

Recurrence of Hepatocellular Carcinoma After Liver Transplantation is Associated with Episodes of Acute Rejections

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Purpose: The impact of acute rejection (AR) after liver transplantation (LT) for hepatocellular carcinoma (HCC) on patient outcome is uncertain. This aim of this study is to investigate whether AR is associated with HCC relapse and overall survival.

Patients and Methods: Patients undergoing LT for HCC between 2001 and 2015 were retrospectively analyzed with regard to histopathological proven AR within the median time until recurrence. Cox's regression analysis was conducted revealing risk factors for HCC recurrence.

Results: HCC recurred in 47 of 252 analyzed patients with a median time to recurrence of 20 months. Patients with AR (28.6%) had a significantly higher frequency of recurrence compared to patients without AR (13.0%, $p=0.002$). Multiple Cox regression analyses identified AR within 20 months to be an independent risk factor for HCC recurrence both as dichotomized (HR=2.91, 95%CI: 1.30–6.53; $p=0.009$) and as a continuous variable (HR=1.81, 95%CI: 1.28–2.54; $p=0.001$). HCC recurrence and AR were associated with higher grades of liver fibrosis one year after LT, when compared to patients without AR ($p=0.019$).

Conclusion: Our results demonstrate an association of AR with HCC recurrence after LT with implications for intervals of monitoring in tumor surveillance. Graft fibrosis and immune mechanisms are potentially related and causal interactions are worth further investigation.

Keywords: histology, protocol liver biopsy, graft fibrosis, immunological mechanisms, predictors of tumor relapse

Introduction

The World Health Organization (WHO) reported hepatocellular carcinoma (HCC) to be the sixth most commonly diagnosed cancer and the fourth leading cause of cancer deaths worldwide in 2018.¹ Potentially curative treatment strategies for HCC include liver transplantation (LT), liver resection (LR) and ablation. Among these options, LT is considered as first-line option for patients fulfilling benchmark criteria, especially for patients, in unsuitable condition for LR.² Excellent long-term outcomes are achieved in selected HCC patients by LT as well as by LR with five-year survival rates between 60% and 70%.^{3–7} However, recurrent disease remains an persisting problem after LT with rates ranging from 9% to 16%.^{8,9} A first peak of intrahepatic recurrence—considered to be due to metastatic spread—is typically seen within the first 24 months after LR, whereas a second peak is postulated to be associated with de novo tumors in fibrotic grafts.¹⁰

Several oncological predictors for HCC recurrence are well known, eg vascular invasion, degree of differentiation, tumor size, number of nodules and satellites.^{8,11–15} During the past years different studies suggested some non-oncological risk factors in association with HCC recurrence, such as age, prolonged cold ischemia time (CIT; >10 h) and warm ischemia time (WIT; >50 min) as well as blood transfusion.^{16–21} Of note, biomarkers seem to play a key role in liver inflammation and ultimately tumor recurrence, such as being able to predict outcomes after LT.²² Particularly the neutrophil lymphocyte ratio (NLR) measured in the peripheral blood was found to be of importance in predicting outcome in several malignancies, including HCC. Several studies found that preoperative elevated NLR is associated with high risk of recurrence and death in patients with HCC undergoing LT.^{8,23–25}

The incidence of acute rejection (AR) varies from 10% to 64% and its implications on patient outcomes after LT remains controversial.^{26–30} Toso et al stated that a history of AR is associated with a better postrecurrence survival after LT possibly linked to anticancer immunity.³⁰ In contrast, other authors reported significantly increased risk of graft failure, mortality, and graft failure-related death regardless of primary liver disease but in association with AR.²⁹ There is increasing evidence for the importance of immune responses in HCC; however, potential implications of AR on tumor recurrence after LT remain uncertain. Consequently, the aim of this study was to investigate associations between episodes of AR and HCC recurrence after LT.

Patients and Methods

Study Population

We analyzed all patients undergoing LT for HCC at the Department of Surgery, Campus Virchow-Klinikum, Charité—Universitätsmedizin Berlin between February 2001 and September 2015. Exclusion criteria were living donor LT (n=16), need for re-LT (n=22) or death within the median time to recurrence (20 months, n=38) and lost to follow-up (n=2).

