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The adverse impact of early postoperative complications after liver transplantation on long-term prognosis in patients with hepatocellular carcinoma

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Abstract

Purpose Liver transplantation (LT) is the main treatment for HCC patients, but postoperative complications such as early allograft dysfunction (EAD), acute rejection (AR), and biliary complications can affect the prognosis of patients. However, whether the occurrence of early complications after transplantation will affect the overall survival and tumor recurrence of patients have not been fully studied. We conducted a retrospective clinical study to evaluate the impact of early complications on overall survival and their relationship with HCC recurrence after liver transplantation.

Methods This retrospective study analyzed 378 patients who underwent liver transplantation for HCC between January 2015 and December 2020 at The First Affiliated Hospital, Sun Yat-sen University. The follow-up data of overall survival (OS) and recurrence-free survival (RFS) were collected.

Results Median recurrence-free survival (RFS) was 92.18 months, with RFS rates of 75.9% at 1 year, 60.8% at 3 year, 56.1% at 5 years. 239 (63.2%) patients remained alive following transplantation, and the median overall survival was 100.75 months, with survival rates of 90.5% at 1 year, 71.2% at 3 year, 66.3% at 5years. Multivariate Cox's regression analysis revealed that postoperative acute rejection (AR) and postoperative early allograft dysfunction (EAD) were independent risk factors for overall survival (OS). AR was the independent risk factors for recurrence-free survival.

Conclusions EAD and AR are independent risk factors for overall survival, and AR is an independent risk factor for recurrence-free survival. Reducing the incidence of EAD, AR and biliary complications after transplantation can effectively improve the overall survival and recurrence-free survival of patients.

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Keywords Liver transplantation, Hepatocellular carcinoma, Tumor recurrence, Acute rejection, Early allograft dysfunction

Introduction

Hepatocellular carcinoma (HCC) is a highly lethal malignancy. In 2020, it was the sixth most commonly diagnosed cancer worldwide, while HCC-related mortality ranked third among all cancer-related deaths [1]. In China, HCC is the fourth most frequently diagnosed malignancy, with a dismal 5-year survival rate of only 18% [2]. Furthermore, HCC has a high propensity for recurrence and metastasis, posing significant challenges for its clinical management [3]. Current treatment options for HCC include targeted therapy, radiofrequency ablation, transcatheter arterial chemoembolization (TACE), surgical resection, and LT [4]. LT is considered a first-line treatment for patients with HCC who are not candidates for surgical resection or who have decompensated liver function.

However, due to the aggressive nature of HCC, tumor recurrence after transplantation remains a major concern. Studies have shown that the 5-year recurrence-free survival rate after liver transplantation is approximately 50–70%, and HCC recurrence significantly impacts post-transplant survival outcomes [5]. At present, there is no conclusive evidence from clinical trials confirming that targeted therapies can prevent early-stage HCC recurrence after surgical resection or liver transplantation. Therefore, accurately predicting tumor recurrence, identifying high-risk patients, and implementing timely and effective interventions are of paramount importance.

Numerous previous studies have established several key predictors of HCC recurrence, including tumor size, tumor number, vascular invasion, histological differentiation, as well as the presence of multiple nodules and satellite lesions [6, 7]. In recent years, increasing attention has been given to the association between ischemia-reperfusion injury (IRI) and HCC recurrence following liver transplantation. IRI has been implicated in various post-transplant complications, including early graft dysfunction (EAD), acute rejection (AR), and biliary complications (BC). The occurrence of these complications can exacerbate liver injury and impair graft function. However, limited research has investigated whether EAD, AR, and BC influence long-term survival and tumor recurrence in patients undergoing liver transplantation for HCC. To address this gap, we conducted a retrospective clinical study to evaluate the impact of EAD, AR, and BC on patient prognosis and their relationship with HCC recurrence after liver transplantation.

Methods

Study population

This was a retrospective cohort study. Adult patients (≥ 18 years old) with HCC who underwent liver transplantation in the First Affiliated Hospital of Sun Yat-sen University from 2015 to 2020 were enrolled. Recipients who underwent split liver transplantation, combined liver-kidney transplantation, or multiple organ transplantation were excluded. Since this study was conducted to investigate the long-term prognosis of patients, those who died within three months after the surgery were excluded. Therefore, a total of 378 HCC patients were included in the study population. All patients underwent CLT. All donors participated in the voluntary program of organ donation after a citizen's death in China, and informed consent was obtained from the relatives of the donors. There were no organ donations from executed prisoners. There were 314 DBD donors and 64 DCD donors. This study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. The patient and family provided signed informed consent forms before surgery. The flowchart of this study is shown in Fig. 1.

The primary endpoint of this study was tumor recurrence. Secondary endpoints included patient overall survival, postoperative peak ALT, AST, EAD, biliary complications, and postoperative acute rejection. Donor variables included donor type, BMI, and cold storage time of the liver graft. Recipient variables included age at transplantation, body mass index (BMI), blood type, gender, preoperative laboratory MELD score, liver disease etiology, preoperative ALT, AST, Child-Pugh grade, preoperative neutrophil count, and preoperative lymphocyte count. Intraoperative data included anhepatic phase, total blood loss, blood transfusion volume, and total operation time. Tumor parameters included alpha-fetoprotein (AFP) before transplantation, maximum tumor diameter, number of lesions, Milan criteria, UCSF criteria, previous hepatectomy, neoadjuvant therapy (RFA or TACE), portal vein tumor thrombus, and microvascular invasion. Postoperative outcome data included EAD, AR, biliary complications, tumor recurrence, recurrence-free survival, and overall survival.

