# Histological predictors of aggressive recurrence of hepatocellular carcinoma after liver resection

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***List of Abbreviations***

*LR, liver resection; HCC, hepatocellular carcinoma; CSPH, clinically significant portal hypertension; OS, overall survival; LT, liver transplantation; MTM, macrotrabecular-massive; TLS, tertiary lymphoid structures; VETC, vessels that encapsulate tumor clusters; US, ultrasound; CT, computed tomography MRI, magnetic resonance imaging; MT, macrotrabecular growth pattern; F-I, primary follicles; F-II, secondary follicles*; *mVI/S, microinvasion and/or satellite nodules; WL, waiting list; AFP, alpha-fetoprotein; RFS, recurrence free survival; TTR, time to recurrence; 95% CI, 95% confidence interval; HR, hazard ratio, sHR, sub-hazard ratios.*

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## ***Impact and Implications***

- Assessment of recurrence risk after liver resection (LR) is crucial in patients with hepatocellular carcinoma (HCC). Patients with a high risk of recurrence are candidates for liver transplantation (LT) as an *ab initio* indication or for the potential use of adjuvant therapy.

- Aggressive recurrences, defined as those exceeding the Milan criteria at first recurrence, have a significant impact on overall survival (OS).
- Fifty-eight percent of patients experienced HCC recurrence. The time to recurrence was 21 (9-51) months, with a prevalence of aggressive recurrence at the first occurrence standing at 35%, and 15.7% for those at the BCLC-C stage.
- After a median follow-up period of 49 (23-85) months, 61 (28%) patients died, and 32 (15%) underwent LT, resulting in a 5-year OS rate of 81%.
- The prevalence of microvascular invasion and/or satellitosis (mVI/S) was 39%, with excellent agreement between pathologists (k coefficients: 0.80).
- In multivariate analysis, mVI/S were the only independent predictors of recurrence, aggressive recurrence, and OS.

## ABSTRACT

**Background and Aims:** Assessment of recurrence risk after liver resection (LR) is critical in hepatocellular carcinoma (HCC), particularly with the advent of effective adjuvant therapy. The aim of the study was to analyze the clinical and pathological factors associated with recurrence, aggressive recurrence, and survival after LR.

**Method:** Retrospective study in which all single HCC (BCLC-0/A) patients treated with LR between February 2000 and November 2020 were included. The main clinical variables were recorded. Histological features were blindly evaluated by two independent pathologists. Aggressive recurrence was defined as those that exceeded the Milan criteria at 1$^{st}$ recurrence.

**Results:** A total of 218 patients were included (30% BCLC 0 and 70% BCLC A), median (IQR) tumor size of 28 (19-42mm). The prevalence of microvascular invasion and/or satellitosis (mVI/S) was 39%, with a kappa-index between both pathologists of 0.8. After a median follow-up of 49 (23-85) months, 61/218 (28%) patients died, 32/218 (15%) underwent LT, 127 (58%) developed HCC recurrence. The prevalence of aggressive recurrence was 35% (44/127 Milan-out, with 20 cases at advanced stage), and the 5-year survival was 81%. The presence of mVI/S was the only independent predictor of recurrence [HR:1.83 (1.28-2.61), p<0.001], aggressive recurrence [HR:3.31(1.74–6.29), p<0.001] and mortality [HR:2.23(1.27- 3.91), p:0.005]. The presence of MTM was significantly associated with a higher prevalence of mVI/S, Edmonson Steiner grade III-IV, AFP values and vessels that encapsulate tumor clusters, but MTM was not significantly associated with recurrence, aggressive recurrence, or OS.

**Conclusion:** The presence of mVI/S was the only independent risk factor for aggressive recurrence and mortality. This has important implications for early-stage patient management, especially in the setting of adjuvant immunotherapy or ab initio LT.

## INTRODUCTION

Liver resection (LR) remains the mainstay therapy for patients with early-stage hepatocellular carcinoma (HCC) [1,2]. The best results are achieved in patients with single lesions and preserved liver function, particularly in those without clinically significant portal hypertension (CSPH). Improvements in the surgical approach, particularly with the advent of laparoscopic and robotic resection, have allowed less invasive resection with fewer postoperative complications [3–5]. In well-selected candidates, LR provides a 5-year survival probability of >70%; however, this excellent outcome is compromised by the high rate of tumor recurrence. Assessment of recurrence risk after LR is critical, and several histological variables, such as tumor size, multifocality, poor differentiation, satellitosis, and the presence of microvascular invasion, have been repeatedly identified as predictors of higher recurrence risk [6–11]. Until recently, no adjuvant therapy has demonstrated a definitive survival benefit [12–15], and the only effective approach has been the *ab initio* liver transplantation (LT) before recurrence [9,10,16]. Nevertheless, the adoption of this strategy is strictly related to donors' availability and the local allocation rules. This scenario could change completely based on the positive results in recurrence free survival (RFS) of the IMbrave050 trial, which demonstrated the efficacy of atezolizumab and bevacizumab in adjuvant treatment after LR [17] and framed the need for an accurate assessment of individual recurrence risk, as well as the risk of presenting an aggressive recurrence pattern that affects the possibility of subsequent treatment and, consequently, overall survival (OS).

