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Original Article

Selection of liver graft from HCV-positive donor and prognosis of liver transplant recipients

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Abstract: Objective: To evaluate the feasibility and safety of using liver grafts from hepatitis C viral (HCV)-positive donors for transplant, and to provide practical and theoretical considerations of using the HCV-positive grafts. Methods: Sixty-five patients were transplanted with diagnosis of HCV-related liver diseases between 2011 and 2013, and 58 of them were enrolled in the present study. All grafts were procured from donors after cardiac death with informed consent. We compared the time of survival between patients received HCV-positive and HCV-negative donor grafts. Pathological examination was performed on liver tissues collected during operation and liver biopsy samples were collected during follow-up. Results: Twelve patients received HCV-positive donor grafts (HCV+group), and 46 received the HCV-negative donor grafts (HCV-group). Pathological examination showed that the HCV-positive donor grafts had higher inflammatory grade and fibrosis stage than the HCV-negative donor grafts. One of the donor grafts had fibrosis at S3, and primary graft non-function was observed after liver transplantation. There were no significant differences in survival time at year 1 and at year 3 post-surgery between HCV+ and HCV-groups. The HCV+group had higher inflammatory grade and fibrosis stage than HCV-group during follow-up period (*inflammatory grade:* 3.0±1.4 vs. 2.6±1.5, *P*=0.49; *fibrosis stage:* 2.3±1.5 vs. 1.2±1.1, *P*=0.04), of which the differences in fibrosis stage was statistically significant. However, when the progression in inflammatory/fibrosis was adjusted to the baseline, there were no significant differences between the HCV+ and HCV-groups. We also assessed liver function at year 1 post-surgery. There were no significant differences in ALB (*P*=0.49), TBIL (*P*=0.71), and INR (*P*=0.26) between HCV+ and HCV-groups, but higher ALT (*P*=0.04) and AST (*P*=0.02) levels were found in HCV+group. The assessment of kidney function showed that creatinine levels increased when compared to the baseline in both HCV+ and HCV-groups, although the increase was not statistically significant. Conclusion: Recipients of liver transplant had similar survival time and fibrosis progression regardless of HCV status of the donors. The HCV-positive grafts could be used for transplantation in patients with HCV-related liver disease, but detailed assessment of the liver donor is needed. Donors with no fibrosis or mild fibrosis (F ≤ stage 1) provide better graft survival. Donor graft with fibrosis (F=2) may still be used for patients with good liver function and low MELD score before surgery. Donor grafts with advanced stage of fibrosis (F ≥ stage 3) should be excluded.

Keywords: HCV-positive donor, HCV-positive grafts, liver transplantation, liver function, fibrosis

Introduction

A significant number of patients are in urgent need of liver transplantation, unfortunately the organ demand exceeds supply. Consequently, some medical centres used grafts from hepatitis C virus (HCV) positive patients. One of the concerns of such procedure is the recurrence of HCV in patients receiving grafts from HCV-positive donors compared with the patients receiving grafts from HCV-negative donors. It is also controversy on whether the HCV-positive donor graft would accelerate the progression of fibrosis and cirrhosis that lead to poor prognosis. There have been studies showing that the use of HCV-positive graft would not affect survival of the recipients [1-4]. However, some studies suggested that recipients of the HCV-positive graft had significant progression of liver fibrosis [5, 6]. The use of HCV-positive graft is thus still controversial.

Materials and methods

Study cohort

Sixty-five patients with HCV-related liver diseases underwent liver transplantation at The

