Hepatobiliary & Pancreatic Diseases International 18 (2019) 517–524

Contents lists available at ScienceDirect

Hepatobiliary & Pancreatic Diseases International

journal homepage: www.elsevier.com/locate/hbpd

Original Article/Transplantation

Hepatocellular carcinoma recurrence after acute liver allograft rejection treatment: A multicenter European experience

Quirino Lai[a,b,*], Samuele Iesari[a,c], Armin Finkenstedt[d], Maria Hoppe-Lotichius[e], Maxime Foguenne[a], Konrad Lehner[d], Gerd Otto[e], Jan Lerut[a]

[a] Starzl Unit of Abdominal Transplantation, University Hospitals Saint Luc, Université catholique Louvain, Brussels, Belgium
[b] Hepato-biliary Surgery and Liver Transplantation Unit, Sapienza University of Rome, Umberto I Hospital, Rome, Italy
[c] Department of Bio-technological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy
[d] Department of Internal Medicine I, Innsbruck Medical University, Innsbruck, Austria
[e] Department of Transplantation and Hepatobiliary Surgery, University of Mainz, Mainz, Germany

ARTICLE INFO

Article history:
Received 10 December 2018
Accepted 10 May 2019
Available online 20 May 2019

Keywords:
Liver transplantation
Recurrence
Steroid
Liver biopsy
Acute rejection

ABSTRACT

Background: During the last decades, several risk factors for the recurrence of hepatocellular carcinoma (HCC) after liver transplantation (LT) have been investigated. However, the impact of two important drivers of oncogenesis, namely the immunosuppression and the treatment of acute cellular rejection (ACR) have been marginally addressed. This study aimed at investigating the impact of ACR treatment on the incidence of tumor recurrence in a large European HCC-LT population.
Methods: Seven hundred and eighty-one adult patients transplanted between February 1, 1985 and June 30, 2016 were retrospectively analyzed. After propensity score match, 116 patients treated for ACR using steroid boluses were compared with 115 patients who did not present any ACR or a histologic but clinical irrelevant ACR.
Results: Steroid boluses treated patients had a 18-fold higher overall incidence of HCC recurrence than those non-treated patients (16.4% vs. 0.9%; $P<0.0001$). At multivariate Cox regression analysis, steroid boluses used to treat ACR were an independent risk factor for HCC recurrence (HR=14.2; 95% CI: 1.8–110.4; $P=0.010$).
Conclusions: The decision to treat ACR as well as to reinforce immunosuppression load should be cautiously taken in view of the presented results. Prospective studies are needed to further elucidate the clinical impact of immunosuppression on HCC recurrence after transplantation.

Introduction

Liver transplantation (LT) is the best curative treatment of hepatocellular carcinoma (HCC) developed in a cirrhotic liver [1]. Unfortunately, about 15%–20% of LT patients experience HCC recurrence [2]. During the last two decades, multiple pre- and post-LT risk factors for recurrence have been investigated [3–5]. Although HCC development and recurrence had already been linked to immunosuppression (IS) more than 30 years ago both in the experimental and clinical settings, the "tumor risk factor IS" has shown controversial results [6–10]. The inconsistency of the studies regarding IS with HCC recurrence is explained by the fact that most investigated cohorts were heterogeneous, receiving very different IS schemes. Moreover, almost all attention in this field of transplant oncology has been given to the morphologic (numbers and diameter of tumor) and biologic (tumor markers and PET-scan tumor uptake) behavior of cancer [11].

Clinical research on HCC recurrence has been primarily done in relation to the detrimental role of the IS load in form of continued steroid use or higher dosage of calcineurin inhibitors (CNI) and the protective role of mammalian target of rapamycin inhibitors (mTORi), with these latter drugs exhibiting both immunosuppressive and anti-angiogenic/proliferative properties [12–17]. The impact of the treatment of acute cellular rejection (ACR) as a possible risk factor for HCC recurrence has never been reported.

The study aimed to investigate the effect of ACR treatment with steroids on HCC recurrence in a broader European population of LT patients using a rigorous statistical approach based on a propensity score matching (PSM).

* Corresponding author at: Starzl Unit of Abdominal Transplantation, University Hospitals Saint Luc, Université catholique Louvain, Brussels, Belgium.
E-mail address: lai.quirino@libero.it (Q. Lai).

https://doi.org/10.1016/j.hbpd.2019.05.006
1499-3872/

Methods

A retrospective analysis was performed including 781 adult patients transplanted between February 1, 1985 and June 30, 2016, and having a pre-LT radiological/pathological diagnosis of HCC. Data were obtained from the prospectively collected databases of three collaborative centers: Brussels ($n = 309$), Innsbruck ($n = 296$), and Mainz ($n = 176$). In order to create two homogeneous and comparable groups, the following exclusion criteria were used: (a) follow-up shorter than two years after LT in non-recurred patients (as this may indicate a too short time for developing a recurrence in these patients) [11] ($n = 134$); (b) HCC recurrence within six months (as this may reflect an inadequate selection process or underestimated tumor load) [18] ($n = 14$) or after five years (as this may be indicative of a possible *de novo* tumor formation) [19] ($n = 14$) from LT; (c) re-LT within six months ($n = 20$); and (d) insufficient data collection ($n = 18$).

This procedure identified 581 patients for PSM analysis. The dependent variable used for the stratification of groups was the use of steroid boluses to treat a clinically relevant ACR.

PSM allowed to identify a total population of 231 cases: 116 patients were treated with steroid boluses (ACR-STER group) and 115 patients without ACR or with a pathologic, but clinically irrelevant ACR (control group). The follow-up of the investigated population was 4.2 years (interquartile ranges [IQR]: 3.0–6.5).

IS regimen after LT

All three centers had similar approaches regarding IS handling consisting of CNI-based IS and low-dose steroid use, sometimes combined with anti-metabolites mycophenolate mofetil (MMF) ($n = 136$, 58.9%) or azathioprine (AZA) ($n = 38$, 16.5%). In the case of reduced renal function at the time of LT, the interleukin-2 receptor antagonist basiliximab (Simulect®, Novartis Basel, CH) was administered to delay the introduction of CNI ($n = 42$, 18.2%). Steroids were gradually tapered and discontinued within 3–6 months from LT. From 2000 onwards, steroids were not used at all or withdrawn within three months: during this period, steroids were removed after >3 months in only 27/200 (13.5%) cases. In the case of CNI-related side effects, some patients were switched to mTORi ($n = 18$, 7.8%). Serum trough levels of CNI or mTORi were regularly monitored at the outpatient clinic and maintained within clinically recommended ranges.