Pathological assessment of surgically resected specimens is crucial for prognostic prediction. A histological subtype termed macrotrabecular-massive (MTM) has been described as an independent predictor of early and overall recurrence [18], which is associated with an aggressive phenotype and biological (elevated alpha-fetoprotein serum levels and a higher prevalence of vascular invasion and satellites) and molecular (G3 transcriptome subset, TP53 mutations, and FGF19 amplifications) features [19]. Although the prognostic value of the MTM subtype has been validated by others [20,21], the inclusion of a subset of patients who are not good candidates for resection according to the guidelines (intermediate BCLC stage, presence of macrovascular invasion, presence of CSPH, or even impaired liver function) may limit the prognostic accuracy of the

MTM subtype when evaluating homogeneous surgical candidates. In addition, the presence of intratumoral tertiary lymphoid structures (TLS) has been associated with a lower risk of early recurrence, and these have been speculated to reflect the presence of in situ effective antitumor immunity [22]. Unfortunately, the clinical applicability of these findings is compromised by the lack of a standardized methodology for assessing the presence, density, and maturity of intratumoral TLS in resected specimens as well as the need for external validation [23]. Finally, vessels that encapsulate tumor clusters (VETC) have recently been described as a histological vascular pattern associated with a novel mechanism of metastasis, often observed in association with the MTM-HCC pattern and microvascular invasion [24]. Although it is an emerging prognostic factor, the absence of a standardized assessment criteria, and the potential collinearity with mVI/S may challenge its prognostic value [24–27].

Accordingly, this study aimed to assess the prognostic role of pathological findings, including the MTM-HCC histological pattern, VETC and the presence of TLS, in terms of overall recurrence of HCC, aggressive recurrence of HCC, and OS after LR.

## MATERIAL AND METHODS

*Study Design and Patient Selection*

This retrospective observational study was conducted at a single center and included consecutive patients with HCC classified as BCLC stage 0 or single A who underwent LR between February 2000 and November 2020. Preoperative diagnosis and staging of HCC were performed using computed tomography (CT), and/or magnetic resonance imaging (MRI). If radiological non-invasive diagnostic criteria, according to the available practice guidelines for HCC management at that time, were not met, a biopsy was performed to establish the diagnosis. None of the patients received neither neoadjuvant nor adjuvant therapies.

Patients with R1 resection, extrahepatic metastasis at the time of surgery, unavailable histological slides or follow-up data, histological diagnosis of combined hepatocellular-cholangiocarcinoma, or inadequate material for histological analysis (necrosis > 90%) were excluded. The main clinical variables were recorded at admission, during surgery, and during the follow-up period.

Preoperative evaluation was performed according to our institutional protocol, as reported in previous studies [16].

*Histological Evaluation*

Histological characteristics were assessed by two pathologists [CF (junior) and AD (senior)]. For each case, both pathologists independently reviewed all available histological slides and were blinded to clinical outcomes. Regarding tumor sampling, different areas of the tumor were sampled depending on the tumor size. All the selected patients had at least 4 tissue blocks, with a mean of 4.6 (median 5, range 4–10) paraffin-embedded tissue blocks per tumor available for evaluation. The macrotrabecular growth pattern (MT) was defined when trabeculae with a thickness exceeding six cells were observed. If the MT pattern constituted >50% of the entire tumor, MTM-HCC was considered based on previously published criteria [18].

Lesion size; tumor grade (according to the Edmondson-Steiner); histological subtypes (trabecular, macrotrabecular, acinar, solid, sclerosing, clear cell, steatotic, steatohepatitic, cholestatic, sarcomatoid) and their percentages within the tumor; presence of satellite nodules; microvascular invasion; percentage of necrosis; perilesional and intratumoral inflammation TLS were evaluated. The TLS were assessed morphologically on hematoxylin & eosin-stained slides using the scale previously published [28]. The TLS were classified as follows: 1. aggregates: vague, ill-defined clusters of lymphocytes; 2) primary follicles (F-I): round-shaped clusters of lymphocytes without germinal center formation; and 3) secondary follicles (F-II): follicles with germinal center formation.

The presence of microvascular invasion was considered when we identified the presence of tumor emboli in the portal vein, hepatic vein, or in large capsule vessels of the surrounding hepatic tissue in the tumoral edge when evaluating the tumoral morphology by hematoxylin and eosin-stained slides. In cases of hesitation, immunohistochemical staining with CD34 to demonstrate the vascular nature of the suspicious lesion was assessed and evaluated [29]. The presence of satellitosis was considered when microscopic nodules of hepatocellular carcinoma separated from the tumor by an interval of non-tumoral liver parenchyma [30]. The presence of VETC was analyzed using a mouse anti-human CD34 monoclonal antibody (clone QBEnd/10, ready-to-use, Ventana

Medical Systems) on 4 µm paraffin-embedded sections for immunohistochemical staining, using Leica Bond max austostainer. The process of deparaffinization, rehydration, and epitope retrieval, as well as the immunostaining was performed using Bond polymer refine detection kit, and the sections were counterstained with hematoxylin. For CD34 assessment, the immunoreactivity of a continuous lining around tumor clusters was defined as VETC. Any percentage of positivity for this vascular pattern was considered positive for VETC, as reported in the literature [24].

In cases of discrepancies in the assessment of MTM-HCC, vascular invasion, and/or satellite nodules and VETC, both pathologists performed a second review to reach a consensus. The agreed results were used for descriptive, univariate, and multivariate analyses. Regarding the non-tumoral parenchyma, the degree of fibrosis according to the METAVIR staging system, steatosis and hepatic inflammation were also recorded.

*Follow-up*

Imaging follow-up included an ultrasound (US) examination one month after LR, followed by an MRI or CT scan at three months, and then a CT scan or MRI every six months during the first two years. If no recurrence was detected within the first 2 years, regular US surveillance was performed every six months after the initial two years from LR [16].