EAD was defined by the presence of one or more of the following after LT: bilirubin of ≥ 10 mg/dL on Day 7, international normalized ratio of ≥ 1.6 on Day 7, and alanine aminotransferase or aspartate aminotransferase > 2000 U/L within the first 7 days [8].

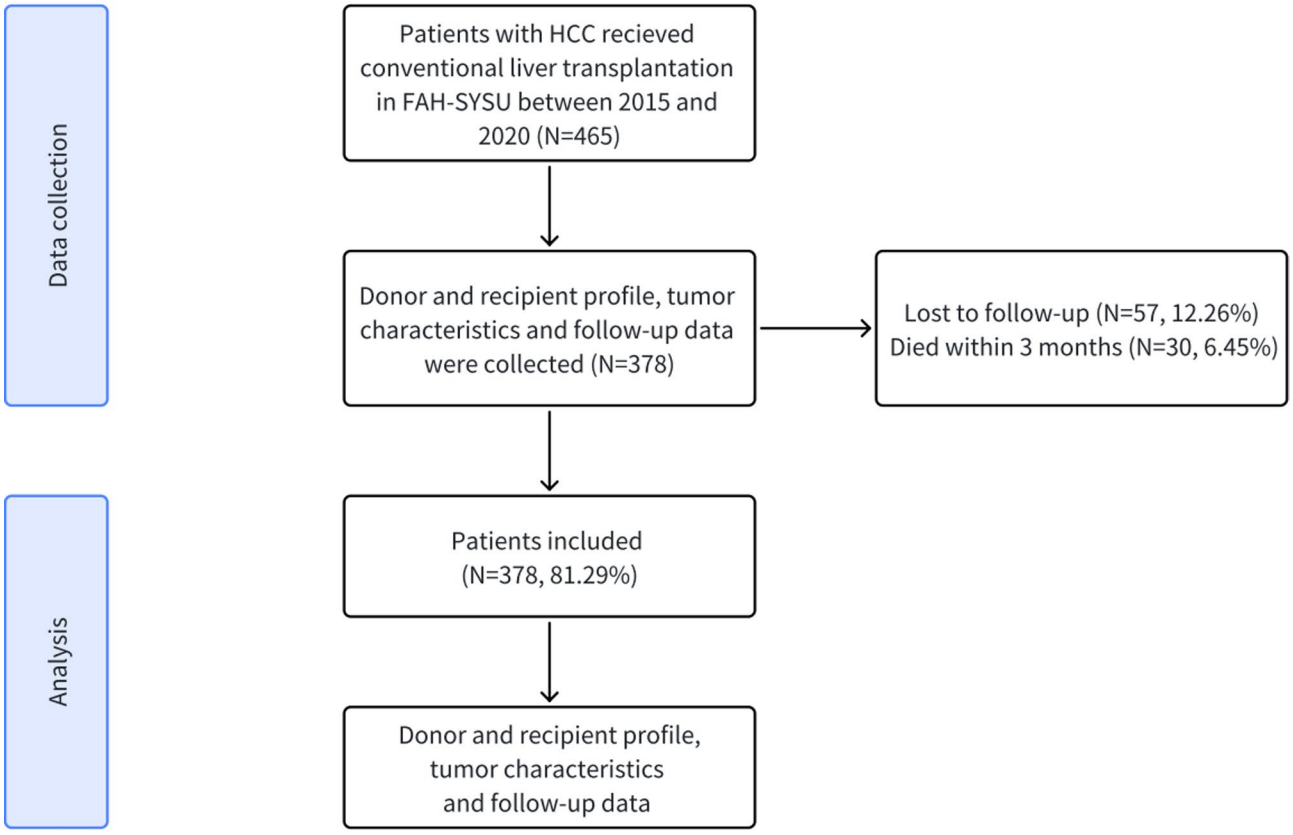

Fig. 1 Retrospective analysis of patient follow-up data collection and analysis flow chart

In this study, the diagnosis of acute rejection was established based on pathological findings or clinical criteria. Informed consent from patients was needed prior to conducting liver biopsies. However, patients frequently refuse liver biopsy following liver transplantation due to various concerns. If liver biopsy was not performed, it was determined according to clinical signs and hematological tests. Clinical acute rejection was defined as an elevation of aminotransferase levels (which should gradually return to normal levels) or a sudden abnormal elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels of 2 or more times the upper limit that could be reversed by modification of the immunosuppressive regimen within 30 days after liver transplantation during recovery of liver function. Hepatic artery or portal vein thrombosis, drug toxicity, or other factors should be ruled out as the cause of liver function impairment [9].

Biliary complications (BC) included anastomotic stenosis, non-anastomotic biliary stricture (NAS), bile leakage, and biliary stones, all of which were diagnosed by magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP). The definition of NAS was as follows: stenosis, dilatation or irregularity of the intrahepatic and extrahepatic bile ducts after the exclusion of isolated bile duct anastomotic stenosis and hepatic artery thrombosis (HAT) [10]. MRCP was conducted within 1 year after transplantation to confirm the status of the biliary tract.