Diagnosis of acute cellular rejection

Unexplained liver test disturbances were thoroughly investigated, always including a liver biopsy. In the case of discordant pathology and clinical findings (e.g., healthy or slightly raised liver tests in patients with histologically proved ACR after protocol biopsy), ACR was considered clinically irrelevant and consequently left untreated ($n = 40$, 17.3%). Only a minority of patients had ACR treatment based on clinical suspicion ($n = 30$, 13.0%). In Brussels, protocol liver biopsies at seven days and one year were done in all patients; whereas the Mainz and Innsbruck centers did not perform protocol biopsies. The severity of ACR was graded following Banff criteria, being stratified as mild (score <5), moderate (5–6), and severe (7–9) rejections [20].

Treatment of acute cellular rejection

ACR treatment consisted in all cases of intra-venous steroid boluses, with a median dosage of 1500 mg (IQR: 1000–1500) followed by steroid maintenance for 3–6 months. Furthermore, IS load was raised by introducing either cyclosporine A (CyA) or tacrolimus (TAC) in CNI-free patients (5/116, 4.3%), switching from CyA to TAC in CyA-treated patients (8/116, 6.9%), higher dosing of CNI-treatment (36/116, 31.0%) and by adding anti-metabolites (51/116, 44.0%).

Statistical analysis

Continuous and dummy variables were reported as medians (IQRs) and as numbers (percentages), respectively. Comparisons for continuous variables were performed using the Mann–Whitney test. For dummy variables, the Chi-square test or the exact Fisher test were used when appropriate. Fifteen different variables recognized in the recent HCC-LT literature as possible confounders for the risk of post-LT HCC recurrence were used for matching the patients in the PSM: transplantation before or after 1996, waiting-time per month, male sex, age per decade, positive hepatitis C virus (HCV)-status, radiological tumor progression, pre-LT alpha-fetoprotein (AFP) value, pre-LT loco-regional treatment (LRT), diameter of the largest lesion >5 cm or number of lesions >3, Milan criteria (MC)-OUT-status, tumor multifocality, poorly differentiated tumor, macro- and microvascular invasion. All morphologic tumor characteristics were based on the final pathology report of the total hepatectomy specimen. PSM was performed using a "nearest neighbor matching" algorithm to match to each ACR-STER group patient a control group patient having the closest propensity score. A caliper of few than 0.20 times the standard deviation of the scores was used [21]. Each pair was used once. Unpaired patients were discarded from the analysis. A final 1:1 match was generated [22].

A multivariate Cox regression analysis was performed on the post-PSM population to identify independent risk factors for HCC recurrence after LT. Initially, 26 different variables were analyzed in the model: male sex, age at LT, HCV-related cirrhosis, waiting time, model for end-stage liver disease (MELD) score >15 at LT, no use of LRT, radiological progression of tumor disease, radiological complete response, $\log_{10}$AFP value (ng/mL), dimension of the largest lesion >5 cm, number of nodules >3, MC–OUT status, poorly differentiated tumor and microvascular invasion at pathology, severity of rejection taking into account Banff score, use of basiliximab, initial use of TAC, initial use of CyA, high exposure to CNI during the first year post-LT, initial use of mTORi, initial use of MMF, initial use of AZA, initial steroid-free therapy, steroid withdrawal, and use of and total dose of steroid boluses per 1000 mg as treatment of ACR. A stepwise backward conditional method was used to exclude step-by-step the covariates with a P value >0.1.

High exposure to CNI was determined by median TAC and CyA serum trough levels at 1, 3, 6, 9, and 12 months after LT or after ACR treatment. Cut-off exposure values were defined as >10 ng/mL for TAC and > 400 ng/mL for CyA, respectively [16].

HCC recurrence rate was presented using Kaplan–Meier statistics and log-rank tests on the entire population. A subgroup analysis was also done on patients with or without pre-ACR steroid withdrawal. Variables with a $P < 0.05$ were considered statistically significant. SPSS statistical package version 23.0 (SPSS Inc., Chicago, IL, USA) was used.

Results

Patient- and tumor-related characteristics before and after PSM are displayed in Tables 1 and 2. After PSM, 116 patients with one or more episodes of ACR treated with steroid boluses were identified and compared to 115 patients consisting of 75 (65.2%) patients who did not present ACR and 40 (34.8%) patients who presented a documented but clinically irrelevant rejection not deserving treatment. When comparing these two groups, no differences were observed concerning patient characteristics, underlying liver

Table 1
Patient- and tumor-related characteristics in two groups before PSM.

Variables	ACR-STER group (n = 116)	Control group (n = 465)	P value
Male sex[§]	94 (81.0%)	389 (83.7%)	0.491
Age at LT (yr)[§]	57 (50–63)	59 (51–64)	0.470
Waiting time (mon)[§]	4.7 (1.7–10.0)	5.7 (3.0–9.4)	0.166
Waiting time ≤4 months	56 (48.3%)	180 (38.7%)	0.072
Underlying liver disease[*]			
HCV-related cirrhosis[§]	40 (34.5%)[**]	172 (37.0%)[**]	0.667
HBV-related cirrhosis	12 (10.3%)	65 (14.0%)	0.360
Alcohol-related cirrhosis	45 (38.8%)	187 (40.2%)	0.832
NASH-related cirrhosis	12 (10.3%)	37 (8.0%)	0.454
Other liver pathology	12 (10.3%)	34 (7.3%)	0.243
Lab-MELD at LT	11 (8–16)	10 (8–14)	0.721
LT before 1996[§]	16 (13.8)	19 (4.1)	**0.0001**
Radiological tumor characteristics before LT			
Major lesion diameter > 5.0 cm	7 (6.0%)	25 (5.4%)	0.334
Number of lesions > 3	20 (17.2%)	66 (14.2%)	**0.002**
Higher value of $\log_{10}$AFP (ng/mL)[§]	1.2 (0.7–1.8)	1.0 (0.7–1.5)	0.102
Radiological response after LRT			
CR	24 (20.7%)	134 (28.8%)	0.081
PR	45 (38.8%)	137 (29.5%)	0.058
SD	12 (10.3%)	73 (15.7%)	0.186
PD[§]	17 (14.7%)	76 (16.3%)	0.777
No LRT[§]	18 (15.5%)	47 (10.1%)	0.102
Tumor pathology			
Major lesion diameter (cm)	2.0 (0.8–3.5)	1.5 (0.5–3.0)	0.107
Major lesion diameter >5.0 cm[§]	11 (9.5%)	29 (6.2%)	0.221
Number of lesions	1 (1–3)	1 (1–2)	0.096
Number of lesions >3[§]	16 (13.8%)	60 (12.9%)	0.760
MC–OUT status[§]	38 (32.8%)	113 (24.3%)	0.076
Multifocality[§]	56 (48.3%)	203 (43.7%)	0.404
Bilobarity	26 (22.4%)	85 (18.3%)	0.503
Poorly differentiated tumor (G3–G4)[§]	10 (8.6%)	55 (11.8%)	0.411
Microvascular invasion[§]	18 (15.5%)	68 (14.6%)	0.772
Macrovascular invasion[§]	5 (4.3%)	5 (1.1%)	**0.031**
Post-LT recurrence	19 (16.4%)	69 (14.8%)	0.666