*Indications for ab-initio LT or recurrence within the Milan* Criteria LT

Patients with vascular microinvasion and/or satellite nodules (mVI/S) were evaluated for inclusion in the waiting list (WL) for LT following the criteria reported in previous studies [9,16]. Patients with comorbidities or psychosocial factors contraindicating LT, those older than the established age cut-off at the time of LR, and those whose initial tumor burden did not meet the Milan criteria were discarded from LT. Similarly, patients without negative histological findings or contraindications for LT who experienced recurrence within the Milan criteria during the follow-up period were evaluated. Since July 2007, the Model of End-Stage Liver Disease (MELD) has been used for organ allocation. Exception points were applied to the LT WL when the initial tumor size exceeded 3 cm, alpha-fetoprotein (AFP) levels were greater than 200 ng/mL, or after recurrence within

the Milan criteria. In such cases, patients were granted 19 points, with an additional point added every 3 months, while on the WL [16].

*Study Definitions:*

- Overall survival (OS): This was assessed as the time elapsed from the date of LR until death or last medical visit. Participants who were lost to follow-up were censored at their last medical visit.
- Recurrence-Free Survival (RFS): The time from the date of LR to the date of the first recurrence of the disease or death. Subjects who did not present with recurrence or died before presentation were censored on the last date of the radiological tumor evaluation.
- Time to Recurrence (TTR): Time from LR to the date of the first radiological disease recurrence. The participants who did not progress were censored on the last radiological evaluation date. Patients who died without recurrence were censored at the date of death.
- Patterns of recurrence after LR were categorized as follows: single nodule <2 cm, single nodule 2-5 cm, two or three nodules <3 cm in size, multifocal outside the Milan criteria, and recurrence with vascular invasion and/or extrahepatic spread.
- Aggressive recurrences were defined as those exceeding the Milan criteria at the first recurrence.

*Ethical considerations*

The study was performed in accordance with the Declaration of Helsinki and the E6 Good Clinical Practice Standards ICH as well as the laws and regulations of Spain. All study data were treated anonymously with restricted access by only authorized personnel for the purposes of the study. This study was approved by the Institutional Review Board of the Hospital Clinic de Barcelona (HCP/2018/1031).

*Statistical analysis:*

Quantitative variables were described using the median and interquartile range (IQR), whereas qualitative variables were reported using the number of cases and percentages. Differences between qualitative variables were assessed using

the chi-square test or Fisher's exact test, as appropriate. Nonparametric tests (Mann-Whitney or Kruskal-Wallis) were used to analyze quantitative variables for the unpaired samples. Inter-observer agreement was determined using Cohen's kappa coefficient. Time-to-event for each outcome was expressed as the median and 95% confidence interval (95% CI), calculated using the Kaplan-Meier method. Survival curves were compared using log-rank tests. Cox regression models were used to estimate Hazard Ratios (HR) and 95% CI to evaluate the factors associated with global recurrence, aggressive recurrence, and death. Fine and Gray competing risk regression analysis (with LT as the competing risk of death) was performed to evaluate variables associated with OS. Estimated risks were presented as sub-hazard ratios (sHR) with 95% CI. The selection of variables for each of the multivariate analyses was based on: 1) the clinical relevance of the variables and their relationship with the study objective, 2) univariate analysis results showing statistically significant association ($p<0.05$), prioritizing the clinical importance of the variable, 3) collinearity, avoiding the inclusion of highly correlated variables, and 4) Confounding variables: including variables that could introduce biases into the relationship between the independent variables of interest and the outcome. All tests were two-tailed, and a p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using the R v. 4.7.1 and SPSS v.29.

## RESULTS

### *Patients*

A total of 218 patients were included in the study (patient flowchart is shown in **Supplementary Figure 1**). A total of 66 (30%) and 152 (70%) patients were BCLC stage 0 and single A, respectively, and histological examination revealed that 71.6% of the patients had cirrhosis. In 84.4% of cases, the lesions were <5 cm before surgery, with a median and IQR size of 28 (19-42) mm. The clinical characteristics of the patients are summarized in **Table 1**. The most frequent histological subtype was trabecular (85%), followed by acinar (57%). The prevalence of mVI/S, VETC and MTM-HCC were 39.4%, 41.8% and 7.8%, respectively. The main histological features of the tumors are presented in **Table 2**.

*Inter-observer agreement between both pathologists*

Interobserver agreement was good for the major histological features with prognostic value. The Kappa coefficients for Edmonson–Steiner degree of differentiation, microvascular invasion, satellitosis, and MTM-HCC were 0.71, 0.81, 0.80, and 0.78, respectively. In contrast, agreement was poor for intratumoral and peritumoral follicle evaluations, with kappa coefficient values of 0.55 and 0.48, respectively. **Table 3** presents the kappa coefficients for each histological feature.

*Recurrence of HCC after LR and* aggressive pattern of recurrence

One hundred twenty-seven patients (58%) experienced HCC recurrence. The RFS was 51 (41-61) months and the TTR was 21 (9-51) months. The prevalence of aggressive recurrence was 35% (44/127), presenting as BCLC-C stage at recurrence in 20 of 44 patients (15.7% of the whole cohort) (**Figure 1**). **Table 4** presents the results of the univariate and multivariate analyses for recurrence and aggressive recurrence. The presence of cirrhosis [HR: 1.89 (1.20 – 2.96), p=0.006], higher ALBI score [HR: 2.15 (1.35 - 3.43), p=0.001], and mVI/S [HR: 1.82 (1.13 - 2.94), p=0.014] were independent predictors for tumor recurrence. In addition, mVI/S was the only independent predictor of aggressive recurrence (HR: 3.08 (1.37 - 6.92), p=0.006). Similarly, global recurrence and aggressive recurrences were more prevalent within the initial two years following LR **(Supplementary Figure 2).** However, no statistically significant difference was found in the prevalence of early aggressive recurrences compared to early recurrences within Milan criteria. The presence of MTM-HCC was not significantly associated with recurrence [HR: 1.57 (0.84 - 2.92), p=0.154] or aggressive recurrence [HR: 1.57 (0.53 - 4.43), p=0.154]. During the post-LR follow-up, 48 of 218 patients were eligible for LT, and at the time of the analysis, 32 had been transplanted. Among these patients, 21 underwent transplantation based on the ab initio indication, 9 due to recurrence after resection, and 2 due to cirrhosis decompensation. Ultimately, information on the treatment and disease course of aggressive recurrences is provided in the **Supplementary Table 1**.