To estimate overall survival (OS), survival time was calculated from the date of LT to death or last known follow-up, and status was recorded at the last point of contact with the patient who died or lived. To estimate recurrence-free survival (RFS), patients with no evidence of recurrence were censored at the time of last follow-up or death.

Conventional liver transplantation

Conventional liver transplantation (CLT) involves a meticulous dissection of the donor liver for the identification of the vessels and bile ducts tract. Subsequently, after heparin injection, cold irrigation is conducted through the abdominal aorta and portal vein using renal preservation solution and UW solution at 4 °C, with drainage via an inferior vena cava cannula. The acquired livers are stored in UW solution at 4 °C and trimmed as necessary to eliminate excess tissue. In the recipient operation, subsequent to liver resection, the inferior vena cava and portal vein of the

donor liver are anastomosed with the recipient vessels to reestablish blood supply, followed by the anastomosis of the hepatic artery and biliary tract. Post-surgery, the patient undergoes intensive care unit (ICU) monitoring and receives immunosuppressive therapy [11].

Statistical analysis

The characteristics of donors and recipients were presented as the mean ± standard deviation (SD) of the measurement parameters and the percentage of the nominal parameters. Continuous variables were compared through the t-test or U-test, and categorical variables were compared by means of the chi-square test or Fisher's exact test. The Kaplan-Meier method and log-rank test were employed to analyze the RFS and OS of HCC patients. Univariate and multivariate Cox proportional hazards regression models were utilized to analyze the independent predictors of RFS and OS. The variables with $P < 0.1$ in the univariate analysis were analyzed via forward stepwise multivariate Cox proportional hazards regression analysis. Results were expressed as hazard ratio (HR) and 95% confidence interval (CI). SPSS 25.0 software was applied for data analysis. $P < 0.05$ was regarded as statistically significant.

Results

Patient characteristics

A total of 378 patients with HCC who underwent LT were included in the study. The baseline clinical and demographic characteristics of 378 recipients and donors comprising the cohort are summarized in Table 1. The mean age of recipients was 51.4 years, 346 (91.5%) were men, and the mean BMI was 23.4. Hepatitis B (314, 83.1%) and Hepatitis C (36, 9.5%) was the most common etiologies in recipients, followed by other (24, 6.4%). Pre-transplantation, patients diagnosed with HBV or HCV infection receive continuous antiviral therapy and undergo regular testing for HBV-DNA and HCV-RNA to ensure the absence of viral replication prior to surgery. Patients will continue to receive the same antiviral therapy post-transplantation as they did pre-transplantation. Post-transplantation patients are routinely monitored through regular testing for eight serological tests including hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis C antibody (anti-HCV), human immunodeficiency virus antibody (anti-HIV), syphilis antibody (TP-Ab), HBV-DNA and HCV-RNA to confirm the absence of viral activity. Serologic assessments following transplantation revealed no indications of viral activity. The median preoperative ALT and AST was 23 U/L (IQR 35–53) and 46 U/L (IQR, 34-74.5), respectively. The number of Child-Pugh grade of A

Table 1 Donor characteristics, recipient characteristics, tumor characteristics, surgical details, and patient outcomes after transplantation

Variables	*N* = 378
Recipient characteristic	
Blood type	
O	106 (28)
A	125 (33.1)
B	120 (31.7)
AB	27 (7.1)
Gender	
Male	346 (91.5)
Female	32 (8.5)
Age (years)	51.4 ± 10.4
BMI (kg/m^2)	23.4 ± 3.4
Liver Disease Etiology	
HBV	314 (83.1)
HCV	36 (9.5)
Other	24 (6.4)
Preoperative ALT (U/L)	23 (35, 53)
Preoperative AST (U/L)	46 (34, 74.5)
MELD score	10 (7.7, 13.8)
Child-Pugh grade	
A	150 (39.7)
B	203 (53.7)
C	25 (6.6)
Neutrophil (10^9/L)	3.5 ± 2.4
Lymphocyte (10^9/L)	1.2 ± 0.7
Tumor characteristics	
AFP (ng/L)	
< 20	176 (46.6)
20 ~ 100	64 (16.9)
100 ~ 400	36 (9.5)
> 400	102 (27)
Maximum tumor diameter (mm)	
0 ~ 30	127 (33.6)
30 ~ 50	104 (27.5)
> 50	147 (38.9)
Tumor number	
1	139 (36.8)
2	81 (21.4)
3	24 (6.3)
> 3	134 (35.4)
Beyond Milan criteria	225 (59.5)
Beyond UCSF criteria	197 (52.1)
MVI	112 (29.6)
TACE history	152 (40.2)
Thermal ablation history	101 (26.7)
Liver resection history	62 (16.4)
Donor characteristic	
Donor type	
DBD	314 (83.1)
DCD	64 (16.9)
BMI	22.3 ± 2.9
Cold ischemia time (hours)	6.8 ± 2.9

Table 1 (continued)

Variables	*N* = 378
Surgical details	
Anhepatic phase (minutes)	56.4 ± 17.4
Total surgery time (minutes)	462 ± 108
Intraoperative blood loss (mL)	1500 (800, 2000)
Early Complications	
EAD	122 (32.3)
AR	121 (32.0)
BC	98 (25.9)