Control group: patients without ACR or with a pathologic, but clinically irrelevant ACR.
* In 36 cases, patients contemporaneously presented multiple pathologies: HCV + alcohol = 16; HCV + HBV = 6; HBV + alcohol = 4; alcohol + other = 4; alcohol + NASH = 2; HCV + other = 3; HBV + other = 1.
** 6 cases in the ACR-STER group and 3 cases in the control group were initally diagnosed as non A non B hepatitis and then updated as HCV after 1991. §: covariates used for the propensity score match. ACR: acute cellular rejection; STER: steroid; LT: liver transplantation; HCV: hepatitis C virus; HBV: hepatitis B virus; NASH: non-alcoholic steato-hepatitis; MELD: model for end-stage liver disease; AFP: alpha-fetoprotein; LRT: loco-regional treatment; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; MC: Milan criteria.

disease, length of waiting time, and the severity of liver disease at the time of LT. The only observed difference was related to the period of LT, with more cases transplanted before 1996 in the group treated with steroid boluses (13.8% vs. 4.4%; $P = 0.010$). No differences were observed between the groups regarding tumor characteristics such as AFP values at LT and pathology of the total hepatectomy specimen. In particular, poorly differentiated tumor (8.6% vs. 8.7%; $P = 1.000$) and microvascular invasion (15.5% vs. 19.1%; $P = 0.491$) were similar in both groups.

CyA-based initial IS was more common in ACR-STER group (25.0% vs. 11.3%; $P = 0.010$) and TAC-based initial IS was more common in the control group (87.0% vs. 68.1%; $P = 0.001$), explaining also the more frequent steroid withdrawal in this group (96.5% vs. 84.5%; $P = 0.003$). No differences were noted when analyzing CNI and mTORi exposure during the first post-LT year (Table 3).

The analysis of ACR characteristics in steroid treated and non-treated patients revealed that almost all the patients in the control group (39/40, 97.5%) had a biopsy-proven ACR (BPACR); this percentage was significantly lower in the ACR-STER group (86/116; 74.1%; $P = 0.001$). The number of patients presenting a severe BPACR was similar in both groups (18.6% vs. 23.1%; $P = 0.631$), as was the severity of ACR (median score: 6 vs. 5; $P = 0.839$) (Table 4).

HCC recurrence was diagnosed in 20 (8.7%) of 231 patients after a median time of 16.6 months (IQR: 10.7–28.1). HCC recurrence was 18-fold higher in the ACR-STER group than in the control group (16.4% vs. 0.9%; $P < 0.0001$). All recurrences in the ACR-STER group were diagnosed after ACR treatment; the median time from first steroid bolus administration to recurrence was 13.9 months (IQR: 10.1–28.0).

With the intent to identify independent risk factors for HCC recurrence, a multivariate Cox regression analysis was constructed. Well-known risk factors for recurrence such as microvascular invasion and poorly differentiated tumor lost their significance during the stepwise procedure; in contrast, the tumor-related variables AFP level (hazard ratio [HR]=2.9; 95% CI: 1.7–4.8; $P < 0.0001$), radiological progression of disease (HR=4.9; 95% CI: 1.7–14.5; $P = 0.004$) and pathological MC–OUT status (HR=4.2; 95% CI: 1.4–12.9; $P = 0.010$) remained significant. HCV-related cirrhosis was a patient-related risk factor (HR=5.8; 95% CI: 1.9–17.2; $P = 0.002$). Steroid withdrawal was protective against the risk of recurrence (HR=0.2; 95% CI: 0.1–0.6; $P = 0.003$), and steroid treatment of ACR was an IS-related risk factor for recurrence (HR=14.2; 95% CI: 1.8–110.4; $P = 0.010$) (Table 5).

One-, three- and five-year HCC recurrence rates in the ACR-STER group and control group were 4.3%, 13.0% and 17.0% versus 0.9%, 0.9% and 0.9%, respectively ($P < 0.0001$) (Fig. 1).

A sub-analysis was also performed to stratify the entire population according to the pre-ACR use of steroids. In patients with pre-ACR steroid withdrawal, a significant difference was again confirmed when comparing the ACR-STER and control groups (five-year recurrence rate: 12.6% vs. 0%; $P = 0.001$). In the subgroup of

Table 2
Patient- and tumor-related characteristics in two groups after PSM.