*Overall Survival: Independent Predictive Factors*

The median follow-up period was 49 (23-85) months. During this period, 61 of 218 (28%) patients died, resulting in a 5-year OS rate of 81% (**Figure 2**). In the univariate analysis, the presence of MTM [HR: 2.50 (1.19-5.29), p=0.016], age [HR: 1.03 (1.01 - 1.06), p=0.034], VETC [HR: 2.00 (1.20 – 3.34), p=0.008] and mVI/S [HR: 2.42 (1.46 - 4.02), p<0.001] were associated with a higher risk of death. In the multivariate analysis, the presence of mVI/S was the only independent predictor of mortality [HR: 1.90 (1.01-3.56), p=0.047]. Similarly, after performing a sensitivity analysis for competitive risk, the only independent predictor of mortality was the presence of mVI/S [sHR: 1.69 (1.25 – 3.05); p=0.045] (**Table 5)**. Finally, the presence of an aggressive pattern of recurrence, such as multifocality beyond the Milan criteria or recurrences at advanced stages (involving vascular invasion or extrahepatic disease), has been consistently linked to significantly lower survival rates. (**Supplementary Figure 3**)

*Clinical and histological characteristics associated with the presence of MTM-HCC*

Of the 218 patients, only 17 (7.8%) presented with MTM-HCC, defined as the presence of the MT pattern comprising > 50% of the tumor. The presence of MTM-HCC histological pattern was significantly associated with a higher prevalence of mVI/S (82.4% vs. 35.8%, p<0.001), Edmonson Steiner grades III-IV (82.4% vs. 36.8%, p<0.001), higher AFP levels [185 (7-664) vs. 6 (3-20) ng/mL, p<0.001], and VETC (100% vs. 36.7%, p<0.001). (**Figure 3**) When the MTM-HCC subtype was defined as the MT pattern comprising 30% of the tumor, according to previous reports [31], the prevalence of MTM-HCC was 25 of 218 (11.5%). Despite this increase in prevalence, all clinical and histological factors continue to be associated with MTM-HCC. Finally, the moderate positive correlation among the different histological features was seen between mVI/S and VETC (ρ=0.525, p<0.001). The correlations amongst the different histological features are described in **Supplementary Figure 4**.

## DISCUSSION

The results of our study, which included a homogeneous cohort of patients with single HCC lesions, well-preserved liver function and without CSPH treated with

LR, showed that the presence of mVI/S was the only prognostic parameter associated with aggressive recurrence patterns and shorter OS. The prevalence of mVI and satellitosis, found in 37% and 19% of cases, respectively, aligns with prior studies on patients with single HCC lesions, well-preserved liver function, and no CSPH who underwent LR [10,14,16]. The reason we have classified it as mVI and/or satellitosis because both entities correspond to the same pathophysiological phenomenon, leading to similar recurrence risks [11,14,32]. Furthermore, MTM-HCC showed a significant correlation with poorer differentiation, presence of mVI/S, VETC and elevated AFP values; however, it was not an independent predictor of overall recurrence, aggressive recurrence, or OS.

Likewise, the interobserver agreement between both pathologists for the detection of the most relevant prognostic parameters, such as mVI, satellites, and differentiation degree, was good, ranging between 0.71 and 0.81, which strengthens the role of the pathological evaluation of surgical specimens in clinical decision-making. In contrast, the assessment of the presence of TLS, another parameter suggested as prognostic in previous studies [22], was unreliable because it was associated with high disagreement between both pathologists (kappa coefficients <0.55), limiting its use for prognostic purposes.

Another relevant finding in our study was the high intratumoral heterogeneity, since two or more histological subtypes coexisted in 92% of the cases, with the trabecular subtype being the most prevalent. The MT pattern was found in 22% of the patients but comprised more than 50% of the tumor, which defines the MTM-HCC subtype, in only 7.8% of the patients. This prevalence was within the range reported in previous studies [33]. The prevalence of VETC was 41.8%, which is consistent with the rates reported in earlier studies using the same cutoff [24]. Although VETC was associated with the three reported outcomes (global recurrence, aggressive recurrence, and overall survival) at the univariate analysis, mVI/S remained as the only histological feature that independently predicted poorer outcome. The intratumoral heterogeneity observed in our analysis limits the use of histological subtyping of tissues obtained by percutaneous biopsy. In the era of potentially effective adjuvant therapy, efforts should be directed at developing molecular signatures, which used alone or in combination with clinical/histological parameters may further refine the prognosis

assessment of early-stage disease. Regrettably, the prognostic molecular signatures reported so far have not been externally validated, and the results derived from the analysis of a single tissue sample may not adequately catch the intra- and intertumor heterogeneity [34–36].

As expected, the presence of cirrhosis and ALBI score were also found as predictors of tumor recurrence in multivariate analysis, but they were not associated with either aggressive recurrence or poorer OS. The ALBI score has emerged as a relevant parameter for outcome assessment in the early stages [37], and several studies have previously demonstrated that the ALBI score is an independent predictor of post LR recurrence [38,39]. This finding is particularly relevant because our cohort included patients with well-preserved liver function and absence of CSPH.