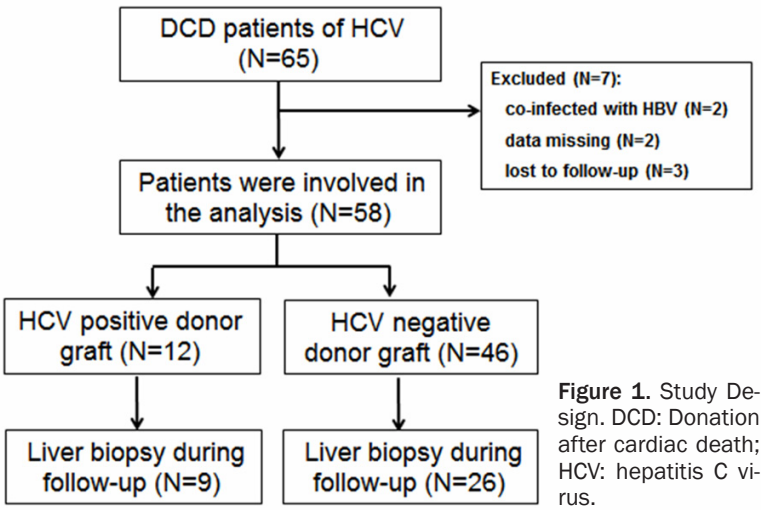

Figure 1. Study Design. DCD: Donation after cardiac death; HCV: hepatitis C virus.

General Hospital of Chinese People's Armed Police Forces or Tianjin First Central Hospital between January 2011 and December 2013. Seven patients were excluded: 2 were co-infected with HBV, 2 had incomplete clinical information, and 3 were lost in follow-up. Fifty-eight patients were enrolled in the current study: 12 of them received HCV-positive graft (8 had primary hepatocellular carcinoma and 4 had end-stage cirrhosis), and 46 received HCV-negative graft (**Figure 1**). As of 30th June 2015, the median follow-up time was 26 months. All grafts were procured from donation cardiac death (DCD), and none was obtained from executed prisoners. The organ donation was complied with the human organ transplant ordinance [7] and guideline published by China Organ Donation Committee [8]. The study was approved by the Ethics Committee of The General Hospital of Chinese People's Armed Police Forces or Tianjin First Central Hospital.

Inclusion criteria of HCV-positive graft

Criteria for accepting the graft for transplantation were as follow: 1) Donors were positive in serum anti-HCV; 2) No systemic infection; 3) No HBV or HIV infection; 4) No drug poisoning; 5) No medical history of malignancy (donors with central nervous system tumours may consider be used); 6) Sodium < 160 mml/L; 7) No fatty liver; 8) Imaging showed no significant liver disease; and 9) Liver function tests < 2X ULN. The HCV-positive donor grafts were transplanted only to patients with HCV-related end-stage liver disease. Before surgery, the risks of receiving HCV-positive grafts were explained, and all the procedures were performed with informed consent of the patients.

Treatments and follow-ups after surgery

Orthotropic liver transplantation was performed. Clinical data such as HCV viremia, liver and kidney functions, cold and warm ischemia time during surgery were collected. After surgery, patients received immunosuppressive regimen of tacrolimus (or Cyclosporin), mycophenolate mofetil, and adrenocortical hormone. The starting dose of hormone was 240 mg and reduced gradually and eventually stopped one-month post-surgery. Tacrolimus trough concentration was 8-10 ng/ml in the first month after surgery, followed by 5-7 ng/ml for one year, and then maintained at 3-4 ng/ml. Cyclosporin peak concentration was 800-1000 ng/ml in the first month after surgery, followed by 500-700 ng/ml for one year, and then maintained at 300-400 ng/ml. Clinical data such as HCV viremia, living and kidney functions, drug concentration, abdominal ultrasound images were collected during regular follow-up visits. Once signs of tumor recurrence were found, follow-up imaging was performed. Adverse events, time and cause of deaths were also recorded.

Pathological analysis

Liver tissues were collected during surgery as a baseline for later comparison. Tissues were paraffin-embedded, and then examined by haematoxylin-eosin (HE) and Masson's staining. Second liver biopsies were collected in thirty-five patients during follow-up. Among them, 9 patients received HCV-positive graft and 26 patients received HCV-negative graft. Tissues were examined by two pathologists in a blinded manner. In case of inconsistent scoring, samples were examined by the third pathologist outside the hospital. Knodell modified histological activity index was used for scoring: inflammatory grade (scale of 0-18), fibrosis stage (scale of 0-6). Changes in inflammation or fibrosis = score at follow-up visit-score at baseline.