The study focused on biopsy-proven AR after LT as potential risk factor for HCC recurrence. For this purpose, all liver biopsies were analyzed including routinely performed per protocol biopsies after LT as well as non-routinely performed biopsies for suspected AR. Episodes of AR occurring within the median time until HCC

recurrence (20 months) were considered. HCC recurrence was defined as any tumor burden independent of the site, eg hepatic recurrence or distant metastases. Charité Local Ethics Committee approved data analysis (EA2/150/13). Medical records were reviewed based on the ethics vote of the IRB without an additional consent from the patients studied. First, all patients signed their informed consent to review their medical records at the time of liver transplantation. However, the retrospective study design was also causally related to this as a period of more than 13 years was studied, during which patients died. The privacy of all patients studied was kept confidential by anonymizing the data and following the Declaration of Helsinki. The owners of the data set are the authors of the study and they have collected the data through a retrospective evaluation. All organs were donated voluntarily with written informed consent and this was conducted in accordance with the Declaration of Istanbul.

Data Collection

Preoperative demographic characteristics and clinical variables included recipient and donor age at time of LT, recipient sex, body mass index (BMI) (in kg/m²), etiology of chronic liver disease, laboratory studies (alpha-fetoprotein (AFP) ≤ 200 $\mu\text{g/L}$ vs >200 $\mu\text{g/L}$, NLR ≤ 4 vs >4 , waiting time for LT ≤ 120 days vs >120 days, model of end-stage liver disease (MELD) score, beyond MILAN criteria (yes vs no) and bridging therapies to LT including LR, radiofrequency ablation (RFA) or transcatheter arterial chemoembolization (TACE) (yes vs no). Analyzed perioperative risk factors were CIT (>10 h) and WIT (>50 min). Oncological factors evaluated in the study were tumor size (>5 cm), >3 tumor nodules, bilobar HCC, T >2 , vascular invasion and poor differentiation (G >2) in the explanted recipient liver (according to TNM classification at time of LT).

For patients undergoing LT with hepatitis C virus (HCV) viremia at time of transplant, duration of viremia after transplantation, type of HCV treatment (interferon, IFN; ribavirin, RBV; direct acting antiviral drugs, DAA) received post-LT and incidence of HCV recurrence were documented.

Immunosuppression

Standard immunosuppression (IS) regimens in our patient cohort consisted of calcineurin inhibitor (CNI)-based IS (FK506 rather than cyclosporine A; CyA) and low-dose steroid usage, with adjustments on an individual basis. Steroids were routinely tapered and stopped within three

months after LT. Combination with antimetabolites as mycophenolate mofetil (MMF) were indicated on an individual basis, eg due to reduced renal function.

Baseline IS was reported in case of continuous application of at least three months in the first year after LT (median of values collected at 1, 3, 6, 9 and 12 months). In addition, usage of mammalian target of rapamycin inhibitors (mTORi—sirolimus, everolimus) was reported before diagnosis of recurrent disease or until last available follow-up (in the no recurrence group).

Post-LT Aftercare

The standardized performed aftercare included per protocol liver biopsies, laboratory AFP level measurement and sonographic examination of liver parenchyma in regard to steatosis, fibrosis and suspicious lesions after six months, 1, 3, 5, 7, 10, and 13 years post-LT. In case of suspected HCC recurrence, CT and/or MRI was performed. Recurrent disease was confirmed by radiological imaging in combination with elevated AFP levels or histopathological confirmation either by biopsy or partial resection.

Histopathology

Expert pathologists routinely analyzed liver biopsies. Paraffin-embedded liver biopsies were stained with H&E, Masson–Golder, iron and periodic-Schiff reagent. Histopathologic evaluation of liver tissue focused on signs of AR, as well as the presence and grading of fibrosis, median inflammation grades (hepatitis), steatosis, and cholangitis. Fibrosis was staged using a scale by Desmet et al (0: absent, 1: mild without septa, 2: moderate with few septa, 3: numerous septa without cirrhosis and 4: cirrhosis).³¹ Inflammation grades were assessed according to the classification by Desmet et al (0: none, 1: minimal, 2: mild, 3: moderate and 4: severe).³¹

Diagnosis and Therapy of AR

All ARs were histopathologically proven. No humoral rejections were included in this study. Common indications for non-protocol liver biopsy were elevated liver enzymes, cholestasis or liver dysfunction. The grading of severity of AR was classified according to Banff criteria and stratified as mild (score <5), moderate (5–6), and severe (7–9).^{32,33} According to histologically proven AR and clinical findings, treatment was initiated. Intravenous application of steroids represented the standard treatment of AR (methylprednisolone of 500 mg over

three days). Moreover, dosage of CyA or FK506 was increased or MMF was added on an individual basis.