While earlier studies have suggested a potential link between microvascular invasion and satellitosis [14,32], and an increased risk of recurrence beyond the Milan criteria, it is crucial to note that the limited sample size prevented interpretation as an independent predictive factor [9,10,16]. The present study confirms the independent prognostic value of mVI/S in predicting the risk of recurrence, aggressive recurrence, and survival after LR, and further strengthens the rationale for considering LT as an *ab initio* indication for detecting this unfavorable histological profile before recurrence. In our cohort, fifty-six out of the 86 patients with mVI/S (65%) experienced HCC recurrence. Among them, 26 (46%) patients had a first recurrence beyond the Milan criteria, with 12 (21.4%) at an advanced stage. Accordingly, salvage LT after tumor recurrence would limit access to LT in a relevant proportion of high-risk patients in whom *ab initio* LT offers long-term survival, as previously demonstrated [9,10,16].

The successful RFS outcome in the IMbrave 050 [17] underscores the urgency of developing robust tools for accurately predicting tumor recurrence following LR. It also amplifies the interest in identifying factors linked to aggressive recurrence patterns. This trial demonstrated a significant reduction in the 12-month RFS within a subgroup of patients at high risk of recurrence who underwent LR or ablation and received atezolizumab plus bevacizumab treatment for 12 months or 17 cycles [17]. In the IMbrave 050 trial, RFS at 12 months was 65% and 78% in the surveillance and treatment groups, respectively, whereas in our cohort of patients with mVI/S, it was ~70% (**Supplementary**

**Figure 5**). Regrettably, any direct comparison is unreliable because in our study, all patients had an ECOG-PS of 0, well-preserved liver function (defined as the absence of compensated portal hypertension), and unifocal HCC (30% BCLC 0 and 70% BCLC A), with a median tumor size of 2.8 cm, and a prevalence of mVI/S of 39%. In the IMbrave050 study, 90% were single, the median tumor size of the biggest lesion was 5.3 cm, with 21% of the patients having an ECOG-PS score of 1 and 67% exhibiting macro/microvascular invasion. Additionally, 11% of patients underwent transarterial chemoembolization following LR, which makes analyzing the results more challenging.

Although many questions remain to be answered regarding the best approach for treating high-risk patients, there is little doubt that the presence of mVI/S is the main independent predictor of recurrence, aggressive recurrence, and OS, and that any adjuvant therapy in the early stage should be compared with the benefit offered by LT according to the *ab initio* indication.

This study has some limitations. Firstly, the retrospective design and the single-center nature, coupled with stringent patient selection criteria, may potentially affect data reproducibility. Nevertheless, the strict patient selection ensures homogeneity and mitigates biases in the assessment of predictive factors. Another limitation is the relatively small number of patients with MTM-HCC, which limited the statistical power to identify significant associations. This highlights the need for further studies with larger sample sizes and multicenter collaboration, being crucial that these studies maintain consistent criteria for patient selection for surgery, post-LR follow-up, and the eligibility criteria for LT.

In summary, the presence of mVI/S emerged as the sole independent risk factor associated with aggressive recurrence and reduced OS rates. This finding holds significant clinical relevance for the management of early-stage patients, particularly in the evolving landscape of adjuvant immunotherapy and consideration of ab-initio LT.

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## Figures

**Figure 1.** Recurrence of HCC after Liver Resection and Pattern of Recurrence. (The Kaplan-Meier method was used to generate survival curves, which were compared using log-rank tests.)

**Figure 2.** Overall Survival after Liver Resection.
(The Kaplan-Meier method was used to generate survival curves, which were compared using log-rank tests.)

**Figure 3.** Clinical and Histological Features Associated with the Presence of MTM-HCC.

**Table 1. Clinical Characteristics**

| Characteristics | | Count (%) / Median (IQR) |
|---|---|---|
| **Sex** | Male | 175 (80.3%) |
| **Age at surgery** | | 63 (54-69) |
| **Size (mm)** | | 28 (19-42) |
| **BCLC 0** | | 66 (30.3%) |
| **BCLC A unifocal** | | 152 (69.7%) |
| **Less than 5 cm** | | 184 (84.4%) |
| **Etiology** | Cryptogenic | 18 (8.3%) |
| | HCV | 120 (55.0%) |
| | HBV | 33 (15.1%) |
| | Alcohol | 18 (8.3%) |
| | NASH | 16 (7.3%) |
| | AIH | 1 (0.5%) |
| | HCV-HBV | 2 (0.9%) |
| | HCV-Alcohol | 6 (2.8%) |
| | HBV-Alcohol | 1 (0.5%) |
| | Healthy Liver | 3 (1.4%) |
| **Cirrhosis** | | 156 (71.6%) |
| **HVPG (mmHg) (n=185)** | | 7 (4.5-8.5) |
| **Liver stiffness (kPa) (n=99)** | | 10.2 (6.8-14.9) |
| **Platelets (/mm$^3$)** | | 169 (125-212) |
| **Creatinine (mg/dL)** | | 0.86 (0.75-0.97) |
| **AST (UI/L)** | | 41 (26-73) |
| **ALT (UI/L)** | | 43 (26-83) |
| **Bilirubin (mg/dL)** | | 0.70 (0.60-1) |
| **Albumin (g/L)** | | 43 (40-45) |
| **INR** | | 1.06 (1-1.12) |
| **Neutrophil-Lymphocyte ratio** | | 2.08 (1.50-2.80) |
| **AFP (ng/mL)** | | 7 (3-23) |
| **AFP ≥ 400 ng/mL** | | 13 (6.2) |
| **Child Pugh** | A5 | 134 (86.5%) |

| | | |
|---|---|---|
| | A6 | 19 (12.3%) |
| | B8 | 2 (1.3%) |
| **ALBI score** | | -2.91 (-3.19 - -2.65) |
| | 1.00 | 172 (80%) |
| **ALBI** | 2.00 | 42 (19.5%) |
| | 3.00 | 1 (0.5%) |

