurrence, the follow-up strategies are still not clearly defined. According to our results, we suggest performing SSM before HCC resection, in order to help the clinician in designing a tailored surveillance program mainly for those patients with SSM >70 kPa.

In conclusion, SSM seems to be an independent predictor of HCC late recurrence after liver resection, since it is directly correlated with the degree of liver disease and PH. In addition, our study confirms that tumor-related factors such as viral etiology, HCC grade, resection margins <1 cm and being beyond the Milan criteria are independently associated with early recurrence.

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Conflict of interest
The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
GM, AC[2], FR, AC[1], MC: collected data. GM, AC[3], GC, MLBR, AC[1]: analysed data. GM, AC[2], AC[3], DF: oversaw the study, wrote the draft and did the final approval of the manuscript.

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Supplementary data
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References
[1] Heimbach J, Kulik LM, Finn R, Sirlin CB, Abecassis M, Roberts LR, et al. Aasld guidelines for the treatment of hepatocellular carcinoma. Hepatology 2017;67:358–380.
[2] Dufour JF, Greten TF, Raymond E, Roskams T, De T, Ducreux M, et al. Clinical Practice Guidelines EASL – EORTC Clinical Practice Guidelines: management of hepatocellular carcinoma European Organisation for Research and Treatment of Cancer. J Hepatol 2012;56:908–943.
[3] Roayaie S, Blume IN, Thung SN, Guido M, Fiel M-I, Hiotis S, et al. A system of classifying microvascular invasion to predict outcome after resection

