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

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Keywords: Hepatocellular carcinoma recurrence, Liver resection, Liver stiffness measurement, Spleen stiffness measurement, Portal hypertension.

Electronic word count: 6681

Number of figures and tables: 6

Financial Support: No grants or other financial support.

Conflict of interest statement: None

Authors' contributions to manuscript: 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.

Acknowledgment: We sincerely thank Dr. Elton Dajti for the valuable advices.

Abstract

Background

Hepatocellular carcinoma (HCC) is a frequent complication of liver disease, and hepatic resection, when feasible, is the first-choice therapy. Tumour recurrence complicates at least 2/3 hepatic resections at 5 years. Early recurrences (<24 months of surgery) are mainly tumour or treatment-related, but predictors of late recurrences (24 months post-surgery) 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 in order to evaluate the predictive role of tumour characteristics, laboratory data, LSM and SSM for both HCC early and late recurrences.

Results

We prospectively enrolled 175 patients. Early HCC recurrence at multivariate analysis was associated with viral (HCV, HBV) 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 (HR 3.321, CI 1.564-7.053,), spleen length (HR 3.123, CI 1.377-7.081), platelet/spleen length ratio if <909 (HR 2.170, CI 1.026-4.587), LSM (HR 1.036, CI 1.005-1.067), SSM (HR 1.046, 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 70kPa (p=0,0002).

Conclusions

SSM seems to be the only predictor of HCC late recurrence, since is directly correlated to liver disease and portal hypertension degree, both 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, since it is directly correlated to 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 tumours and a preserved liver function [1,2]. Tumour 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 tumours (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 tumour- or treatment-related (i.e. tumour size, tumour 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 HCC late 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 PH degree 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) as a non-invasive marker of PH and its complications has been investigated. In addition, our research group also identified spleen 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 also identify patients at risk of recurrence after resection. In fact, two 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 been investigated yet. The aim of this study was to evaluate the role of liver (LSM) and spleen stiffness measurements 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 subjects.

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 tumour-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 (TE) within one week before surgery using a FibroScan® (Echosens, Paris, France) after an overnight fasting and a complete abdominal ultrasound (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]. A 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 three 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 [inter-quartile range (IQR) or 25^{th}-75^{th} 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 steato-hepatitis (NASH)], HCC grade tumour, 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 (<12cm vs ≥12cm), 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 MELD (Model for End-Stage Liver Disease) score, these two 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 six months starting from the 24th month in order to detect late recurrences. We performed two separate analyses: the first considering the first 24 months and the second a follow up of at least a further six months. We assumed that if a patient has an early recurrence, he/she cannot have a late recurrence.

In order to identify the factors associated with early and late HCC recurrence, two 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 an early HCC recurrence, a similar Cox regression analysis was performed to assess the ability of the same variables to predict late HCC recurrences. In order to provide an exemplification of the meaning and possible use of our results in clinical practice, we selected a cut-off value that allowed to optimize the ability in predicting HCC late recurrences. According to this cut off value, the included patients were divided into two sub-groups; then using the Kaplan-Meier approach the risk of late HCC recurrence was estimated and compared between the two 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 two 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 in 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 in 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% confidence intervals (CI) were calculated. P values less than 0.05, (two 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 at follow-up. Table 1 shows the characteristics of the remaining 157 patients, followed up for at least 24 months from the inclusion or until HCC

recurrence. 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) (see **Fig. 1**).

Of the 157 patients evaluated, one patient who did not develop HCC recurrence underwent liver transplantation within 24 months from liver resection, six 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 one 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. 3.8% of our population had a F1 grade of liver fibrosis on the biopsy specimen sampled during surgery, according to the Metavir classification[33], 8.3% had a grade F2, 31.2 % had a grade F3, and 56.7% had a grade F4. Seventy-two (45.9%) patients underwent atypical liver resection and 85 patients (54.1%) underwent anatomical resection. All patients underwent a R0 resection with absence of tumour 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, nine patients developed early HCC recurrence and only one patient developed late HCC recurrence**.** Of the six patients with invalid LSM, four patients developed early HCC recurrence and none of these six patients developed late HCC recurrence.