Statistical Analysis

For univariate analysis, continuous variables were reported as median (range), and categorical data as counts and percentages. Comparison of categorical data was performed, by using Pearson's chi-squared test or Fisher's exact test. Mann–Whitney *U*-test or Student's *t* test were used for continuous variables. OS and HCC recurrence rates were calculated by using Kaplan–Meier survival analysis with the log rank test for between-group comparison. Time until tumor recurrence was defined as the date of LT until tumor recurrence and censored at date of last available follow-up or death from nontumor-related causes. A sub-analysis was used to identify differences between patients with and without recurrent disease in regard to occurrence of AR. Therefore patients were divided into AR and no AR groups and again divided into HCC recurrence and no HCC recurrence. In addition, patients with HCC recurrence were separated into early (within 24 months) and late recurrence (>24 months) and compared within these groups. A subgroup analysis for HCV patients only was performed (see [Supplemental Table 1](#)).

To evaluate the association between potential risk factors for HCC recurrence, we conducted a multiple Cox proportional hazard regression with variables that were significant on the univariate analysis ($p < 0.05$). Cox proportional regression analysis was used to develop a multiple model adjusting for the combined effects of pretransplant AFP >200 µg/L (yes vs no), preoperative NLR >4 (yes vs no), beyond MILAN (yes vs no), bilobar HCC expression (yes vs no), T grade >2 (yes vs no), grading >2 (yes vs no), and AR after LT (yes vs no) (model 1) or frequency of AR within 20 months (model 2). HRs were presented with 95%CI.

The statistical significance was set at p -value <0.05. All statistical analysis was performed using IBM SPSS Statistics version 22.

Results

Of 330 patients undergoing LT for HCC, 252 patients fulfilled the defined criteria and were subsequently analyzed ([Figure 1](#)). The main underlying etiology for chronic liver disease was alcoholic liver disease ($n=107$, 42.5%), followed by chronic HCV ($n=83$, 32.9%). During a mean follow-up time of 94.5 ± 47.5 months, 47 patients (18.7%) developed

recurrent disease. Of those one, two, three, and five-year recurrence rates were 21.3% (n=10), 57.4% (n=27), 70.2% (n=33), and 83.0% (n=39), respectively, with a median time until recurrence of 20 months (range: 5–108). Eight patients (17.0%) developed HCC recurrence in the liver graft, 16 patients (34.0%) had extrahepatic metastases and 23 patients (48.9%) had intra- and extrahepatic recurrence.

Histopathological proven AR occurred in 91 patients (36.1%) within the first 20 months after LT. The frequency of disease recurrence was significantly higher in patients with AR (28.6%) compared to patients without AR (13.0%, $p=0.002$). AR episodes within the first 20 months were more frequent in patients with HCC recurrence, when compared to patients without HCC recurrence ($p=0.001$, Figure 1). Furthermore, there was a tendency toward more frequent ARs within the first 30 days after LT in the HCC recurrence group ($p=0.137$, Figure 1). Of patients with AR, those with recurrent disease revealed significantly more episodes of AR ($p=0.014$) and were characterized by significantly higher grades of fibrosis one year after LT when compared to patients without recurrence ($p=0.019$). When comparing patients with and without AR, CIT >10 h were less frequent, whereas hepatitis was more frequent in patients with AR (Table 1).

With regard to immunosuppression, there was no difference between the percentage of FK506 and CyA baseline usage (FK506: AR, n=85, 93.4%; no AR, n=150, 93.2%;

$p=0.942$; CyA: AR, n=5, 5.5%; no AR, n=10, 6.2%, $p=0.817$) or between FK506 trough levels in patients with and without rejection (AR: 7.0, 1.3–12.3; no AR: 7.1, 1.0–13.5; $p=0.711$). Furthermore, there were no correlations between FK506 level and number of rejections (Spearman, $r=-0.39$; $p=0.546$). Correspondingly, no difference between percentage of FK506 usage on the one hand and recurrence on the other hand was found (no HCC recurrence, n=190, 92.7%; HCC recurrence, n=45, 95.7%, $p=0.450$). Usage of mTORi before HCC recurrence was also comparable between all groups (sirolimus: AR, n=18, 19.8%; no AR, n=28, 17.4%, $p=0.637$; everolimus: AR, n=5, 5.5%; no AR, n=13, 8.1%, $p=0.445$). Patients with recurrent disease received in 61.5% steroid bolus for first AR and patients without HCC recurrence in 61.9% of the cases ($p=0.397$). Only MMF was administered slightly more frequently in the group with rejections as opposed to the group without rejections (AR, n=55, 60.4%; no AR, n=65, 40.4%, $p=0.002$). Clinical and oncological features as well as histopathologic biopsy parameters are shown in Table 1 and therapy regimens for patients with AR are listed in Table 2.