Variables	ACR-STER group ($n = 116$)	Control group ($n = 115$)	P value
Male sex	94 (81.0%)	95 (82.6%)	0.865
Age at LT (yr)	57 (50–63)	60 (54–64)	0.128
Waiting time (mon)	4.7 (1.7–10.0)	6.0 (3.0–9.7)	0.275
Waiting time ≤4 months	56 (48.3%)	44 (38.3%)	0.145
Underlying liver disease*			
HCV-related cirrhosis	40 (34.5%)**	40 (34.8%)**	1.000
HBV-related cirrhosis	12 (10.3%)	13 (11.3%)	0.836
Alcohol-related cirrhosis	45 (38.8%)	54 (47.0%)	0.233
NASH-related cirrhosis	12 (10.3%)	12 (10.4%)	1.000
Other liver pathology	12 (10.3%)	10 (8.7%)	0.823
Lab-MELD at LT	11 (8–16)	10 (8–14)	0.576
LT before 1996	16 (13.8%)	5 (4.4%)	**0.010**
Radiological tumor characteristics before LT			
Major lesion diameter > 5.0 cm	7 (6.0%)	14 (12.2%)	0.700
Number of lesions > 3	20 (17.2%)	19 (16.5%)	**0.040**
Higher value of $\log_{10}$AFP (ng/mL)	1.2 (0.7–1.8)	1.0 (0.7–1.5)	0.227
Radiological response after LRT			
CR	24 (20.7%)	30 (26.1%)	0.354
PR	45 (38.8%)	38 (33.0%)	0.411
SD	12 (10.3%)	11 (9.6%)	1.000
PD	17 (14.7%)	28 (24.3%)	0.069
No LRT	18 (15.5%)	18 (15.7%)	0.100
Tumor pathology			
Major lesion diameter (cm)	2.0 (0.8–3.5)	2.0 (1.0–3.0)	0.956
Major lesion diameter > 5.0 cm	11 (9.5%)	9 (7.8%)	0.816
Number of lesions	1 (1–3)	1 (1–3)	0.896
Number of lesions >3	16 (13.8%)	20 (17.4%)	0.474
MC–OUT status	38 (32.8%)	28 (24.3%)	0.190
Multifocality	56 (48.3%)	56 (48.7%)	1.000
Bilobarity	26 (22.4%)	27 (23.5%)	1.000
Poorly differentiated tumor (G3–G4)	10 (8.6%)	10 (8.7%)	1.000
Microvascular invasion	18 (15.5%)	22 (19.1%)	0.491
Macrovascular invasion	5 (4.3%)	1 (0.9%)	0.213
Post-LT recurrence	19 (16.4%)	1 (0.9%)	**<0.0001**

Control group: patients without ACR or with a pathologic, but clinically irrelevant ACR.
* In 19 cases, patients contemporaneously presented multiple pathologies: HCV + alcohol = 8; HBV + alcohol = 3; alcohol + other = 2; alcohol + NASH = 2; HCV + other = 2; HCV + HBV = 2.
** 5 cases in the ACR-STER group and one case in the CONTROL group were initally diagnosed as non A non B hepatitis and then updated as HCV after 1991. ACR: acute cellular rejection; STER: steroid; LT: liver transplantation; HCV: hepatitis C virus; HBV: hepatitis B virus; NASH: non-alcoholic steato-hepatitis; MELD: model for end-stage liver disease; AFP: alpha-fetoprotein; LRT: locoregional treatment; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; MC: Milan criteria.

Table 3
Immunosuppression regimen used in two patient groups after PSM.

Induction drugs use	ACR-STER group ($n = 116$)	Control group ($n = 115$)	P value
Basiliximab	18 (15.5%)	24 (20.9%)	0.311
mTORi use			
Drug introduction at any moment	26 (22.4%)	14 (12.2%)	0.055
Initial (<3 mon) introduction	8 (6.9%)	4 (3.5%)	0.375
Continuous use >1 year	21 (18.1%)	11 (9.6%)	0.085
Still ongoing use	13 (11.2%)	9 (7.8%)	0.502
Interruption for any cause	8 (6.9%)	3 (2.6%)	0.215
CNI use			
Initial TAC use	79 (68.1%)	100 (87.0%)	**0.001**
TAC exposure during first LT year (ng/mL)	5.8 (4.9–7.1)	5.7 (4.8–6.8)	0.369
Initial CyA use	29 (25.0%)	13 (11.3%)	**0.010**
CyA exposure during first LT year (ng/mL)	242 (158–316)	185 (124–271)	0.281
Anti-metabolites use			
Initial MMF use	66 (56.9%)	70 (60.9%)	0.593
Initial azathioprine use	25 (21.6%)	13 (11.3%)	0.050
Steroids use			
Steroid-free initial approach	2 (1.7%)	1 (0.9%)	1.000
Steroid withdrawal	98 (84.5%)	111 (96.5%)	**0.003**

Control group: patients without ACR or with a pathologic, but clinically irrelevant ACR. ACR: acute cellular rejection; STER: steroid; mTORi: mammalian target of rapamycin inhibitor; TAC: tacrolimus; CyA: cyclosporine A; MMF: mycophenolate mofetil.

patients having continuous steroid administration, the ACR-STER group patients having both continuous steroid administration and steroid bolus treatment further raised the five-year HCC recurrence rate to a remarkable 42.9%. Interestingly, the five-year recurrence rate was also markedly elevated to 25% in patients of the control group continuing steroids (Table 6).

When analyzing the modifications of IS after treatment in the ACR-STER group, two main differences were related to HCC recurrence: recurring patients were more commonly kept on steroids during the first post-LT year (57.9% vs. 32.0%; P=0.04) and on reinforced IS by adding CNI or by switching CNI from CyA to TAC (68.4% vs. 37.1%; $P = 0.020$) (Table 7).

Table 4
Specific aspects correlated with acute rejection in the post-PSM population.

Variables	ACR-STER group ($n = 116$)	ACR-non-treated group ($n = 40$)*	P value
Biopsy-proved ACR	86 (74.1%)	39 (97.5%)	**0.001**
Banff score§	6 (4–7)	5 (4–7)	0.839
Mild (score <5)	46 (53.5%)	22 (56.4%)	0.847
Moderate (score 5–6)	24 (27.9%)	8 (20.5%)	0.508
Severe (score 7–9)	16 (18.6%)	9 (23.1%)	0.631

* On 115 patients. §Including 86 patients in ACR-STER group and 39 ACR-non-treated patients from control group. ACR: acute cellular rejection, STER: steroid.

Table 5
Multivariable Cox regression analysis for the risk of HCC recurrence after liver transplantation in the post-PSM population.