The median length of follow up in the patients without late recurrence was 1871 days, and in patients with recurrences, it was 951 days. The post-operative and 90-day mortality rate from surgery were null.

Risk factors for early (<24 months) HCC recurrence

Among all variables evaluated in the univariate Logistic Cox Regression Analysis (see Table 2), considering 157 patients at risk of early recurrences, early recurrences were 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 in **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 (four patients excluded due to incomplete follow up, in particular one patient underwent liver transplantation within 24 months of surgery, and three 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 as optimal cut-off value for SSM 70 kPa which allowed 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 significative difference (p=0.0002), **Fig.3**.

Post-hoc analyses

We performed two 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 at univariate analysis a statistically significant result: 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 at univariate analysis a statistically significant result 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 recurrences after surgery in cirrhotic patients. The most significant result was that an increase in SSM was identified as the only predictor of late recurrences of HCC. In addition we found that viral etiology and tumour 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 one of the most common malignancies worldwide [1,2]. Tumour recurrence complicates 70% of cases of hepatic resection at 5 years, and is the expression of both intrahepatic metastasis (known as early recurrence) or the development of de novo tumours (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 tumour [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 tumour characteristics. In fact, late 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. On the other hand, HCC early recurrence was associated with viral etiology, HCC diameter, HCC grade, resection margins < 1cm, satellitosis and being beyond the Milan criteria. These results suggest that late recurrence could be regarded as de novo tumour[37,38] with a different tumour biology compared to early HCC recurrences [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%.

At best of our knowledge this is the first study documenting that SSM is an independent predictor of HCC late recurrence. SSM is a non-invasive accurate method for the assessment of PH degree, as previously demonstrated by our group[18] and others [39]. It is widely accepted that PH degree 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 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 (HIF-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 to note 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 grading 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 predictor 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, tumour differentiation (grading), resection margins < 1cm, 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 alfa-fetoprotein in our series for the prediction of HCC early 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 and colleagues [47] showed that HCV patients had a 2-5% higher incidence of HCC recurrence compared with HBV patients 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 tumour 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 micro-vascular invasion (MVI) [51], which are all observed in the presence of larger tumours.

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

By identifying completely different predictors among the early and late recurrences, our results may indirectly confirm previous studies which showed that two 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 HCC late recurrences 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. On the other hand, we designed the study just in one center in order 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 recurrences. In fact, all our patients were enrolled in a pre-era DAAs era and only 39 out of 93 (41.9%) HCV patients reached DAAs era without HCC recurrences; 38 out of 39 patients (97,4%) reached sustained virological response and five of them developed HCC late 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 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 HCC late 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, EASL recently published HCC recommendations advise a 3–4 month interval of follow-up in the first year after resection for primary HCC. For HCC late recurrence, the follow-up strategies are still not clearly defined. According to our results, we suggest to perform 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, spleen stiffness measurement seems to be an independent ~~only~~ predictor of HCC late recurrence after liver resection, since it is directly correlated to the degree of liver disease and portal hypertension. In addition, our study confirms that tumour-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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Table 1. Demographics and clinical data of study population.

	Whole cohort (No. = 157)	No HCC Recurrence (No.=64)	Early HCC recurrence (No.=66)	Late HCC Recurrence (No.=27)
Patient demographics				
Male, No. (%)	138 (87.9)	54 (84.4)	58 (87.9)	26 (96.3)
Female, No. (%)	19 (12.1)	10(15.6)	8 (12.1)	1 (3.7)
Age at HCC diagnosis, yrs (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, No. (%)	39 (24.8)	9 (14.1)	18 (27.3)	12 (44.4)
Spleen length ≥12cm, No. (%)	83 (52.9)	23 (35.9)	41 (62.1)	19 (70.4)
Etiology , No. (%)				
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, No. (%)				
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, No. (%)				
< 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 tumour, No. (%)				
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, No. (%)				
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, No. (%)				
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, No. (%)				
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, No. (%)				
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, No. (%)				
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, No. (%)				
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, No. (%)				
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)

Abbreviations: No., number; HCC, hepatocellular carcinoma; IQR, inter quartile range; BMI, body mass index; ALT, alanine amino-transferase; HCV, hepatitis C virus; HBV, hepatitis B virus; NASH, non-alcoholic steato-hepatitis; MELD, model for End-Stage Liver Disease; kPa, kilopascals.