When comparing patients with early and late recurrent disease no significant differences with regard to preoperative and postoperative course as well as histopathologic features were found (Table 3). There was a tendency toward higher preoperative AFP levels in early HCC recurrence patients ($p=0.067$), whereas late recurrence

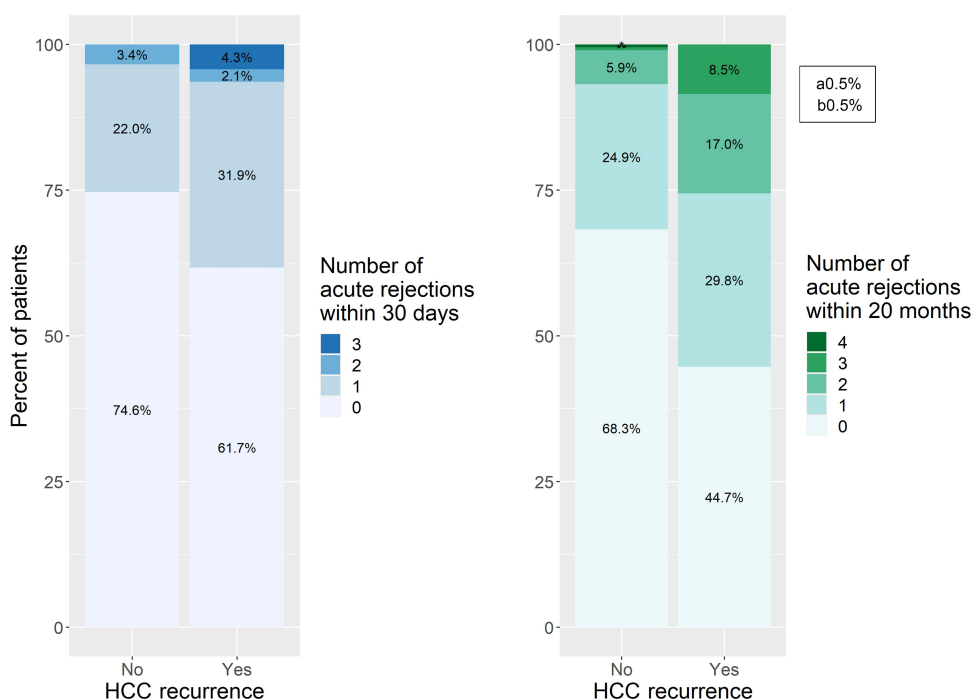


Figure 1 Frequency of acute rejections (AR) within 30 days and 20 months after OLT compared between no HCC recurrence and HCC recurrence groups.

Notes: ^a0.5% of patients had three episodes of acute rejections within 20 months. ^b0.5% of patients had four episodes of acute rejections within 20 months.

Table I Patient Characteristics and Perioperative Outcomes Compared Between Patients with Acute Rejection (AR) and without AR (No AR) Within 20 Months