Variables	Beta coefficient	HR	95%CI	P value
HCV-related cirrhosis	1.749	5.8	1.9–17.2	**0.002**
Radiological progression of disease	1.591	4.9	1.7–14.5	**0.004**
$\log_{10}$AFP value (ng/mL)	1.055	2.9	1.7–4.8	**<0.0001**
Pathological MC–OUT status	1.436	4.2	1.4–12.9	**0.010**
Steroid withdrawal	−1.524	0.2	0.1–0.6	**0.003**
Use of steroid bolus for ACR treatment	2.655	14.2	1.8–110.4	**0.010**

-2Log likelihood: 146.4. Variables initially introduced in the model and then removed using a backward conditional method: male sex, age at LT (per year), waiting time (per month), MELD > 15 at LT, no use of locoregional treatments, radiological complete response, pathological dimension of the target lesion > 5 cm, pathological number of nodules >3, poorly differentiated tumor, microvascular invasion, severe Banff score, use of basiliximab, initial use of tacrolimus, initial use of cyclosporine, high exposure to CNI during the first year after LT, initial use of mTORi, initial use of MMF, initial use of azathioprine, steroid-free initial therapy, total dose of steroid boluses for the treatment of ACR (per 1000 mg). HR: hazard ratio; 95% CI: 95% confidence intervals; HCV: hepatitis C virus; AFP: alpha-fetoprotein; MC: Milan criteria; ACR: acute cellular rejection; LT: liver transplantation; MELD: model for end-stage liver disease; CNI: calcineurin inhibitor; mTORi: mammalian target of rapamycin inhibitor; MMF: mycophenolate mofetil.

Discussion

The majority of studies dealing with HCC recurrence focus on the well-established tumor-related variables morphology (number and diameter), biology (evolution of tumor and inflammatory markers in the absence or presence of LRT and tumor uptake at PET-scan) [23,24] and pathology (microvascular invasion, tumor differentiation, satellite lesions) of the hepatectomy specimen.

Conversely, the role of IS has been less extensively clarified, although several studies have been published on this topic. Indeed, Virchow already demonstrated as early as 1863 that inflammation and tumor formation are closely linked [25,26]. This early observation has been confirmed later on, both in countless experiments and in clinical experiences. It is well known from all different fields of oncology that an immunosuppressed state is a significant trigger of oncogenesis [3–5,7,27,28] and that cancer is a price to pay for immunosuppressive therapy [29–31]. This price has been demonstrated for all different categories of immunosuppressive drugs [9,32–35].

A possible explanation for the inconsistency of the studies focused on IS and HCC recurrence lies in the fact that investigating the correlation between IS *per se* and reinforced IS (such as applied in the treatment of ACR), and HCC recurrence after LT is difficult due to the presence of numerous pre- and post-LT confounders. With the intent to adequately study the link between IS and ACR treatment, it is of importance to construct a model based on the comparison of patients with similar tumor-related characteristics.

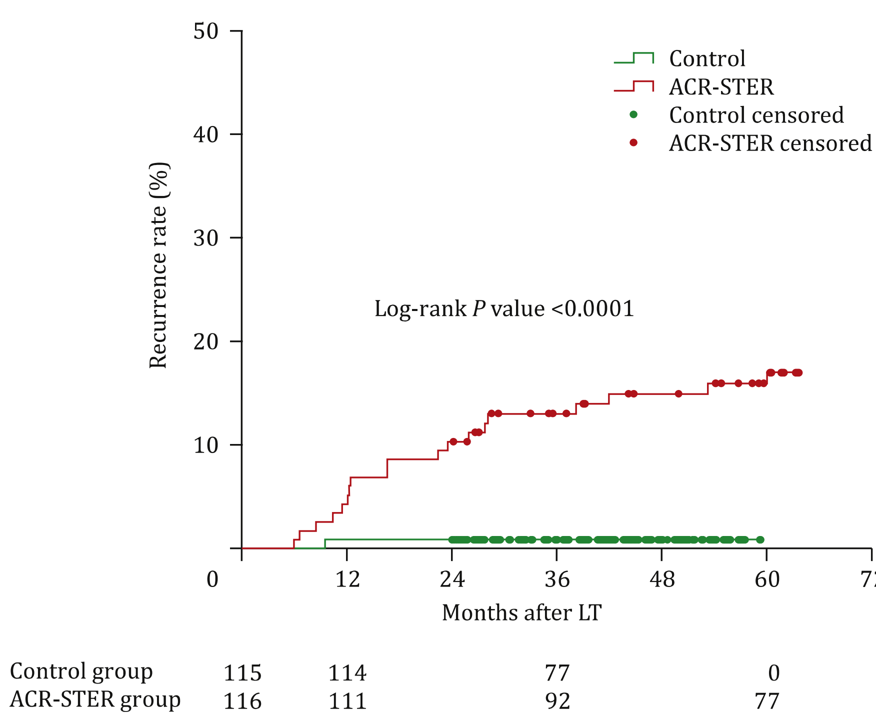

Fig 1. HCC recurrence rates observed in two post-PSM groups of patients with or without acute cellular rejection treatment.

Table 6
Recurrence rates of the post-PSM population and the two sub-groups of patients with and without pre-ACR steroid withdrawal.

Groups	*n*	Recurrence rate			*P* value
		1-year	3-year	5-year	
Entire population					
ACR treated	116	4.3%	13.0%	17.0%	**<0.0001**
No treatment	115	0.9%	0.9%	0.9%	
No pre-ACR steroid withdrawal					
ACR treated	18	22.2%	33.3%	42.9%	0.727
No treatment	4	25.0%	25.0%	25.0%	
Pre-ACR steroid withdrawal					
ACR treated	98	1.0%	9.2%	12.6%	**0.001**
No treatment	111	0	0	0	

ACR: acute cellular rejection.

Table 7
Immunosuppression regimen used in the ACR-treated group, differences between recurring and not recurring cases.

Variables	No recurrence ($n = 97$)	HCC recurrence ($n = 19$)	*P* value
Second treatment with steroid boluses	13 (13.4%)	2 (10.5%)	1.000
Post-ACR steroid continuation >6 months	39 (40.2%)	11 (57.9%)	0.206
Post-ACR steroid continuation >12 months	31 (32.0%)	11 (57.9%)	**0.040**
CNI add or switch to a stronger CNI	36 (37.1%)	13 (68.4%)	**0.020**
MMF introduction	45 (46.4%)	6 (31.6%)	0.314
mTORi introduction	3 (3.1%)	0	1.000
Any post-ACR IS change	78 (80.4%)	17 (89.5%)	0.519
High CNI exposure 1 year after ACR	16 (16.5%)	6 (31.6%)	0.196

HCC: hepatocellular cancer; ACR: acute cellular rejection; CNI: calcineurin inhibitor; MMF: mycophenolate mofetil; mTORi: mammalian target of rapamycine inhibitor; IS: immunosuppression.