*IQR, Interquartile Range; HCV: Hepatitis C virus; HBV: Hepatitis B virus; NASH: Non-alcoholic steatohepatitis; AIH: Autoimmune hepatitis; HVPG, hepatic venous pressure gradient; AFP, alpha-fetoprotein.*

**Table 2. Histopathological Features**

| Characteristics | Count (%) / Median (IQR) |
|---|---|
| **Microvascular invasion** | 81 (37.2%) |
| **Satellitosis** | 41 (18.8%) |
| **Microvascular invasion and/or satellitosis** | 86 (39.4%) |
| **Vessels that encapsulate tumor clusters*** | 89 (41.8%) |
| **Grade Ed-Steiner differentiation** | |
| Well | 37 (17%) |
| Moderately | 93 (42.7%) |
| Poorly | 79 (36.2%) |
| Undifferentiated | 9 (4.1%) |
| **Macrotrabecular pattern** | 47 (21.6%) |
| **Percentage of macrotrabecular pattern** | 30 (10-55) |
| **Macrotrabecular-massive>50%** | 17 (7.8%) |
| **Macrotrabecular-massive>=30** | 25 (11.5%) |
| **Trabecular** | 186 (85.3) |
| **Acinar** | 124 (56.9) |
| **Solid** | 95 (43.6%) |
| **Scirrhous** | 5 (2.3%) |
| **Clear cell type** | 52 (23.9%) |
| **Steatotic** | 67 (30.7%) |
| **Steatohepatitic** | 20 (9.2%) |
| **Sarcomatoid** | 2 (0.9%) |
| **Necrosis** | 50 (22.9%) |
| **Pseudocapsule** | 142 (65.1%) |
| **Intra-tumoral tertiary lymphoid structures** | |
| No | 71 (32.6%) |
| Yes, aggregates. | 78 (35.8%) |
| Yes, F-l | 51 (23.4%) |

| | |
|---|---|
| Yes, F-II | 18 (8.3%) |
| **Peri-tumoral tertiary lymphoid structures** | |
| No | 35 (16.1%) |
| Yes, aggregates. | 73 (33.5%) |
| Yes, F-I | 89 (40.8%) |
| Yes, F-II | 21 (9.6%) |

*Evaluated in 213 patients.

**Table 3. Concordance of histological features between pathologists**

(Inter-observer agreement was determined using Cohen's kappa coefficient)

| Characteristics | Cohen's Kappa | P |
|---|---|---|
| **Degree of Differentiation by Edmonson Steiner** | 0.711 | <0.001 |
| **Trabecular** | 0.696 | <0.001 |
| **Macrotrabecular** | 0.775 | <0.001 |
| **Acinar** | 0.849 | <0.001 |
| **Solid** | 0.694 | <0.001 |
| **Scirrhous** | 0.491 | <0.001 |
| **Clear cell type** | 0.856 | <0.001 |
| **Steatotic** | 0.746 | <0.001 |
| **Steatohepatitic** | 0.721 | <0.001 |
| **Cholestasic** | 0.493 | <0.001 |
| **Sarcomatoid** | 1 | |
| **Necrosis** | 0.881 | <0.001 |
| **Pseudocapsule** | 0.783 | <0.001 |
| **Satellitosis** | 0.795 | <0.001 |
| **Microvascular invasion** | 0.813 | <0.001 |
| **Intra-tumoral tertiary lymphoid structures** | 0.550 | <0.001 |
| **Peri-tumoral tertiary lymphoid structures** | 0.481 | <0.001 |

**Table 4. Univariate and Multivariate Analyses for Recurrence and Aggressive Recurrence.**

(Cox regression analysis was conducted to estimate Hazard Ratios (HR) and 95% Confidence Intervals (CI) for factors associated with overall recurrence and aggressive recurrence.)

**a. Global Recurrence**

| | | Global Recurrence | | | |
|---|---|---|---|---|---|
| | | **No (n=91)** **n (%) / Median (IQR)** | **Yes (n=127)** **n (%) / Median (IQR)** | **HR (95% CI)** | **p value** |
| Sex | Male | 68 (74.7) | 107 (84.3) | 1.55 (0.96-2.51) | 0.059 |
| Age at surgery | | 64 (59-68) | 63 (53-70) | 1.002 (0.98-1.02) | 0.822 |
| Size (mm) | | 26 (20-40) | 30 (18-43) | 1.004 (0.99-1.01) | 0.267 |
| BCLC 0 | | 24 (26.4) | 42 (33.1) | 0.88 (0.61-1.28) | 0.503 |
| BCLC A unifocal | | 67 (73.6) | 85 (66.9) | 1.14 (0.78-1.65) | 0.506 |
| Less than 5 cm | | 79 (86.8) | 105 (82.7) | 0.84 (0.53-1.33) | 0.445 |
| Cirrhosis | | 54 (59.3) | 102 (80.3) | **2.08 (1.34 - 3.23)** | **0.001** |
| Platelets (/$mm^3$) | | 172 (137-224) | 163 (117-202) | 0.99 (0.99-1.01) | 0.346 |
| Bilirubin (mg/dL) | | 0.70 (0.50-0.90) | 0.80 (0.60-1) | **1.58 (1.07-2.34)** | **0.022** |