	AR (n=91)			No AR (n=161)			*p-value
	HCC Recurrence (n=26)	No HCC Recurrence (n=65)	p-value	HCC Recurrence (n=21)	No HCC Recurrence (n=140)	p-value	
Gender (male:female)	22:4	51:14	0.506	20:1	108:32	0.055	0.892
Recipient age (in years)	60 (33–72)	56 (21–70)	0.171	59 (45–70)	58.5 (40–70)	0.876	0.277
Donor age (in years)	55 (12–84)	59 (8–91)	0.527	57 (22–78)	55 (19–84)	0.853	0.589
Alcohol	12 (46.2)	29 (44.6)		12 (57.1)	54 (38.6)		
HCV	9 (34.6)	22 (33.8)		4 (19.0)	48 (34.3)		
HBV	2 (7.7)	4 (6.2)		1 (4.8)	19 (13.6)		
Other	3 (11.5)	10 (15.3)		4 (19.2)	19 (13.6)		
AFP >200 µg/L	6 (26.1)	9 (14.3)	0.202	6 (28.6)	11 (8.3)	0.006^a	0.168
Time to transplant >120 days	11 (42.3)	35 (56.5)	0.226	8 (38.1)	69 (50.4)	0.295	0.595
Pretransplant MELD	11 (6–21)	11 (6–36)	0.654	14 (7–32)	11 (1–40)	0.174	0.112
Preoperative treatment	20 (76.9)	42 (64.6)	0.255	10 (47.6)	79 (56.8)	0.428	0.052
Mortality (%)	24 (92.3)	17 (26.2)	<0.001^a	20 (95.2)	45 (32.1)	<0.001^a	0.470
OS (months)	39 (20–165)	98 (34–212)	<0.001^a	48 (20–157)	104 (21–219)	<0.001^a	0.210
Time until recurrence (months)	20 (5–120)	–		24 (8–99)	–		0.840
NLR >4	6 (37.5)	12 (24.0)	0.291	10 (66.7)	26 (26.5)	0.002^a	0.519
NLR >5	3 (18.8)	5 (10.0)	0.351	7 (46.7)	17 (17.3)	0.010^a	0.125
CIT (min)	552 (120–1118)	518 (147–846)	0.215	569 (18–1119)	572 (77–1274)	0.333	0.186
CIT >10 h	10 (38.5)	15 (23.1)	0.137	10 (47.6)	54 (39.1)	0.460	0.042^a
WIT (min)	42 (31–65)	45 (16–80)	0.663	47 (35–70)	46 (18–115)	0.528	0.056
WIT >50 min	4 (20.0)	10 (19.2)	0.941	5 (29.4)	35 (31.3)	0.879	0.076
T3/T4 stage	7 (26.9)	5 (7.8)	0.016^a	11 (52.4)	14 (10.1)	<0.001^a	0.610
HCC bilobar	13 (50.0)	14 (21.5)	0.007^a	9 (42.9)	33 (23.7)	0.063	0.560
Poor differentiation (G >2)	9 (36.0)	6 (11.1)	0.009^a	8 (38.1)	18 (14.9)	0.011^a	0.901
Vascular invasion	8 (34.8)	9 (16.4)	0.072	11 (55.0)	18 (14.9)	<0.001^a	0.831
Within MILAN	11 (42.3)	44 (67.7)	0.025^a	4 (19.0)	94 (68.6)	<0.001^a	0.804
Number of AR within 20 months	1 (1–3)	1 (1–4)	0.014^a	–	–	–	
Extrahepatic recurrence	11 (42.3)			5 (23.8)			0.002^a
Intrahepatic recurrence	0 (0.0)			8 (38.1)			
Both extra- and intrahepatic recurrence	15 (57.5)			8 (38.1)			
Biopsy after 1 year							
Fibrosis	17 (89.5)	36 (80.0)	0.359	12 (92.3)	80 (77.7)	0.220	0.570
Stage, median	2 (0–3)	1 (0–3)	0.019^a	1 (0–2)	1 (0–3)	0.518	0.105
Steatosis	13 (72.2)	40 (80.0)	0.495	8 (57.1)	74 (66.7)	0.480	0.074
-In %	20 (0–80)	12.5 (0–80)	0.931	25 (0–50)	10 (0–90)	0.601	0.505
Hepatitis	16 (88.9)	39 (78.0)	0.314	9 (64.3)	75 (67.0)	0.841	0.036^a
Grade, median	1 (0–3)	1 (0–3)	0.158	1 (0–1)	1 (0–4)	0.425	0.010^a

Notes: ^aSignificant results. *p-value comparing HCC recurrence with AR and HCC recurrence without AR.

Abbreviations: AR, acute rejection; HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; MELD, model for end-stage liver disease; OS, overall survival; NLR, neutrophil lymphocyte ratio; CIT, cold ischemia time; WIT, warm ischemia time.