Table 1. Baseline demographic and clinicopathological characteristics of liver transplant recipients and donors

	HCV+graft (n=12)	HCV-graft (n=46)	*P* values
Recipient			
Age (years), mean ± SD	54±14	52±10	0.55
Gender (male), n (%)	8 (67%)	34 (74%)	0.72
HCV-RNA (positive), n (%)	10 (83%)	42 (91%)	0.59
MELD score, mean ± SD	14±4	13±6	0.90
Etiology (HCC), n (%)	9 (75%)	17 (37%)	0.02
Cold ischemic time (minutes), mean ± SD	452±171	426±124	0.12
Warm ischemic time (minutes), mean ± SD	44±19	40±13	0.34
Donor			
Age (years), mean ± SD	45±16	49±23	0.21
Gender (male), n (%)	9 (75%)	44 (96%)	0.15
Histological inflammatory grade	2.1±1.6	0.9±0.9	0.01
Fibrosis stage	0.9±1.0	0.1±0.3	< 0.01

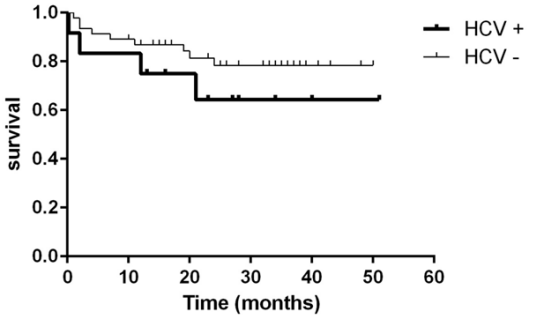

Figure 2. Graft survival in recipients of HCV (+) and HCV (-) donor graft. There was no statistical difference between graft survival of recipients who received HCV (+) and HCV (-) grafts by Kaplan-Meier survival estimate and log-rank test (P=0.29).

Statistical analysis

Continuous data were expressed in mean ± standard deviation. T-test analysis was performed to compare differences. When data were not normally distributed, non-parametric tests were used. Chi-square test was used for categorical data. Kaplan-Meier survival curves were compared with the log-rank test to evaluate the impact of donor HCV status on survival. Mann-Whitney Exact test was used to evaluate changes in inflammation/fibrosis between HCV positive and negative groups. Independent t-test was used to compare differences in liver function. Paired t-test was used to compare differences in kidney function between baseline and in follow-up, while independent t-test was used in comparison between HCV positive and negative groups. *P*-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 19.0.

Results

Clinical profiles of donors and recipients

Table 1 showed the clinicopathological characteristics of the donors and recipients. There were no significant differences in age, sex, model for end-stage liver disease (MELD) score, % of positive HCV RNA, cold and warm ischemic time in patients receiving HCV+ or HCV-grafts (*P* > 0.05) (**Table 1**). Among the 12 patients receiving HCV-positive grafts, 9 of them had HCV associated hepatocellular carcinoma (HCC) and 3 of them had non-compensated liver cirrhosis before surgery. The HCC patients had no metastasis observed in the liver or in other organs. Among the 46 HCV-negative graft recipients, 17 of them had HCV associated HCC (37%) (*P*=0.02).

There were no significant differences in age and sex between HCV-positive and -negative donors. The HCV-positive donor, however, had higher histological inflammatory grade (P=0.01) and fibrosis stage (P < 0.01). The fibrosis stages of the donors were: 5 donors at Stage 0; 4 at Stage 1; 2 at Stage 2; and 1 at Stage 3. The graft from the donor at Stage 3 fibrosis was found non-functional after transplantation.

Impact of donor HCV status on graft survival

There were no significant differences in overall survival between the HCV-positive and -negative graft recipients. The survival rates of HCV-positive and HCV-negative grafts were 75% vs. 84% at year-1, and 64% vs. 78% at year-3 after transplantation (*P*=0.29) (**Figure 2**). HCV+group had 4 deaths, including 1 died from non-functional graft after transplantation, 1 from abdominal infection, and 2 from recurrent of HCC. HCV-group had 9 deaths, in which 2 were died from infection, and 7 were died from recurrence of tumours.