Removing during the stepwise multivariate Cox regression analysis several well-identified risk factors of HCC recurrence confirms the validity of the here presented approach. Before constructing a PSM which aimed to minimize selection biases, 200 patients had already been removed from analysis because of short (<2 years) post-LT follow-up or too early (<6 months) or too late (>5 years) recurrence or occurrence [18–20]. The decision to remove all the not recurred cases with a follow-up <2 years was connected with the necessity to exclude cases without enough time to develop a recurrence. On the opposite, we decided to preserve the subjects recurring between 6 and 24 months because they met the event of interest (i.e., recidivism). We can argue that this approach should "artificially" alter the number of recurred cases in the study, but we considered our approach methodologically robust. We felt even a worse bias to introduce in the PSM patients experiencing a premature death and, consequently, not enough time for developing a recurrence.

The final PSM model allowed comparing two patient groups well balanced for the variables used for constructing the PSM. Although one could argue that this methodology results in the loss of many data, mainly regarding numerosity of events of interest (e.g., tumor recurrence), PSM appears to be the best method to obtain statistical reliability in this challenging field of clinical research.

Despite this rigorous approach, it is important to point out that a higher number of patients transplanted before 1996 belonged to the ACR-STER group, a period during which prophylactic and therapeutic IS regimen was more frequently CyA-based, a regimen known to have a higher incidence of rejection when compared to TAC-based IS [36,37]. This possible bias related to the CNI choice has however been reduced by the similar median CyA and TAC exposure during the first post-LT year [16,38]. Moreover, this parameter lost its significance in the multivariate analysis.

Conversely, the use of steroids plays an essential role in tumor recurrence. Previous studies have reported that steroid withdrawal during the post-LT period was protective against the risk of recurrence [39,40]. The Chinese Liver Transplant Registry study including 368 patients treated without and 736 with steroids showed that MC-IN HCC HBV-infected patients with steroid-including IS displayed a significantly higher recurrence rate ($P<0.05$) [39]. The Guangzhou center study including 502 LT patients also correlated recurrence rate and delay of steroid withdrawal. One-year recurrence rates were 5.1%, 7.8%, 14.1% and 21.4% in patients never on steroids or stopping steroids after 0.5, 3 and 6 months, respectively ($P = 0.01$). Three-year recurrence rates were also significantly different (12.8%, 15.6%, 23.9% and 35.7%; $P = 0.04$) [40]. Of note, a recent meta-analysis including 1980 LT patients showed that rapid steroid discontinuation in TAC-based IS did not result in increased risk of rejection nor of morbidity [41]. The Japanese Sendai study including 144 living donor LT showed that strongly immunosuppressed patients had worse results compared to those given less immunosuppression (five-year disease-free survival rate: 20.0% vs. 76.2%; $P = 0.046$) and that steroid use in the first 6 months was a risk factor for recurrence in univariate analysis ($P = 0.026$) [42]. A randomized prospective multicenter trial coming from the US confirmed the safety of a steroid-free regimen in HCV-positive patients regarding rejection rates and overall survivals, further suggesting that the benefits deriving from early steroid withdrawal are more significant than the possible adverse effects coming from withdrawal [43].

In the presented series, the use of steroid boluses for ACR treatment led to an 18-fold-increase in overall risk for HCC recurrence. Moreover, a temporal correlation was identified between ACR treatment and HCC recurrence, with all cases recurring within one to two years after ACR treatment. Our findings strongly contrast with the absence of information about this topic in the transplant oncology literature although the impact of steroids, both direct and indirect, on oncogenesis has been documented since more than two decades. The direct action consists of anti-apoptotic and proliferative promoting effects on cancer cells [44]. The indirect action comprises in a decrease in cancer immunosurveillance by inactivating T and B cells (leading to reduced exposure of cancer antigens to the major histocompatibility complexes) and in the inhibition of the neutrophil-mediated tumor cell cytostasis by decreasing the binding of neutrophils to cancer cells [45]. Of note also the finding that steroid receptor-free HCC patients have higher survival rates [46]. All these findings are in line with our data, and they further clarified the link between oncogenesis and inflammation (read rejection) in the context of transplantation [47,48]. Firstly, the ACR-related inflammation might favor recurrence, and secondly, the following immunosuppressive treatment using steroids can be seen as a "double hit phenomenon" further fertilizing the soil for tumorigenesis. It should be mentioned that the sub-analysis focusing on ACR severity was not able to correlate the severity of Banff score and tumor recurrence.

It is of course hard to determine, based on data obtained from a retrospective study, if it is the use of steroid boluses *per se* or the subsequent higher IS load that promotes tumor recurrence. Vivarelli et al. [16,49,50] and afterward, Rodríguez-Perálvarez et al. [38] reported in a retrospective study a higher incidence of HCC recurrence in case of higher IS load. In our study, the total dose of steroid boluses did not correlate with recurrence, whereas post-ACR IS regimen changes such as delayed (>1 year) steroid discontinuation and switch to a reinforced IS regimen did in ACR treated cases. All these observations corroborate the idea that both uses of steroid boluses to manage rejection and exposure to long-standing increased IS after ACR treatment promote HCC recurrence.

Despite the design of a rigorous statistical model, this report still has some limitations. Firstly, the study is a retrospective one, resulting in the incompleteness of some data such as detailed information about inflammation due to HCV allograft re-infection before ACR. This argument is counteracted by the fact that HCV allograft reinfection is universal in the absence of anti-viral treatment (all patients were transplanted in the pre-direct acting anti-viral agents era) and occurs later than ACR. Another aspect impossible to be retrospectively evaluated is represented by an HCV recurrence being confused with a histologically proved ACR, with steroids given to manage recurrent HCV rather than ACR [51]. Secondly, the study is a tri-centric one, implying some, albeit minor, differences related to IS- and pre-LT HCC handling. A study spanning three decades is prone to the biases deriving from the significant changes observed regarding IS management, with early heavy-handed use of CNI followed over time by lower IS trough levels and the added use of adjunctive agents. However, this tri-centric collaboration was set up with the intent to merge similar philosophies regarding tumor as well as IS handling. The third one is the seemingly low number of recurrences in the investigated population after the artificial censoring by PSM. This rigorous PSM construct was, however, necessary to create two very homogeneous and comparable patient groups. Lastly, although several variables were selected for trying to balance the two cohorts thanks to the PSM, some differences were not completely solved. As an example, adverse HCC-specific risk factors like multifocality, bilobarity, MC–OUT status or macrovascular invasion were more commonly observed, although not statistically significant, in the ACR-STER group. The present result was observed although all of these covariates were adopted for constructing the PSM, showing the limits of a retrospective analysis even after use of a sophisticated statistical analysis like the PSM.