in patients with hepatocellular carcinoma. Gastroenterology 2009;137:850–855.
[4] Poon RTP, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, et al. Extended hepatic resection for hepatocellular carcinoma in patients with cirrhosis: is it justified? Ann Surg 2002;236:602–611.
[5] Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, Falck-Ytter Y. Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. Ann Intern Med 2013;158:329–337.
[6] Mazzaferro V, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, et al. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. Hepatology 2006;44:1543–1554.
[7] Ishizawa T, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, et al. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. Gastroenterology 2008;134:1908–1916.
[8] Pawlik TM, Esnaola NF, Vauthey J-N. Surgical treatment of hepatocellular carcinoma: similar long-term results despite geographic variations. Liver Transpl 2004;10:S74–S80.
[9] Zhou J-Y, Zhang L, Li L, Gu G-Y, Zhou Y-H, Chen J-H. High hepatitis B virus load is associated with hepatocellular carcinomas development in Chinese chronic hepatitis B patients: a case control study. Virol J 2012;9:16.
[10] Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. Cancer 2000;89:500–507.
[11] Tung-Ping Poon R, Fan ST, Wong J. Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. Ann Surg 2000;232:10–24.
[12] Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. J Hepatol 2003;38:200–207.
[13] Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis 2005;25:181–200.
[14] Ripoll C, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. Gastroenterology 2007;133:481–488.
[15] Ripoll C, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A, et al. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. J Hepatol 2009;50:923–928.
[16] EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63:237–264.
[17] Berzigotti A. Non invasive evaluation of portal hypertension using ultrasound elastography. J Hepatol 2017;67:399–411.
[18] Colecchia A, Montrone L, Scaioli E, Bacchi-Reggiani ML, Colli A, Casazza G, et al. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. Gastroenterology 2012;143:646–654.
[19] Colecchia A, Colli A, Casazza G, Mandolesi D, Schiumerini R, Reggiani LB, et al. Spleen stiffness measurement can predict clinical complications in compensated HCV-related cirrhosis: a prospective study. J Hepatol 2014;60:1158–1164.
[20] Stefanescu H, Grigorescu M, Lupsor M, Procopet B, Maniu A, Badea R, et al. Spleen stiffness measurement using Fibroscan for the noninvasive assessment of esophageal varices in liver cirrhosis patients. J Gastroenterol Hepatol 2011;26:164–170.
[21] Jung KS, Kim SU, Choi GH, Park JY, Park YN, Kim DY, et al. Prediction of recurrence after curative resection of hepatocellular carcinoma using liver stiffness measurement (FibroScan®). Ann Surg Oncol 2012;19:4278–4286.
[22] Jung KS, Kim JH, Kim SU, Song K, Kim BK, Park JY, et al. Liver stiffness value-based risk estimation of late recurrence after curative resection of hepatocellular carcinoma: development and validation of a predictive model. PLoS ONE 2014;9 e99167.
[23] Bruix J, Sherman M. Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.
[24] Bonino F, Arena U, Brunetto MR, Coco B, Fraquelli M, Oliveri F, et al. Liver stiffness, a non-invasive marker of liver disease: a core study group report. Antivir Ther 2010;15:69–78.
[25] Cosgrove D, Piscaglia F, Bamber J, Bojunga J, Correas J-M, Gilja O, et al. Guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: clinical applications. Ultraschall Med 2013;34:238–253.
[26] Bamber J, Cosgrove D, Dietrich CF, Fromageau J, Bojunga J, Calliada F, et al. guidelines and recommendations on the clinical use of ultrasound elastography. Part 1: basic principles and technology. Ultraschall Med 2013;34:169–184.
[27] Cucchetti A, Piscaglia F, Caturelli E, Benvegnù L, Vivarelli M, Ercolani G, et al. Comparison of recurrence of hepatocellular carcinoma after resection in patients with cirrhosis to its occurrence in a surveilled cirrhotic population. Ann Surg Oncol 2009;16:413–422.
[28] Portolani N, Coniglio A, Ghidoni S, Giovanelli M, Benetti A, Tiberio GAM, et al. Early and late recurrence after liver resection for hepatocellular carcinoma. Ann Surg 2006;243:229–235.
[29] Giannini EG, Marenco S, Borgonovo G, Savarino V, Farinati F, Del Poggio P, et al. Alpha-fetoprotein has no prognostic role in small hepatocellular carcinoma identified during surveillance in compensated cirrhosis. Hepatology 2012;56:1371–1379.
[30] Tateishi R, Shiina S, Yoshida H, Teratani T, Obi S, Yamashiki N, et al. Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. Hepatology 2006;44:1518–1527.
[31] Yang S-L, Liu L-P, Yang S, Liu L, Ren J-W, Fang X, et al. Preoperative serum α-fetoprotein and prognosis after hepatectomy for hepatocellular carcinoma. Br J Surg 2016;103:716–724.
[32] Shimoda M, Tago K, Shiraki T, Mori S, Kato M, Aoki T, et al. Risk factors for early recurrence of single lesion hepatocellular carcinoma after curative resection. World J Surg 2016;40:2466–2471.
[33] Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology 1994;20:15–20.
[34] Belghiti J, Panis Y, Farges O, Benhamou JP, Fekete F. Intrahepatic recurrence after resection of hepatocellular carcinoma complicating cirrhosis. Ann Surg 1991;214:114–117.
[35] Xia H, Chen J, Shi M, Gao H, Sekar K, Seshachalam VP, et al. EDIL3 is a novel regulator of epithelial-mesenchymal transition controlling early recurrence of hepatocellular carcinoma. J Hepatol 2015;63:863–873.
[36] Koh YW, Park Y-S, Kang HJ, Shim JH, Yu E. MET is a predictive factor for late recurrence but not for overall survival of early stage hepatocellular carcinoma. Tumor Biol 2015;36:4993–5000.
[37] Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711.
[38] Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999;19:329–338.
[39] Colecchia A, Marasco G, Taddia M, Montrone L, Eusebi LH, Mandolesi D, et al. Liver and spleen stiffness and other noninvasive methods to assess portal hypertension in cirrhotic patients: a review of the literature. Eur J Gastroenterol Hepatol 2015;27.
[40] Rappaport AM, MacPhee PJ, Fisher MM, Phillips MJ. The scarring of the liver acini (Cirrhosis). Tridimensional and microcirculatory considerations. Virchows Arch A Pathol Anat Histopathol 1983;402:107–137.
[41] Onori P, Morini S, Franchitto A, Sferra R, Alvaro D, Gaudio E. Hepatic microvascular features in experimental cirrhosis: a structural and morphometrical study in CCl4-treated rats. J Hepatol 2000;33:555–563.
[42] Song J, Huang J, Huang H, Liu S, Luo Y. Performance of spleen stiffness measurement in prediction of clinical significant portal hypertension: a meta-analysis. Clin Res Hepatol Gastroenterol 2017.
[43] Cheng Z, Yang P, Qu S, Zhou J, Yang J, Yang X, et al. Risk factors and management for early and late intrahepatic recurrence of solitary hepatocellular carcinoma after curative resection. HPB 2015;17:422–427.
[44] Du Z-G, Wei Y-G, Chen K-F, Li B. Risk factors associated with early and late recurrence after curative resection of hepatocellular carcinoma: a single institution's experience with 398 consecutive patients. Hepatobiliary Pancreat Dis Int 2014;13:153–161.
[45] Li T, Fan J, Qin L-X, Zhou J, Sun H-C, Qiu S-J, et al. Risk factors, prognosis, and management of early and late intrahepatic recurrence after resection of primary clear cell carcinoma of the liver. Ann Surg Oncol 2011;18:1955–1963.
[46] Utsunomiya T, Shimada M, Kudo M, Ichida T, Matsui O, Izumi N, et al. A Comparison of the surgical outcomes among patients with HBV-positive, HCV-positive, and non-B non-C hepatocellular carcinoma. Ann Surg 2015;261:513–520.
[47] Sasaki K, Shindoh J, Nishioka Y, Margonis GA, Sugawara T, Andreatos N, et al. Impact of viral etiology on postoperative de novo recurrence after hepatectomy for hepatocellular carcinoma in cirrhotic patients. J Gastrointest Surg 2017;21:487–495.