Values are presented as a number (% of total sample) or median (P25, P75) or mean ± SD. Abbreviations: HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; MELD, Model for End-Stage Liver Disease; AFP, Alpha-fetoprotein; MVI, microvascular invasion; TACE, Transarterial Chemoembolization; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; BMI, Body Mass Index; EAD, early allograft dysfunction; AR, acute rejection; BC, Biliary complication

(39.7%) recipients was 150, and 203 (53.7%) had a grade of B, while 25 (6.6%) patients had a grade of C. Most of donors were DBD (314, 83.1%). Additionally, the mean cold ischemia time was 6.8 h. For surgery details, the mean anhepatic phase was 56.4 min, the mean total surgery time was 462 min and the median intraoperative blood loss was 1500 ml (Table 1).

Tumor characteristics and patient outcomes

In this cohort, 225 (59.5%) patients were beyond Milan criteria at diagnosis, and 197 (52.1%) were beyond UCSF criteria. The AFP values, tumor size and number of HCC are shown in Table 1. A total of 102 (27%) patients had AFP levels greater than 400 ng/ml, 134 (35.4%) patients had more than 3 tumors, and 147 (38.9%) patients had tumor sizes greater than 50 mm. Some patients underwent TACE (152, 40.2%), ablation (101, 26.7%), and liver resection (62, 16.4%) before transplantation. On explant pathology, 112 (29.6%) patients had tumor microvascular invasion (MVI). HCC recurrence was observed in 165 (43.7%) patients. Median RFS was 92.18 months (IQR, 11.8–66.2), with recurrence-free rates of 75.9% at 1 year, 60.8% at 3 years, 56.1% at 5 years (Fig. 2B). 239 (63.2%) patients remained alive following transplantation, and the median overall survival was 100.75 months, with survival rates of 90.5% at 1 year, 71.2% at 3 years, 66.3% at 5 years (Fig. 2A).

Univariate analysis of overall survival and recurrence-free survival

Univariate cox regression analysis showed that pretransplant AST > 100 U/L (HR: 1.933, 95%CI, 1.312–2.849, $P = 0.001$), AFP > 400 ug/L (HR: 2.649, 95%CI, 1.891–3.711, $P < 0.001$), and maximum tumor diameter > 30 mm (HR: 2.095, 95%CI, 1.398–3.139, $P < 0.001$) were risk factors affecting the overall survival of patients, and the overall survival of patients after transplantation would also be affected when the tumor stage was beyond the UCSF standard (HR: 1.417, 95%CI, 1.011–1.988, $P = 0.043$) and the tumor MVI (HR: 3.130, 95%CI, 2.241–4.371, $P < 0.001$). At the same time, the postoperative complications of EAD (HR: 2.805, 95%CI, 2.008–3.919, $P < 0.001$), AR (HR: 5.517, 95%CI, 3.901–7.803, $P < 0.001$), and BC (HR: 2.569, 95%CI, 1.831–3.604, $P < 0.001$) were associated with the overall survival of patients. Tumor downstaging by preoperative neoadjuvant therapy is a protective factor for long-term survival of patients (Table 2).

Cox regression analysis was performed to examine RFS in the entire cohort. Univariate cox regression analysis showed that AST > 100 umol/L (HR: 2.362, 95%CI, 1.665–3.351, $P < 0.001$), AFP > 400 ug/L (HR: 2.676, 95%CI, 1.960–3.655, $P < 0.001$), maximum tumor size > 30 mm (HR: 2.823, 95%CI, 1.890–4.217, $P < 0.001$), tumor stage beyond UCSF criteria (HR: 1.603, 95%CI, 1.172–2.193, $P = 0.003$) and MVI (HR: 3.435, 95%CI, 2.524–4.675, $P < 0.001$) were also risk factors for recurrence-free survival. Similarly, tumor staging beyond Milan criteria (HR: 1.631, 95%CI, 1.174–2.267, $P = 0.004$), donor liver cold storage time > 10 h (HR: 1.576, 95%CI, 1.054–2.357, $P = 0.027$), and AR (HR:5.375, 95%CI, 3.563–8.110, $P < 0.001$) after transplantation will lead to an increased risk of tumor recurrence (Table 2). The survival analysis of patients with EAD, AR and BC after liver transplantation showed that the overall survival and recurrence-free survival of patients with liver cancer were significantly affected by EAD ($P < 0.0001$), AR ($P < 0.0001$) and BC ($P < 0.001$) (Fig. 3A and F).