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

Early HCC recurrence No.=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≥12cm	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 Right Left Bilateral	 1 0.774 (0.355 - 1.686) 0.908 (0.395 - 2.085)	0.7348		
HCC max diameter (mm)*	1.010 (1.001-1020)	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)	<.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

* HR for one unit increase.

Abbreviations: HCC, hepatocellular carcinoma; No., number; HR, hazard ratio; CI, confidence intervals; BMI, body mass index; ALT, alanine amino-transferase.

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

Late HCC recurrence	Univariate		Multivariate		Post-hoc Multivariate	
No.=87	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 ≥12cm	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 Right Left Bilateral	 1 1.352 (0.331 - 5.514) 2.055 (0.483 - 8.743)	0.4709				
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				

* HR for one unit-increase.

Abbreviations: HCC, hepatocellular carcinoma; No., number; HR, hazard ratio; CI, confidence intervals; BMI, body mass index; ALT, alanine amino-transferase.

Figure legends:

- **Figure 1. Study flow-chart.** Abbreviations: *HCC, Hepatocellular carcinoma.*
- **Figure 2. Early HCC recurrence free survival:** Kaplan Meier curve for early HCC recurrence-free survival. Abbreviations: *HCC, Hepatocellular carcinoma.*
- **Figure 3. Late HCC recurrence free survival.** Survival free of HCC late recurrence according to SSM≤70 kPa or SSM>70 kPa. Abbreviations: *HCC, Hepatocellular carcinoma; SSM, spleen stiffness measurement.*

Figure 1
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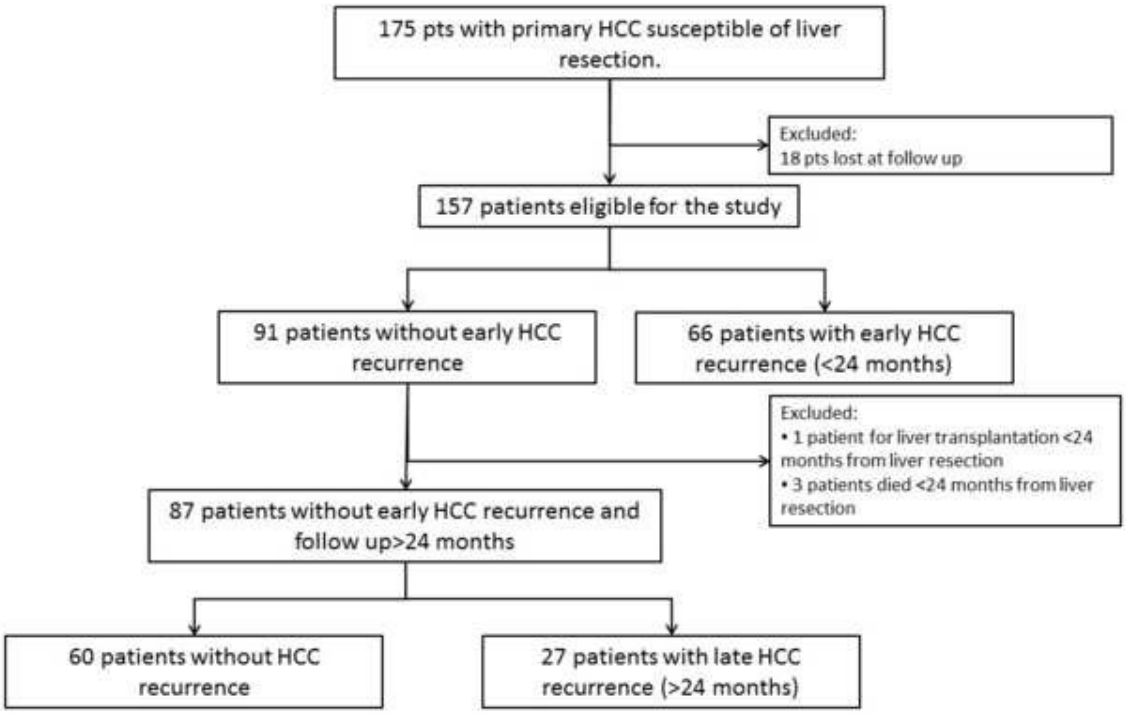