In conclusion, steroids play an essential role in the tumor recurrence after LT. This role was reinforced when treating ACR using steroid boluses raising the risk for recurrence to 18 fold. Conversely, steroid withdrawal decreased HCC recurrent rate. Prospective studies are needed to validate our results.

Contributors

LQ, IS, FA and LJ were responsible for the conception, design and analysis of the study and for writing the paper. LQ, IS, FA, HLM, FM, and LK were involved with the collection and interpretation of data. FA, HLM and OG participated in data management and manuscript review. All authors contributed to the design and interpretation of the study and to further drafts. LQ is the guarantor.

Funding

None.

Ethical approval

Not needed.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

[1] Lai Q, Lerut JP. Hepatocellular cancer: how to expand safely inclusion criteria for liver transplantation. Curr Opin Organ Transpl 2014;19:229–234.
[2] Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, et al. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. Liver Transpl 2011;17:S44–S57.
[3] Toso C, Meeberg G, Hernandez-Alejandro R, Dufour JF, Marotta P, Majno P, et al. Total tumor volume and alpha-fetoprotein for selection of transplant candidates with hepatocellular carcinoma: a prospective validation. Hepatology 2015;62:158–165.
[4] Lai Q, Nicolini D, Inostroza Nunez M, Iesari S, Goffette P, Agostini A, et al. A novel prognostic index in patients with hepatocellular cancer waiting for liver transplantation: time-radiological-response-alpha-fetoprotein-inflammation (TRAIN) score. Ann Surg 2016;264:787–796.
[5] Mazzaferro V, Sposito C, Zhou J, Pinna AD, De Carlis L, Fan J, et al. Metroticket 2.0 model for analysis of competing risks of death after liver transplantation for hepatocellular carcinoma. Gastroenterology 2018;154:128–139.
[6] Yokoyama I, Carr B, Saitsu H, Iwatsuki S, Starzl TE. Accelerated growth rates of recurrent hepatocellular carcinoma after liver transplantation. Cancer 1991;68:2095–2100.
[7] Lerut J, Iesari S, Foguenne M, Lai Q. Hepatocellular cancer and recurrence after liver transplantation: what about the impact of immunosuppression? Transl Gastroenterol Hepatol 2017;2:80.
[8] Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 1999;397:530–534.
[9] Shinozuka H, Warty VS, Masuhara M, Murase N, Iwatsuki S. Effect of FK 506 on experimental liver carcinogenesis. Transplant Proc 1991;23:3197–3199.
[10] Andrassy J, Graeb C, Rentsch M, Jauch KW, Guba M. mTOR inhibition and its effect on cancer in transplantation. Transplantation 2005;80:S171–S174.
[11] Lai Q, Vitale A, Iesari S, Finkenstedt A, Mennini G, Spoletini G, et al. Intention-to-treat survival benefit of liver transplantation in patients with hepatocellular cancer. Hepatology 2017;66:1910–1919.
[12] Chen ZS, He F, Zeng FJ, Jiang JP, Du DF, Liu B. Early steroid withdrawal after liver transplantation for hepatocellular carcinoma. World J Gastroenterol 2007;13:5273–5276.
[13] Xing T, Huang L, Yu Z, Zhong L, Wang S, Peng Z. Comparison of steroid-free immunosuppression and standard immunosuppression for liver transplant patients with hepatocellular carcinoma. PLoS One 2013;8:e71251.
[14] Lerut J, Mathys J, Verbaandert C, Talpe S, Ciccarelli O, Lemaire J, et al. Tacrolimus monotherapy in liver transplantation: one-year results of a prospective, randomized, double-blind, placebo-controlled study. Ann Surg 2008;248:956–967.
[15] Lerut JP, Pinheiro RS, Lai Q, Stouffs V, Orlando G, Juri JM, et al. Is minimal, [almost] steroid-free immunosuppression a safe approach in adult liver transplantation? Long-term outcome of a prospective, double blind, placebo-controlled, randomized, investigator-driven study. Ann Surg 2014;260:886–892.
[16] Vivarelli M, Dazzi A, Zanello M, Cucchetti A, Cescon M, Ravaioli M, et al. Effect of different immunosuppressive schedules on recurrence-free survival after liver transplantation for hepatocellular carcinoma. Transplantation 2010;89:227–231.
[17] Geissler EK, Schnitzbauer AA, Zülke C, Lamby PE, Proneth A, Duvoux C, et al. Sirolimus use in liver transplant recipients with hepatocellular carcinoma: a randomized, multicenter, open-label phase 3 trial. Transplantation 2016;100:116–125.
[18] Adler M, De Pauw F, Vereerstraeten P, Fancello A, Lerut J, Starkel P, et al. Outcome of patients with hepatocellular carcinoma listed for liver transplantation within the Eurotransplant allocation system. Liver Transpl 2008;14:526–533.
[19] Trevisani F, Garuti F, Cucchetti A, Lenzi B, Bernardi M. De novo hepatocellular carcinoma of liver allograft: a neglected issue. Cancer Lett 2015;357:47–54.
[20] Demetris AJ, Adeyi O, Bellamy CO, Clouston A, Charlotte F, et al., Banff Working Group Liver biopsy interpretation for causes of late liver allograft dysfunction. Hepatology 2006;44:489–501.