Multivariable predictors of HCC patient overall survival and recurrence-free survival outcomes

Multivariable regression analysis was performed to identify independent risk factors for overall survival and recurrence-free survival after LT. Among the evaluated factors, AR (HR: 3.439, 95%CI, 2.244–5.270, $P < 0.001$) had the largest hazard ratio for the risk of overall survival among all risk factors. AFP > 400 ug/L (HR: 1.667, 95%CI, 1.152–2.416, $P = 0.007$), MVI (HR: 1.749, 95%CI, 1.218–2.511, $P = 0.002$), and EAD after transplantation (HR: 1.635, 95%CI, 1.143–2.340, $P = 0.013$) were independent risk factors associated with patient overall survival (Table 3). Results of other univariate analyses, including AST > 100 umol/L, UCSF criteria, tumor size, were not significantly associated with overall survival. However, in the multivariate analysis of recurrence-free survival of tumor patients, AR after transplantation (HR: 5.141, 95%CI, 3.425–7.718, $P < 0.001$), AFP > 400 ug/L (HR: 1.642, 95%CI, 1.117–2.299, $P = 0.004$), MVI (HR: 1.890, 95%CI, 1.357–2.632, $P < 0.001$) and maximum tumor

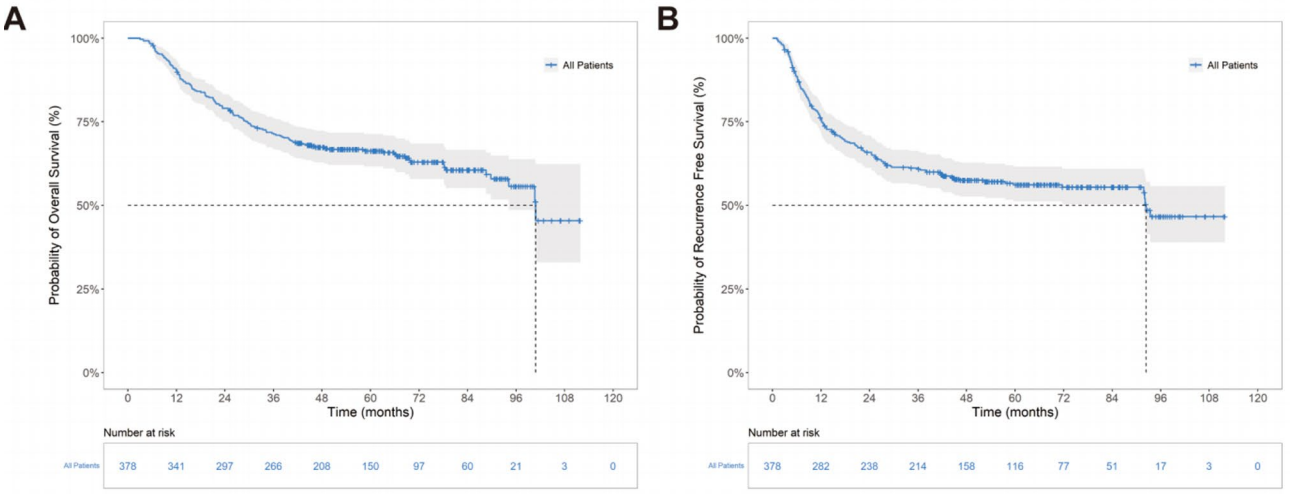

Fig. 2 Overall survival and recurrence-free survival of HCC patients after liver transplantation. (**A**) Overall survival. (**B**) Recurrence-free survival

diameter > 30 mm (HR: 1.564, 95%CI, 1.010–2.422, $P = 0.045$) were the independent risk factors for RFS (Table 3).

Discussion

According to retrospective analysis of the follow-up data of 378 patients after liver transplantation, we confirmed that postoperative acute rejection, AFP > 400 ug/L, MVI, and maximum tumor diameter > 30 mm were independent risk factors for tumor recurrence. Multivariate cox regression analysis demonstrated that EAD, AR, AFP > 400 ug/L, and MVI were independent risk factors for the overall survival of HCC patients after liver transplantation.

Previous studies have established that tumor recurrence and metastasis following transplantation are closely associated with various factors, including tumor size and number, microvascular invasion, and elevated AFP levels [12–15]. Published data regarding the impact of acute rejection on graft and patient survival are currently mixed. While earlier reports found acute rejection was not significantly associated with worse outcomes [16]. Ding et al. demonstrate AR after liver transplantation may indicate a better prognosis in HCC patients [17]. However, recently, there are some studies have found acute rejection is associated with tumor recurrence [18, 19]. Moritz et al. demonstrate an association of AR with HCC recurrence after LT. Patients with AR (28.6%) had a significantly higher frequency of tumor recurrence compared to patients without AR (13.0%, $p = 0.002$) [20]. In our study, we confirmed that AR were independent risk factors for tumor recurrence and overall survival. The occurrence of AR can be attributed to multiple factors. First, ABO blood group incompatibility between donors and recipients may trigger rejection. Second, inadequate immunosuppressive therapy post-transplantation may lead to AR. Third, the increasing use of marginal liver donors, such as aged livers, fatty livers, and donation after circulatory death (DCD) livers, may contribute to AR [21–23]. The inflammatory microenvironment play critical roles on orchestrating cancer cells together with immune cells to facilitate tumor recurrence post-LT [24]. Previous studies have shown that NETs levels are positively correlated with liver enzymes and AR incidence in liver transplant patients [25, 26]. The release of NETs leads to the appearance of AR. NETs can induce M1 polarization and the intracellular translocation of HMGB1 in Kupffer cells. At the same time, HMGB1 can activate TLR-4/MAPK signaling pathway, leading to the formation of NETs. This positive feedback loop between neutrophils and Kupffer cells further amplifies inflammatory signaling and graft injury [27]. NET also plays an important role in cancer immune editing and immune cell interaction in tumor development. NETs are able to activate dormant cancer cells, which can lead to tumor recurrence [28, 29]. Therefore, excessive immune activation leads to AR and subsequent tumor recurrence. Reducing the incidence of AR after transplantation may improve overall survival and lower the risk of tumor recurrence in HCC patients.