Figure 2

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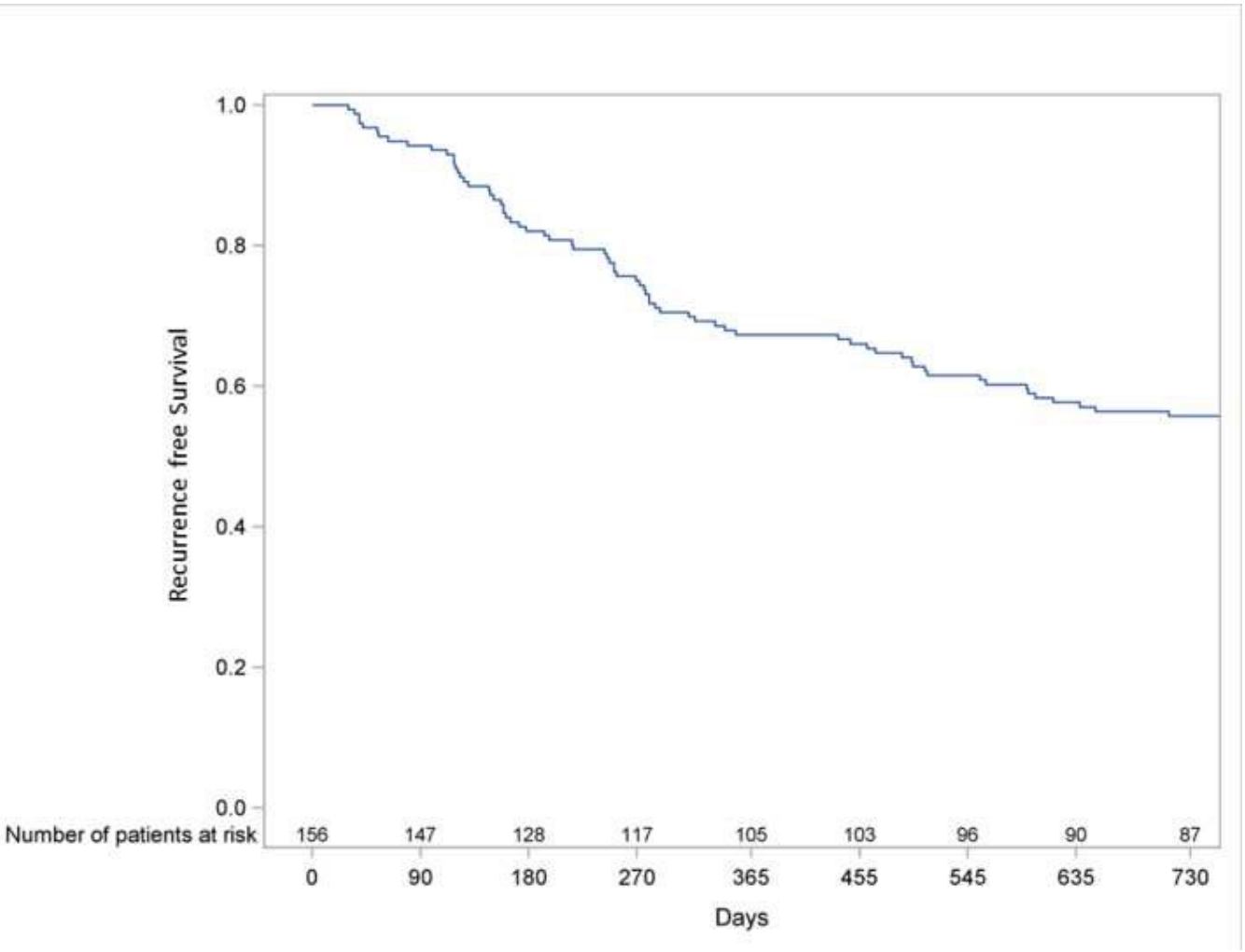