| | | | | | |
|---|---|---|---|---|---|
| Albumin (g/L) | | 45 (42-46) | 42 (40-45) | **0.93 (0.89-0.98)** | **0.002** |
| INR | | 1.05 (0.99-1.11) | 1.07 (1.02-1.12) | 0.67 (1.52-2.93) | 0.591 |
| Neutrophil-Lymphocyte ratio | | 2.12 (1.56-2.83) | 2.07 (1.44-2.79) | 0.91 (0.80-1.03) | 0.157 |
| AFP ≥ 400 ng/mL | | 5 (5.7) | 8 (6.6) | 1.40 (0.68-2.87) | 0.361 |
| Child Pugh | A5 | 47 (87) | 87 (86.1) | | |
| | A6 | 6 (11.1) | 13 (12.9) | 0.96 (0.59-1.56) | 0.852 |
| | B8 | 1 (1.9) | 1 (1) | | |
| ALBI score | | -3.07 (-3.25 - -2.87) | -2.82 (-3.08 - -2.57) | **2.26 (1.43 -3.58)** | **<0.001** |
| ALBI | 1 | 81 (91) | 91 (72.2) | | |
| | 2 | 7 (7.9) | 35 (27.8) | **2.01 (1.37-2.95)** | **<0.001** |
| | 3 | 1 (1.1) | 0 | | |
| Microvascular invasion and/or satellitosis | | 30 (33) | 56 (44.1) | **1.73 (1.21 -2.46)** | **0.002** |
| Degree of Differentiation by Edmonson Steiner | Well | 17 (18.7) | (15.7) | Ref | |
| | Moderately | 40 (44) | 53 (41.7) | 1.17 (0.70-1.96) | 0.552 |
| | Poorly | 30 (33) | 49 (38.6) | 1.55 (0.92-2.61) | 0.104 |
| | Undifferentiated | 4 (4.4) | 5 (3.9) | 1.34 (0.50-3.57) | 0.562 |
| Macrotrabecular-massive | | 6 (6.6) | 11 (8.7) | 1.57 (0.84-2.92) | 0.154 |
| Vessels that encapsulate tumor clusters | | 35 (38.5) | 54 (44.3) | **1.53 (1.07-2.20)** | **0.021** |

| *Multivariate* | *HR* | *p* |
|---|---|---|
| Vessels that encapsulate tumor clusters | 1.10 (0.68 - 1.78) | 0.686 |
| **Cirrhosis** | **1.89 (1.20 – 2.96)** | **0.006** |
| **ALBI** | **2.15 (1.35 - 3.43)** | **0.001** |
| **Microvascular invasion and/or satellitosis** | **1.82 (1.13 – 2.94)** | **0.014** |

**b. Aggressive Recurrence**

| | | Aggressive Recurrence | | | |
|---|---|---|---|---|---|
| | | No (n=83) | Yes (n=44) | HR (95% CI) | p value |
| | | n (%) / Median (IQR) | n (%) / Median (IQR) | | |
| Sex | Male | 68 (81.9) | 39 (88.6) | 0.56 (0.22-1.43) | 0.227 |
| Age at surgery | | 63 (53-72) | 63 (53-69) | 0.99 (0.97-1.03) | 0.904 |
| Size (mm) | | 30 (18-45) | 29 (19-43) | 1.01 (0.99-1.01) | 0.949 |
| BCLC 0 | | 27 (32.5) | 15 (34.1) | 0.79 (0.42-1.48) | 0.460 |
| BCLC A unifocal | | 56 (67.5) | 29 (65.9) | 1.27 (0.67-2.39) | 0.460 |
| Less than 5 cm | | 67 (80.7) | 38 (86.4) | 1.19 (0.50-2.85) | 0.688 |
| Cirrhosis | | 65 (78.3) | 37 (84.1) | 1.70 (0.75-3.86) | 0.202 |
| Platelets (/mm$^3$) | | 162 (125-198) | 168 (112-213) | 0.92 (0.43-1.99) | 0.841 |
| Bilirubin (mg/dL) | | 0.80 (0.60-1.10) | 0.75 (0.60-1.00) | 1.01 (0.99-1.01) | 0.181 |

| | | | | | |
|---|---|---|---|---|---|
| Albumin (g/L) | | 42 (40-45) | 42 (40-45) | 1.06 (0.97-1.15) | 0.197 |
| INR | | 1.07 (1.03-1.13) | 1.05 (1.01-1.11) | 0.50 (0.01-3.27) | 0.160 |
| Neutrophil-Lymphocyte ratio | | 1.95 (1.31-2.85) | 2.24 (1.65-2.76) | 0.98 (0.81-1.18) | 0.350 |
| AFP ≥ 400 ng/mL | | 5 (6.3) | 3 (7.1) | 1.88 (0.57 -6.17) | 0.300 |
| Child Pugh | A5 | 52 (80) | 35 (97.2) | 0.13 (0.02-1.01) | 0.056 |
| | A6 | 12 (18.5) | 1 (2.8) | | |
| | B8 | 1 (1.5) | 0 | | |
| ALBI score | | -2.82 (-3.03 - -2.57) | -2.82 (-3.21 - -2.57) | 0.68 (0.30-1.52) | 0.342 |
| ALBI | 1 | 60 (72.3) | 91 (72.2) | 1.11 (0.56-2.17) | 0.768 |
| | 2 | 23 (27.7) | 35 (27.8) | | |
| Microvascular invasion and/or satellitosis | | 29 (34.9) | 27 (61.4) | **3.61 (1.94 - 6.69)** | **<0.001** |
| Degree of Differentiation by Edmonson Steiner | Well | 17 (20.5) | 3 (6.8) | Ref | |
| | Moderately | 35 (42.2) | 18 (40.9) | **4.03 (1.4 - 11.6)** | **0.010** |
| | Poorly | 27 (32.5) | 22 (50) | **5.58 (1.79 - 17.43)** | **0.003** |
| | Undifferentiated | 4 (4.8) | 1 (2.3) | 4.18 (0.46 - 37.80) | 0.204 |
| Macrotrabecular-massive | | 7 (8.4) | 4 (9.1) | 1.57 (0.53 - 4.43) | 0.154 |
| Vessels that encapsulate tumor clusters | | 30 (38.5) | 24 (54.5) | **2.28 (1.25 - 4.15)** | **0.007** |