In our study, it was proved that EAD and AR after transplantation have a negative impact on the long-term prognosis and survival of patients. At the same time, for HCC patients, our study found that the AFP > 400 ug/L preoperative and tumor microvascular invasion also affected the survival of patients. At present, the diagnostic criteria of EAD are based on the level of liver enzymes, bilirubin levels and INI. The elevated level of liver enzymes is due to the liver suffering from ischemia-reperfusion injury, the elevated

Table 2 Univariate analyses of risk factors for overall survival and Recurrence-free survival in the entire cohort

Overall survival	**HR**	***p***	**Recurrence-free survival**	**HR**	***p***
variable			**variable**		
Blood type			Blood type		
O			O		
A	0.778	0.234	A	1.002	0.992
B	0.763	0.208	B	0.976	0.905
AB	0.710	0.347	AB	0.665	0.262
Gender			Gender		
Male			Male		
Female	1.080	0.799	Female	1.182	0.537
Age			Age		
≤ 60			≤ 60		
>60	0.997	0.990	>60	0.956	0.819
HBV			HBV		
negative			negative		
positive	0.854	0.467	positive	0.896	0.586
HCV			HCV		
negative			negative		
positive	1.329	0.286	positive	1.107	0.699
Preoperative ALT			Preoperative ALT		
≤ 100			≤ 100		
>100	1.639	0.064	>100	1.516	0.096
Preoperative AST**			Preoperative AST***		
≤ 100			≤ 100		
>100	1.933	0.001	>100	2.362	< 0.001
MELD score	0.979	0.250	MELD score	0.988	0.441
Child-Pugh grade			Child-Pugh grade		
A			A		
B, C	0.782	0.150	B, C	0.781	0.115
Preoperative NLR			Preoperative NLR		
≤ 5			≤ 5		
>5	1.326	0.172	>5	1.233	0.278
AFP***			AFP***		
≤ 400			≤ 400		
>400	2.649	< 0.001	>400	2.676	< 0.001
Maximum tumor diameter***			Maximum tumor diameter***		
≤ 30			≤ 30		
>30	2.095	< 0.001	>30	2.823	< 0.001
Tumor number			Tumor number		
Single			Single		
Multiple	0.920	0.633	Multiple	1.169	0.342
Milan criteria			Milan criteria**		
Within			Within		
Beyond	1.409	0.055	Beyond	1.631	0.004
UCSF criteria*			UCSF criteria**		
Within			Within		
Beyond	1.417	0.043	Beyond	1.603	0.003
MVI***			MVI***		
No			No		
Yes	3.130	< 0.001	Yes	3.435	< 0.001
Neoadjuvant therapy* (Thermal ablation or TACE)			Neoadjuvant therapy (Thermal ablation or TACE)		
No			No		
Yes	0.699	0.038	Yes	0.861	0.337

Table 2 (continued)

Overall survival	HR	*p*	Recurrence-free survival	HR	*p*
variable			variable		
Liver resection history			Liver resection history		
No			No		
Yes	0.805	0.370	Yes	0.876	0.536
Donor type			Donor type		
DBD			DBD		
DCD	1.356	0.140	DCD	1.396	0.080
Cold ischemia time			Cold ischemia time*		
≤600			≤600		
>600	1.187	0.465	>600	1.576	0.027
Anhepatic phase			Anhepatic phase		
≤50			≤50		
>50	1.041	0.820	>50	1.102	0.545
Total surgery time			Total surgery time		
≤10			≤6		
>10	0.910	0.748	>10	1.274	0.310
Intraoperative blood loss			Intraoperative blood loss		
≤2000			≤2000		
>2000	0.813	0.321	>2000	1.030	0.871
EAD***			EAD		
No			No		
Yes	2.805	<0.001	Yes	1.188	0.319
AR***			AR***		
No			No		
Yes	5.517	<0.001	Yes	5.375	<0.001
BC***			BC		
No			No		
Yes	2.569	<0.001	Yes	1.055	0.773

The left side of the table represents the univariate analysis of risk factors for recurrence-free survival, The right side of the table represents the univariate analysis of risk factors for overall survival (*, **, *** represent $P<0.05$, $P<0.01$, and $P<0.001$, respectively)

Abbreviations: HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; MELD, Model for End-Stage Liver Disease; NLR, Neutrophil to Lymphocyte Ratio; AFP, Alpha-fetoprotein; MVI, microvascular invasion; TACE, Transarterial Chemoembolization; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; EAD, early allograft dysfunction; AR, acute rejection; BC, Biliary complication

level of bilirubin is often due to the injury of the biliary tract, and the elevated level of INR is due to the dysfunction of liver bleeding and coagulation, which may be the poor function of the liver itself [30]. Although EAD is not a risk factor for tumor recurrence in published papers, our survival analysis demonstrated that the presence of EAD significantly impacts patient survival and tumor recurrence. Current research has shown that independent risk factors for EAD include donor BMI, donor steatosis, CIT ≥ 10 h, thermal ischemia time ≥ 50 min, and recipient MELD score [31, 32]. Therefore, we need to pay attention to these EAD risk factors, do a good preoperative evaluation and care for the donor, shorten the cold storage time of organs, choose the donor with less thermal ischemia time, reduce the occurrence of EAD, and improve the prognosis of patients.