Figure 3 revised

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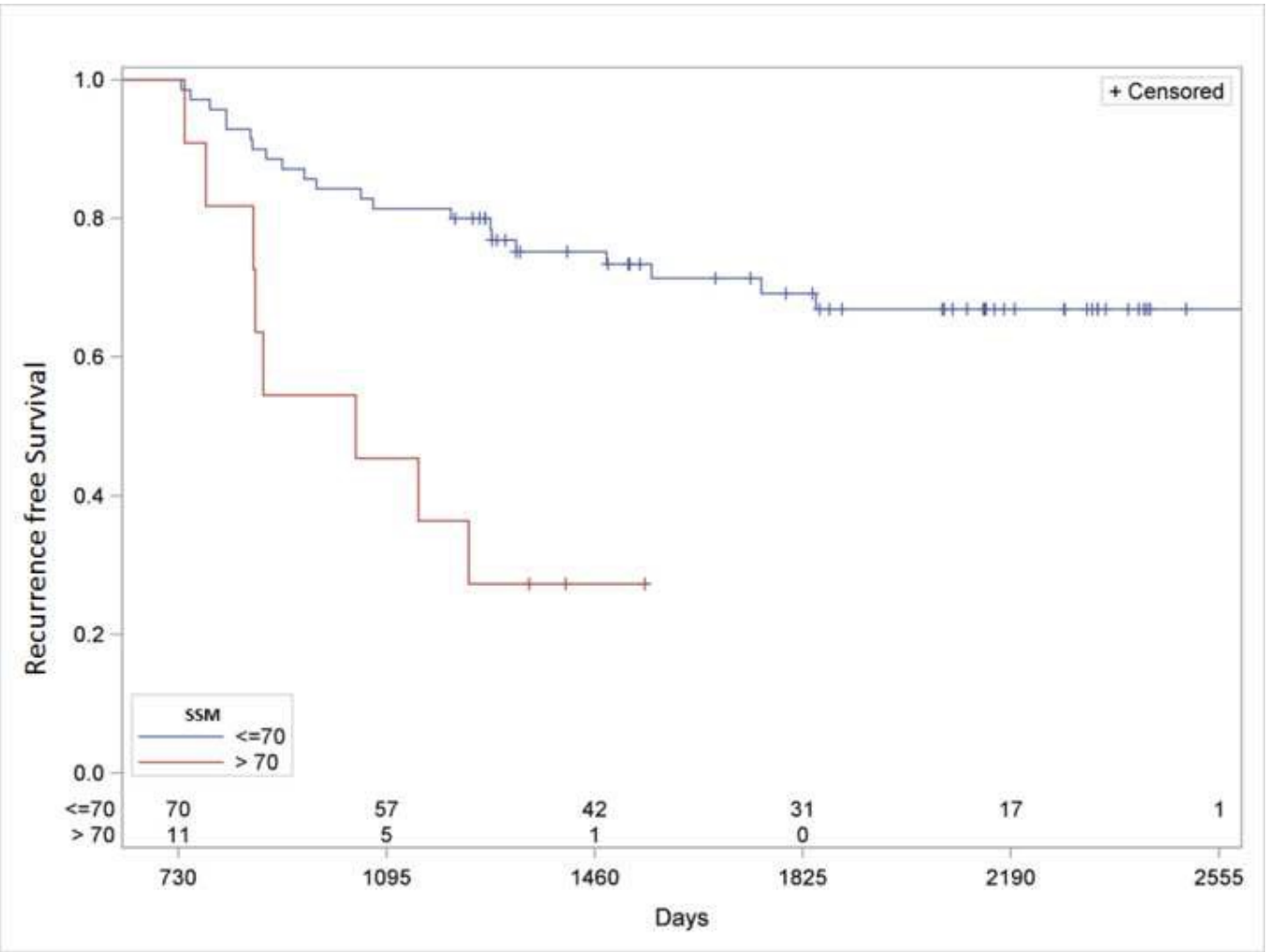

*Graphical Abstract