Table 2 Therapy of First AR Compared Between HCC Recurrence and No HCC Recurrence

	AR (n=91)		p-value
	HCC Recurrence n=26n (%)	No HCC Recurrence n=65n (%)	
Banff Score			
Mild (score <5)	22 (84.6)	50 (76.9)	0.563
Moderate (score 5–6)	4 (15.4)	13 (20.0)	
Severe (Score 7–9)	0 (0.0)	2 (3.1)	
Therapy of first AR			
Methylprednisolone	16 (61.5)	39 (61.9)	0.397
Elevation of immunosuppression	7 (26.9)	10 (15.9)	
Additional immunosuppression	3 (11.5)	5 (7.9)	
Methylprednisolone and immunosuppression changes	0 (0.0)	4 (6.3)	
Other medication	0 (0.0)	1 (1.6)	
None	0 (0.0)	4 (6.3)	

Abbreviations: AR, acute rejection; HCC, hepatocellular carcinoma.

tended toward prolonged cold ischemia times ($p=0.078$). Out of 47 patients with recurrent disease 33 (70.2%) were histologically proven, whereas 14 cases were radiologically confirmed in combination with elevated AFP levels with a median of 7063 $\mu\text{g/L}$.

Risk Factor Analysis for HCC Recurrence

In univariate analysis, AFP >200 $\mu\text{g/L}$, NLR >4, HCC beyond MILAN, tumor size >5 cm and >3 nodules, bilobar HCC, poor differentiation (G >2), T3 and T4 stage and vascular invasion were associated with tumor recurrence (Table 4). AR within the first 20 months after LT were associated with recurrent disease as a continuous variable (HR=1.78, 95%CI: 1.35–2.35, $p<0.001$) as well as a dichotomized variable (HR=2.39, 95%CI: 1.34–4.25, $p=0.003$). Therefore, two Cox regression models were established: model 1 with AR as a dichotomized variable (yes vs no) and model 2 with AR as a continuous variable (number of AR events) (Table 4).

Statistically significant risk factors on univariate analysis were considered in multiple Cox analyses. Instead of taking tumor size, number, and vascularisation into account, the T stage was used.

AR within 20 months as a dichotomized variable (HR=2.91, 95%CI: 1.30–6.53, $p=0.009$) and as a continuous variable (HR=1.81, 95%CI: 1.28–2.54, $p=0.001$) were found to be statistically significant predictors for HCC-recurrence after LT. Other factors associated with HCC recurrence were baseline NLR >4, T3 or T4 stage of the tumor and bilobar HCC.

Discussion

Herein we report on the association between histopathological proven AR and HCC recurrence after LT for HCC. We showed that the occurrence of AR within the first 20 months after LT is an independent risk factor for HCC recurrence. While reported incidence rates of AR after LT vary, the 36.1% incidence we observed is generally in accordance with other studies.^{26–28,34} It has been described that younger recipient age, lack of renal impairment, higher aspartate aminotransferase (AST) levels, fewer human leukocyte antibody (HLA)-DR matches, longer cold ischemia times, and older donors were independently associated with an increased incidence of AR.²⁷ Other reports on AR after LT discuss biliary complications as a potential cause and summarize that most ARs respond to bolus corticosteroid therapy.²⁸ Our study revealed that overall mortality and survival regardless of the occurrence of AR was comparable in patients without recurrent disease.²⁹

We hypothesize that the interplay of three key factors eventually determines the relationship between AR and tumor recurrence:

First, associations between immunosuppression and the risk of tumor recurrence after LT for HCC have been demonstrated previously.³⁵ In our study cohort baseline FK506 usage during the first year post-LT was comparable between patients with and without HCC recurrence. This point is important because one could have imagined that associated number of rejections had more to do with the tailored immunosuppressive games in HCC patients at high oncological risk than with HCC recurrence itself. Of note, there were no correlations between FK506 level and number of rejections, respectively. MMF was indeed administered slightly more frequently in the group with rejections as opposed to the group without rejections, however, the clinical relevance is considered low in relation to the available literature.^{36,37} With regard to the use of mTORi before HCC recurrence, also no differences were found between both groups. This is important to

Table 3 Patient Characteristics and Perioperative Outcomes Compared Between Patients with Early HCC Recurrence and Late HCC Recurrence