Our survival analysis demonstrated that EAD, AR, and BC significantly affected both overall survival and recurrence-free survival. Notably, ischemia-reperfusion injury (IRI) plays a crucial role in the development of these complications.

IRI is an inevitable challenge in liver transplantation, promoting tumor cell proliferation through the initiation of a potent inflammatory cascade and subsequent tissue repair, leading to microvascular dysfunction, immune cell infiltration, and the release of pro-inflammatory mediators [33]. Additionally, IRI regulates the hepatic inflammatory microenvironment, exacerbating graft injury and contributing to complications such as EAD, AR, and BC [9, 34].

Our study suggests that both preoperative tumor characteristics and postoperative complications significantly influence prognosis. Therefore, efforts should be made to minimize postoperative complications through optimized surgical techniques and perioperative management. However, many complications, such as AR and EAD, are influenced by factors present before and during transplantation. Hence, it is essential to mitigate risk factors associated with

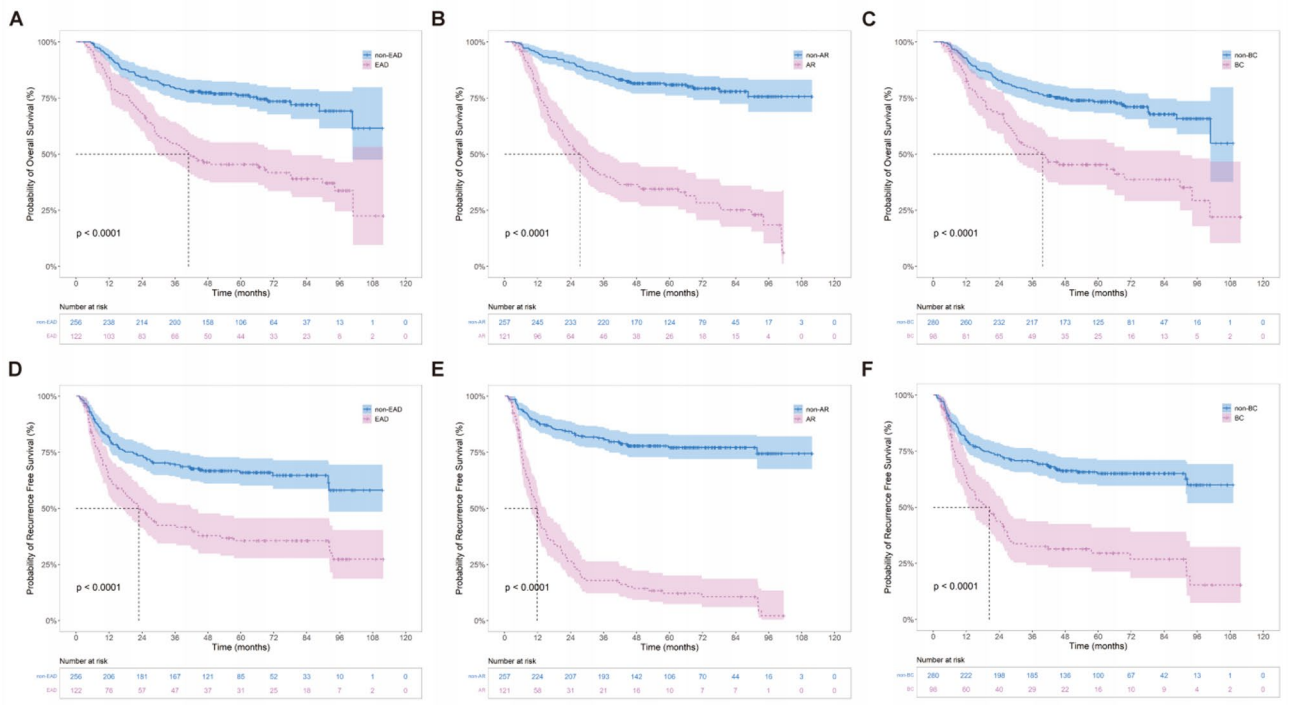

Fig. 3 Effects of EAD, AR and BC on overall survival and recurrence-free survival after liver transplantation. (**A**) Impact of EAD on overall survival (**B**) Impact of AR on overall survival (**C**) Impact of BC on overall survival (**D**) Impact of EAD on recurrence-free survival (**E**) Impact of AR on recurrence-free survival (**F**) Impact of BC on recurrence-free survival

these complications. The occurrence of AR and EAD often indicates severe inflammatory injury, significant immune alterations, and an increased likelihood of tumor recurrence. Thus, patients experiencing these complications require close monitoring and timely pharmacological intervention to prevent tumor recurrence and prolong survival.

However, for the occurrence of acute rejection, we should not only focus on ischemia-reperfusion injury. Due to the activation of the immune system, some patients with liver cancer treated with PD-1 before transplantation without adequate preparation for drug withdrawal will also cause acute rejection after transplantation [35]. Therefore, for patients with AR after transplantation, a comprehensive multivariate analysis should be conducted to assess risk factors, and immunosuppressive regimens should be promptly adjusted to reduce tumor recurrence risk. Patients who received immunotherapy prior to transplantation should undergo careful perioperative management to minimize the incidence of AR and related complications.