[48] Zhang Z-M, Zhang Y-M, Yao F, Yi P, Huang S, Liu J-Y, et al. Analysis on postoperative efficacy of radical hepatectomy for patients with non-HBV/HCV hepatocellular carcinoma. Asian Pac J Cancer Prev 2015;16:3479–3483.
[49] Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Tahara M, Kakisaka T, et al. Analysis of the risk factors for early death due to disease recurrence or progression within 1 year after hepatectomy in patients with hepatocellular carcinoma. World J Surg Oncol 2012;10:107.
[50] Liu L, Miao R, Yang H, Lu X, Zhao Y, Mao Y, et al. Prognostic factors after liver resection for hepatocellular carcinoma: a single-center experience from China. Am J Surg 2012;203:741–750.
[51] Kaibori M, Ishizaki M, Matsui K, Kwon A-H. Predictors of microvascular invasion before hepatectomy for hepatocellular carcinoma. J Surg Oncol 2010;102:462–468.
[52] Lammer J, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, et al. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. Cardiovasc Intervent Radiol 2010;33:41–52.
[53] Colecchia A, Scaioli E, Montrone L, Vestito A, Di Biase AR, Pieri M, et al. Pre-operative liver biopsy in cirrhotic patients with early hepatocellular carcinoma represents a safe and accurate diagnostic tool for tumor grading assessment. J Hepatol 2011;54:300–305.
[54] Pawlik TM, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy. Ann Surg 2007;245:435–442.
[55] Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol 2016;65:727–733.
[56] Cabibbo G, Petta S, Calvaruso V, Cacciola I, Cannavò MR, Madonia S, et al. Is early recurrence of hepatocellular carcinoma in HCV cirrhotic patients affected by treatment with direct-acting antivirals? A prospective multicentre study. Aliment Pharmacol Ther 2017;46:688–695.
[57] Ikeda K, Kawamura Y, Kobayashi M, Kominami Y, Fujiyama S, Sezaki H, et al. Direct-acting antivirals decreased tumor recurrence after initial treatment of hepatitis C virus-related hepatocellular carcinoma. Dig Dis Sci 2017;62:2932–2942.
[58] Fraquelli M, Rigamonti C, Colombo M. Spleen stiffness measured by transient elastography accurately predicts esophageal varices in liver cirrhosis. Gastroenterology 2012;143.
[59] Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul J-L, et al. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. J Hepatol 2018;69:182–236.