	Early HCC Recurrence n=27n (%)	Late HCC Recurrence n=20n (%)	p-value
Gender (male:female)	25:2	17:3	0.404
Recipient age (in years)	60 (33–72)	59 (51–70)	0.575
Donor age (in years)	53 (12–84)	58 (24–78)	0.505
Alcohol	14 (51.9)	10 (50.0)	
HCV	6 (22.2)	7 (35.0)	
HBV	3 (11.1)	0 (0.0)	
Other	4 (14.8)	3 (15.0)	
AFP >200 µg/L	10 (37.0)	2 (11.8)	0.067
Time to transplant >120 days	11 (40.7)	8 (40.0)	0.959
Pretransplant MELD	10 (6–32)	12.5 (7–23)	0.358
Preoperative treatment	19 (70.4)	11 (55.0)	0.278
Mortality (%)	27 (100.0)	17 (85.0)	0.070
OS (months)	31 (20–82)	76 (28–165)	<0.001 ^a
NLR >4	9 (52.9)	7 (50.0)	0.870
CIT (min)	550 (18–1020)	712 (340–1119)	0.078
CIT >10 h	9 (33.3)	11 (55.0)	0.137
WIT (min)	45 (31–65)	47 (35–70)	0.191
WIT >50 min	4 (18.2)	5 (33.3)	0.292
T3/T4 stage	11 (40.7)	7 (35.0)	0.689
HCC bilobar	15 (55.6)	7 (35.0)	0.163
Poor differentiation (G >2)	9 (33.3)	8 (42.1)	0.544
Vascular invasion	12 (44.4)	7 (43.8)	0.965
Within MILAN	9 (33.3)	6 (30.0)	0.808
Acute rejection (Yes)	16 (59.3)	10 (50.0)	0.528
Extrahepatic recurrence	7 (25.9)	9 (45.0)	0.248
Intrahepatic recurrence	4 (14.8)	4 (20.0)	
Both extra- and intrahepatic recurrence	16 (59.3)	7 (35.0)	
Number of AR within 20 months	1 (0–3)	0.5 (0–3)	0.680

Note: ^aSignificant results.

Abbreviations: HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; MELD, model for end-stage liver disease; OS, overall survival; NLR, neutrophil lymphocyte ratio; CIT, cold ischemia time; WIT, warm ischemia time.

emphasize, as it excludes a bias of antiproliferative immunosuppression on tumor recurrence.

Lai et al recently reported for the first time on the association between higher overall incidence of HCC recurrence and treatment of AR with steroid boluses.³⁸ They hypothesized that the usage of steroid boluses to manage AR and exposure to longstanding immunosuppression after AR treatment promotes HCC recurrence.³⁸ Based on our descriptive data, we cannot confirm or exclude causal links of steroid pulse therapy in episodes of AR and the risk of tumor recurrence. Interestingly, 65% of their matched control group consisted of patients without occurrence of AR.³⁸ Therefore, their hypothesis is not controversial to our assumption that AR itself as inflammatory immune response could be triggering oncogenesis. Nonetheless, our findings seem to elucidate possible links

between modulating immune responses and HCC recurrence after LT. Furthermore, Decaens et al found histologically proven acute rejections to be associated with significantly worsened five-year HCC recurrence-free survival after LT.³⁹

Second, AR might trigger the development of fibrosis in grafts through sustained sterile inflammation.⁴⁰ In pediatric patients an association between antibody-mediated AR and progress of graft fibrosis leading to liver graft damage after LT was reported.^{41,42} Furthermore, development and severity grade of liver fibrosis after LT is discussed as a potential predictor for death and graft loss in recipients.⁴³ The chronically inflamed environment may promote carcinogenesis and may contribute to the increased frequency of tumor recurrence. This is supported by the papers suggesting

Table 4 Model 1 (AR as a Dichotomized Variable) and Model 2 (AR as a Continuous Variable): Analysis of Risk Factors for HCC Recurrence After OLT