Furthermore, our study further substantiated that liver ischemia exerts a significant influence on tumor recurrence and overall survival of patients subsequent to transplantation. Patients encountering severe IRI injury are more prone to tumor recurrence, and liver ischemia constitutes an independent risk factor for tumor recurrence [36]. In recent years, with the development of perfusion technology, the ischemic time of the liver is shortened, and the ischemia-reperfusion injury is mitigated [37]. A number of randomized controlled trial (RCT) have proved that ischemia-free liver transplantation (IFLT) [38], normothermic machine perfusion (NMP) [39], and hypothermic oxygenated perfusion (HOPE) [40] can reduce the occurrence of IRI-related complications and improve the prognosis of patients. Therefore, the emergence of perfusion technology is expected to improve the prognosis of HCC patients and reduce the recurrence rate of tumor after liver transplantation in the future.

Nevertheless, our study has several limitations. As a single-center retrospective study, selection bias may exist. Although we identified AFP > 400 μg/L, MVI, and early postoperative complications as independent risk factors for tumor recurrence, further validation through large-scale, multicenter randomized controlled trials is necessary. Additionally, AR in our study was primarily diagnosed based on clinical criteria, with limited pathological confirmation. Future research should incorporate histopathological data and explore the effects of novel therapeutic strategies, including machine perfusion technology, in reducing tumor recurrence after liver transplantation.

In conclusion, the biological characteristics of liver tumors, including tumor size, number, stage, and microvascular invasion, significantly influence

Table 3 Multivariate analyses of risk factors for overall survival and recurrence-free survival in the entire cohort

Overall survival	HR	*p*	Recurrence-free survival	HR	*p*
variable			variable		
Preoperative ALT			Preoperative ALT		
≤100			≤100		
>100	1.021	0.946	>100	0.730	0.276
Preoperative AST			Preoperative AST		
≤100			≤100		
>100	1.026	0.914	>100	1.446	0.085
AFP**			AFP**		
≤400			≤400		
>400	1.667	0.007	>400	1.642	0.004
Milan criteria			Milan criteria		
Within			Within		
Beyond	1.048	0.882	Beyond	0.944	0.847
UCSF criteria			UCSF criteria		
Within			Within		
Beyond	0.972	0.924	Beyond	1.034	0.907
Maximum tumor diameter			Maximum tumor diameter*		
≤30			≤30		
>30	1.176	0.473	>30	1.564	0.045
Neoadjuvant therapy (Thermal ablation or TACE)			MVI***		
No			No		
Yes	0.817	0.272	Yes	1.890	<0.001
MVI**			Donor type		
No			DBD		
Yes	1.749	0.002	DCD	1.036	0.858
EAD**			Cold ischemia time		
No			≤600		
Yes	1.635	0.007	>600	1.455	0.756
AR***			EAD		
No			No		
Yes	3.439	<0.001	Yes	1.215	0.259
BC			AR***		
No			No		
Yes	1.117	0.587	Yes	5.141	<0.001
			BC		
			No		
			Yes	1.099	0.607

The left side of the table represents the multivariate analysis of risk factors for overall survival, The right side of the table represents the multivariate analysis of risk factors for recurrence-free survival (*, **, *** represent $P<0.05$, $P<0.01$, and $P<0.001$, respectively). Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; AFP, Alpha-fetoprotein; MVI, microvascular invasion; TACE, Transarterial Chemoembolization; EAD, early allograft dysfunction; AR, acute rejection; BC, Biliary complication

post-transplant prognosis. Additionally, IRI-related complications such as EAD, AR, and BC are closely associated with tumor recurrence and serve as independent risk factors. Therefore, greater attention should be directed toward managing postoperative complications, implementing early interventions, and integrating novel strategies to effectively reduce tumor recurrence and improve long-term survival in HCC patients undergoing liver transplantation.

Abbreviations

AFP Alpha-fetoprotein;
ALT Alanine transaminase;
AR Acute rejection;
AST Aspartate aminotransferase;
BC Biliary complications;
BMI Body mass index;
CI Confidence interval;
CLT Conventional liver transplantation;
DBD Donation after brain death;
DCD Donation after circulatory death;
EAD Early allograft dysfunction;
ERCP Endoscopic retrograde cholangiopancreatography
HAT Hepatic artery thrombosis;
HCC Hepatocellular carcinoma;
HOPE Hypothermic oxygenated perfusion;
HR Hazard ratio;
ICU Intensive care unit;
IFLT Ischemia-free liver transplantation;

IRI	Ischemia-reperfusion injury;
LT	Liver transplantation;
MRCP	Magnetic resonance cholangiopancreatography;
MVI	Microvascular invasion;
NAS	Non-anastomotic biliary stricture;
NMP	Normothermic machine perfusion;
OS	Overall survival;
RCT	Randomized controlled trial;
RFS	Recurrence-free survival;
TACE	Transcatheter arterial chemoembolization;

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures involving human participants performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All donors participated in the voluntary program of organ donation after a citizen's death in China, and informed consent was obtained from the relatives of the donors. There were no organ donations from executed prisoners. This study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. All patients and their families signed an ethical authorization form and informed consent before surgery.

Competing interests
The authors declare no competing interests.

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