[21] Lunt M. Selecting an appropriate caliper can be essential for achieving good balance with propensity score matching. Am J Epidemiol 2014;179:226–235.
[22] Caliendo M, Kopeinig S. Some practical guidance for the implementation of propensity score matching. J Econ Surv 2008;22:31–72.
[23] Hsu CC, Chen CL, Wang CC, Lin CC, Yong CC, Wang SH, et al. Combination of FDG-PET and UCSF criteria for predicting HCC recurrence after living donor liver transplantation. Transplantation 2016;100:1925–1932.
[24] Halazun KJ, Najjar M, Abdelmessih RM, Samstein B, Griesemer AD, Guarrera JV, et al. Recurrence after liver transplantation for hepatocellular carcinoma: a new MORAL to the story. Ann Surg 2017;265:557–564.
[25] Schnitzbauer AA, Schlitt HJ, Geissler EK. Influence of immunosuppressive drugs on the recurrence of hepatocellular carcinoma after liver transplantation: a gap between basic science and clinical evidence. Transplantation 2011;91:1173–1176.
[26] Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545.
[27] Ciccarelli O, Lai Q, Goffette P, Finet P, De Reyck C, Roggen F, et al. Liver transplantation for hepatocellular cancer: UCL experience in 137 adult cirrhotic patients. Alpha-foetoprotein level and locoregional treatment as refined selection criteria. Transpl Int 2012;25:867–875.
[28] Lai Q, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, et al. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. Liver Transpl 2013;19:1108–1118.
[29] Schneck SA, Penn I. De-novo brain tumours in renal-transplant recipients. Lancet 1971;1:983–986.
[30] Penn I, Hammond W, Brettschneider L, Starzl TE. Malignant lymphomas in transplantation patients. Transplant Proc 1969;1:106–112.
[31] Penn I. Occurrence of cancers in immunosuppressed organ transplant recipients. Clin Transpl 1998;7:147–158.
[32] Alexander P. Some immunologically based reactions that can cause the regression of large tumor masses. Natl Cancer Inst Monogr 1976;44:105–108.
[33] Zeidman I. The fate of circulating tumor cells. II. A mechanism of cortisone action in increasing metastases. Cancer Res 1962;22:501–503.
[34] Hellmann K, Hawkins RI, Whitecross S. Antilymphocytic serum and tumour dissemination. Br Med J 1968;2:533–535.
[35] Shimizu T, Martin MS, Pelletier H, Lagadec P, Martin F. Effects of cyclosporin A on progressive and regressive tumors induced by two cancer lines derived from a single colon carcinoma chemically induced in the rat. Immunobiology 1989;178:401–415.
[36] Venkataramanan R, Jain A, Warty VW, Abu-Elmagd K, Furakawa H, Imventarza O, et al. Pharmacokinetics of FK 506 following oral administration: a comparison of FK 506 and cyclosporine. Transplant Proc 1991;23:931–933.
[37] O'Grady JG, Burroughs A, Hardy P, Elbourne D, Truesdale AUK and Republic of Ireland Liver Transplant Study Group. Tacrolimus versus microemulsified ciclosporin in liver transplantation: the TMC randomised controlled trial. Lancet 2002;360:1119–1125.
[38] Rodríguez-Perálvarez M, Tsochatzis E, Naveas MC, Pieri G, García-Caparrós C, O'Beirne J, et al. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. J Hepatol 2013;59:1193–1199.
[39] Wei Q, Gao F, Zhuang R, Ling Q, Ke Q, Wu J, et al. A national report from China Liver Transplant Registry: steroid avoidance after liver transplantation for hepatocellular carcinoma. Chin J Cancer Res 2017;29:426–437.
[40] Hu AB, Wu LW, Tai Q, Zhu XF, He XS. Safety and efficacy of four steroid-minimization protocols in liver transplant recipients: 3-year follow-up in a single center. J Dig Dis 2013;14:38–44.
[41] Gu J, Wu X, Lu L, Zhang S, Bai J, Wang J, et al. Role of steroid minimization in the tacrolimus-based immunosuppressive regimen for liver transplant recipients: a systematic review and meta-analysis of prospective randomized controlled trials. Hepatol Int 2014;8:198–215.
[42] Miyagi S, Kawagishi N, Sekiguchi S, Akamatsu Y, Sato K, Takeda I, et al. The relationship between recurrences and immunosuppression on living donor liver transplantation for hepatocellular carcinoma. Transplant Proc 2012;44:797–801.
[43] Klintmalm GB, Davis GL, Teperman L, Netto GJ, Washburn K, Rudich SM, et al. A randomized, multicenter study comparing steroid-free immunosuppression and standard immunosuppression for liver transplant recipients with chronic hepatitis C. Liver Transpl 2011;17:1394–1403.
[44] Brattsand R, Linden M. Cytokine modulation by glucocorticoids: mechanisms and actions in cellular studies. Aliment Pharmacol Ther 1996;10:81–92.
[45] Yazawa H, Kato T, Nakada T, Sendo F. Glucocorticoid hormone suppression of human neutrophil-mediated tumor cell cytostasis. Int J Cancer 1999;81:74–80.
[46] Ho WL, Wu CC, Yeh DC, Chen JT, Huang CC, Lin YL, et al. Roles of the glucocorticoid receptor in resectable hepatocellular carcinoma. Surgery 2002;131:19–25.
[47] Lai Q, Castro Santa E, Rico Juri JM, Pinheiro RS, Lerut J. Neutrophil and platelet-to-lymphocyte ratio as new predictors of dropout and recurrence after liver transplantation for hepatocellular cancer. Transpl Int 2014;27:32–41.
[48] Pinato DJ, Stebbing J, Ishizuka M, Khan SA, Wasan HS, North BV, et al. A novel and validated prognostic index in hepatocellular carcinoma: the inflammation based index (IBI). J Hepatol 2012;57:1013–1020.
[49] Vivarelli M, Cucchetti A, Piscaglia F, La Barba G, Bolondi L, Cavallari A, et al. Analysis of risk factors for tumor recurrence after liver transplantation for hepatocellular carcinoma: key role of immunosuppression. Liver Transpl 2005;11:497–503.
[50] Vivarelli M, Cucchetti A, La Barba G, Ravaioli M, Del Gaudio M, Lauro A, et al. Liver transplantation for hepatocellular carcinoma under calcineurin inhibitors: reassessment of risk factors for tumor recurrence. Ann Surg 2008;248:857–862.
[51] Petrovic LM, Villamil FG, Vierling JM, Makowka L, Geller SA. Comparison of histopathology in acute allograft rejection and recurrent hepatitis C infection after liver transplantation. Liver Transpl Surg 1997;3:398–406.