	Univariate		Multivariable (Model 1)		Multivariable (Model 2)	
	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
Recipient age	1.023 (0.982–1.066)	0.278				
Donor age	0.998 (0.981–1.016)	0.833				
Male recipient	0.447 (0.177–1.129)	0.088				
Recipient BMI	0.997 (0.935–1.062)	0.915				
Time to transplant >120 days	0.638 (0.356–1.144)	0.131				
Previous hepatic resection	0.504 (0.212–1.200)	0.122				
Previous RFA	0.706 (0.300–1.663)	0.426				
Previous TACE	0.849 (0.476–1.515)	0.579				
AFP >200 µg/L	3.007 (1.547–5.845)	0.001^a	1.667 (0.587–4.737)	0.338	1.543 (0.520–4.579)	0.435
NLR >4	2.695 (1.331–5.454)	0.006^a	3.735 (1.754–7.954)	0.001^a	3.765 (1.757–8.069)	0.001^a
CIT >10 h	1.349 (0.757–2.406)	0.310				
WIT >50 min	0.708 (0.409–1.836)	0.708				
Beyond MILAN	3.982 (2.155–7.357)	<0.001^a	1.357 (0.531–3.468)	0.523	1.178 (0.458–3.032)	0.734
Tumor size >5 cm	4.206 (2.322–7.619)	<0.001^a				
>3 tumor nodules	2.153 (1.114–4.161)	0.022^a				
Bilobar	2.695 (1.519–4.782)	0.001^a	2.527 (1.194–5.346)	0.015^a	2.761 (1.305–5.840)	0.008^a
T3/T4	4.690 (2.599–8.463)	<0.001^a	10.025 (4.121–24.388)	<0.001^a	9.181 (3.892–21.654)	<0.001^a
Vascular invasion	3.492 (1.911–6.381)	<0.001^a				
Poor differentiation (G >2)	2.928 (1.607–5.335)	<0.001^a	1.750 (0.747–4.101)	0.197	1.897 (0.816–4.408)	0.137
AR (Yes)	2.389 (1.344–4.249)	0.003^a	2.913 (1.299–6.532)	0.009^a		
Number of AR within 20 months	1.780 (1.348–2.350)	<0.001^a			1.805 (1.282–2.542)	0.001^a

Note: ^aSignificant results.

Abbreviations: BMI, body mass index; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; AFP, alpha-fetoprotein; NLR, neutrophil lymphocyte ratio; CIT, cold ischemia time; WIT, warm ischemia time; AR, acute rejection.

a subclass of HCC tumors is linked to markers of immune invasion.⁴⁴ Furthermore, liver fibrosis might be a factor not only associated with late, but also early phase recurrence after curative liver treatment.¹⁰ Until today no reports on the correlation of AR related inflammation as potential stimulus for fibrosis and furthermore to HCC recurrence were found.

Third, our results suggest a potential immunological role of frequent AR on HCC recurrence. As Li et al already discussed, the inflammatory microenvironment may play critical roles on orchestrating cancer cells together with immune cells to facilitate tumor recurrence post-LT.²³ Interestingly, Motomura et al reported preoperative higher IL17 levels to be significantly associated with higher NLR, leading to shorter recurrence free survival, whereas IL17 also seems to be associated with the prediction of AR after LT.^{23,45} Moreover, high expression of IL17 was identified to enhance the proliferation in HBV-related HCC.^{46,47} In order to develop new markers for early phase liver graft injury leading to late phase tumor recurrence, we are in need of an

improved understanding of the interplay between liver ischemia and inflammatory markers (eg IL17). In this regard, the role of immunoregulatory cells should be taken under focus.⁴⁸ Of note, in patients with AR, hepatitis was found more frequently one year after LT, possibly contributing to development of fibrosis and recurrent disease.

As described widely in literature, patients with HCV need to be taken under special focus, since first IFN therapy can lead to more frequent episodes of AR and second, distinction between AR and HCV recurrence might be crucial.⁴⁹ Therefore, we conducted a sub-analysis for this cohort, identifying no differences in regard to HCV viremia at the time of LT, HCV viremia duration post-LT, HCV recurrence and therapy of HCV (data not shown).

Our patient cohort was homogeneous, with a minimum length of follow-up of 20 months, with a sufficient observation time and regular post-LT check-ups. However, the results cannot be fully interpreted without considering specific limitations. The exclusion of patients who died before 20 months may potentially create a selection bias.

Nevertheless, as shown in our supplemental analysis, no differences were noticed regarding the main hypothesis, that AR is associated with HCC recurrence. Of note, the study period of over 14 years at a single center must be reported as limitation. Therefore, in the long-term, results should be verified in a prospective study to mitigate the biases associated with a single center retrospective design. Nevertheless, serious consideration should be placed on the presence and frequency of AR within the first 20 months after LT as an early clinical marker for HCC recurrence. Therefore, those patients should be taken under special surveillance with the question of HCC recurrence.

Conclusion

The occurrence and frequency of episodes of AR within 20 months after LT is associated with HCC recurrence. Our results have implications for monitoring and tumor surveillance after LT. Although we cannot prove causal links to graft fibrosis and immunological mechanisms, hypothesized interactions are worth further investigation.

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Authorship

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors have approved the final article.

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