| ***Multivariate*** | ***HR*** | ***p*** |
|---|---|---|
| *Degree of differentiation* | | |
| Well Differentiated | **Ref** | |
| Moderately Differentiated | 1.49 (0.42 – 5.21) | 0.537 |
| Poorly Differentiated | 1.72 (0.45 – 6.57) | 0.427 |
| Undifferentiated | 0.36 (0.03 – 3.81) | 0.393 |
| Vessels that encapsulate tumor clusters | 1.23 (0.61 – 2.49) | 0.561 |
| **Microvascular invasion and/or satellitosis** | **3.08 (1.37 - 6.92)** | **0.006** |

**Table 5. Univariate and Multivariate Analyses for Overall Survival**

(Cox regression analysis was conducted to estimate Hazard Ratios (HR) and 95% Confidence Intervals (CI) for factors associated with mortality.)

| | | Death | | | |
|---|---|---|---|---|---|
| | | **No (n=157)** | **Yes (n=61)** | **HR (95% CI)** | **p value** |
| | | **n (%) / Median (IQR)** | **n (%) / Median (IQR)** | | |
| Sex | Male | 125 (79.6) | 50 (82) | 0.77 (0.40-1.48) | 0.425 |
| Age at surgery | | 62 (54-68) | 66 (55-72) | **1.03 (1.01 - 1.06)** | **0.034** |
| Size (mm) | | 26 (18-40) | 34 (20-43) | 1.001 (0.99 - 1.013) | 0.817 |
| BCLC 0 | | 50 (31.8) | 16 (26.2) | 0.68 (0.39-1.21) | 0.193 |
| BCLC A unifocal | | 107 (68.2) | 45 (73.8) | 1.46 (0.83-2.59) | 0.193 |
| Less than 5 cm | | 132 (84.1) | 52 (85.2) | 1.47 (0.72 - 2.99) | 0.295 |
| Cirrhosis | | 109 (69.4) | 47 (77) | 1.77 (0.98 – 3.17) | 0.057 |
| Platelets (/mm$^3$) | | 169 (124-214) | 169 (125-197) | 1.01 (0.99-1.02) | 0.830 |
| Bilirubin (mg/dL) | | 0.70 (0.60-1.00) | 0.70 (0.60 -1.01) | 0.82 (0.41-1.63) | 0.570 |
| Albumin (g/L) | | 43 (41-46) | 42 (40-45) | 0.97 (0.91-1.04) | 0.367 |
| INR | | 1.06 (1.00-1.12) | 1.07 (1.00-1.09) | 0.34 (0.02- 5.53) | 0.452 |
| Neutrophil-Lymphocyte ratio | | 2 (1.57-2.73) | 2.15 (1.31-2.87) | 0.89 (0.74-1.01) | 0.257 |

| | | | | | |
|---|---|---|---|---|---|
| AFP ≥ 400 ng/mL | | 9 (5.9) | 4 (6.9) | 1.20 (0.44 -3.31) | 0.722 |
| Child Pugh | A5 | 93 (85.3) | 41 (89.1) | | |
| | A6 | 14 (12.8) | 5 (10.9) | 0.66 (0.26-1.66) | 0.378 |
| | B8 | 2 (1.8) | 0 | | |
| ALBI score | | -2.95 (-3.19 - .2,76) | -2.82 (-3.14 - -2.55) | 1.23 (0.61 - 2.49) | 0.566 |
| ALBI | 1.00 | 129 (83.2) | 43 (71.7) | | |
| | 2.00 | 25 (16.1) | 17 (28.3) | 1.49 (0.84-2.64) | 0.178 |
| | 3.00 | 1 (0.6) | 0 | | |
| Microvascular invasion and/or satellitosis | | 55 (35) | 31 (50.8) | **2.42 (1.46 - 4.02)** | **<0.001** |
| Degree of Differentiation by Edmonson Steiner | Well | 26 (16.6) | 11 (18) | Ref | |
| | Moderately | 75 (47.8) | 18 (29.5) | 0.79 (0.37-1.67) | 0.536 |
| | Poorly | 49 (31.2) | 30 (49.2) | 1.82 (0.91-3.65) | 0.091 |
| | Undifferentiated | 7 (4.5) | 2 (3.3) | 1.46 (0.32-6.60) | 0.626 |
| Macrotrabecular-massive | | 9 (5.7) | 8 (13.1) | **2.50 (1.19 - 5.29)** | **0.016** |
| Vessels that encapsulate tumor clusters | | 59 (38.3) | 30 (50.8) | **2.00 (1.20 – 3.34)** | **0.008** |

| *Multivariate* | *HR* | *p* |
|---|---|---|
| **Microvascular invasion and/or satellitosis** | **2.23 (1.27 - 3.91)** | **0.005** |
| Age | 1.03 (0.99 - 1.06) | 0.069 |
| MTM-HCC (>50%) | 1.32 (0.49 -3.52) | 0.573 |
| Vessels that encapsulate tumor clusters | 1.26 (0.66 – 2.39) | 0.489 |