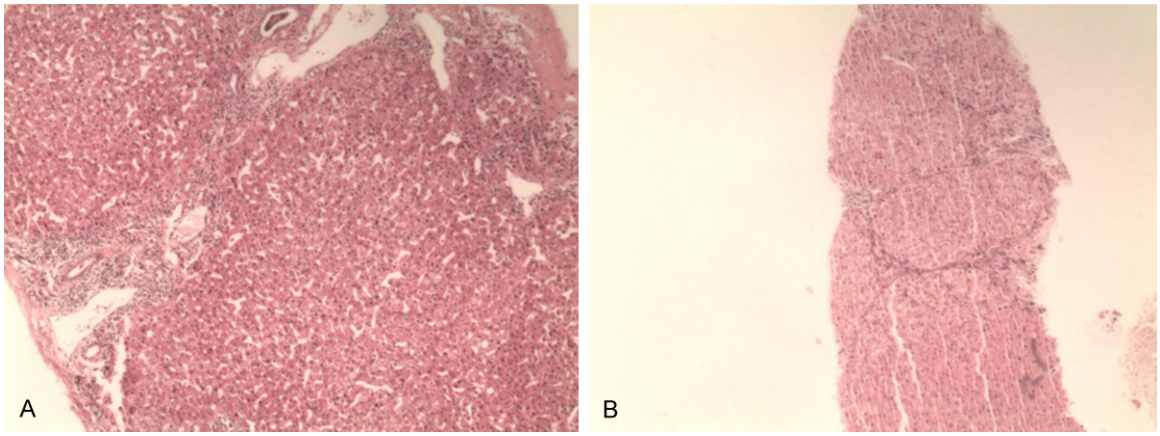

Figure 3. Shown are representative pictures of the pathological examination of liver tissue. A: Intraoperative biopsy; B: Follow-up.

Table 2. Changes in inflammatory grade and fibrosis stage in HCV-positive and HCV-negative graft recipients

	HCV+ n=9	HCV- n=26	*P*
Inflammatory grade			
Intraoperative	1.9±1.5	0.8±0.8	0.03
Follow-up	3.0±1.4	2.6±1.5	0.49
P	0.031	< 0.001	
Fibrosis stage			
Intraoperative	0.8±0.8	0.1±0.3	0.04
Follow-up	2.3±1.5	1.2±1.1	0.04
P	0.010	< 0.001	
Change-Inflammatory	1.1±1.2	1.8±1.4	0.18
Change-Fibrosis	1.5±1.9	1.2±1.1	0.17

Effect of HCV-positive graft on liver pathology of recipients

Liver tissues were collected in all patients for pathological examination during surgery (**Figure 3**). Nine HCV-positive graft recipients, who survived for more than 1 year, had liver biopsy collected at follow-up. There were 31 HCV-negative graft recipients survived for more than 1 year, and 26 of them had liver biopsy performed at follow-up. Higher inflammatory and fibrosis stages were found in both HCV+ and HCV- groups, in which the differences in fibrosis were significant. The mean inflammatory grades at follow-up were: 3.0±1.4 in HCV+group vs. 2.6±1.5 in HCV-group (P=0.49); fibrosis stages were 2.3±1.5 in HCV+group vs. 1.2±1.1 in HCV- group (P=0.04) (**Table 2**). Further analysis showed that there were no significant differences in the changes of inflammatory and fibrosis stages (score at follow-up visit-score at baseline). The changes in inflammatory stage were 1.1±1.2 in HCV+group vs. 1.8±1.4 in HCV- group (P=0.18); the changes in fibrosis stage were 1.5±1.9 in HCV+group vs. 1.2±1.1 in HCV- group (P=0.17) (**Figure 4**).

Evaluation of liver function 1 year after surgery

Liver function was evaluated at year 1 post-surgery by measuring levels of alanine transaminase (ALT), aspartate transaminase (AST), albumin (ALB), bilirubin (TBIL), and international normalized ratio (INR) at follow-up visit. We found the levels of ALB (P=0.49), TBIL (P=0.71), and INR (P=0.26) had no significant differences between HCV+ and HCV- groups. However, ALT (P=0.04) and AST (P=0.02) levels were significantly higher in HCV+group (**Table 3**).

Evaluation of kidney function in recipients

There were 9 patients in HCV+group and 31 patients in HCV-group that survived more than 1 year. To evaluate the kidney function of these patients, we compared the creatinine levels at the follow-up visit to the levels at baseline (before surgery). We found both HCV+ and HCV- groups had a significantly higher creatinine level at follow-up than at the baseline (HCV+group: 54±25 vs. 73±24, P=0.002; HCV- group: 68±23 vs. 84±17, P=0.001) (**Figure 5**). The creatinine levels were similar between two groups at the baseline and at the follow-up visit.

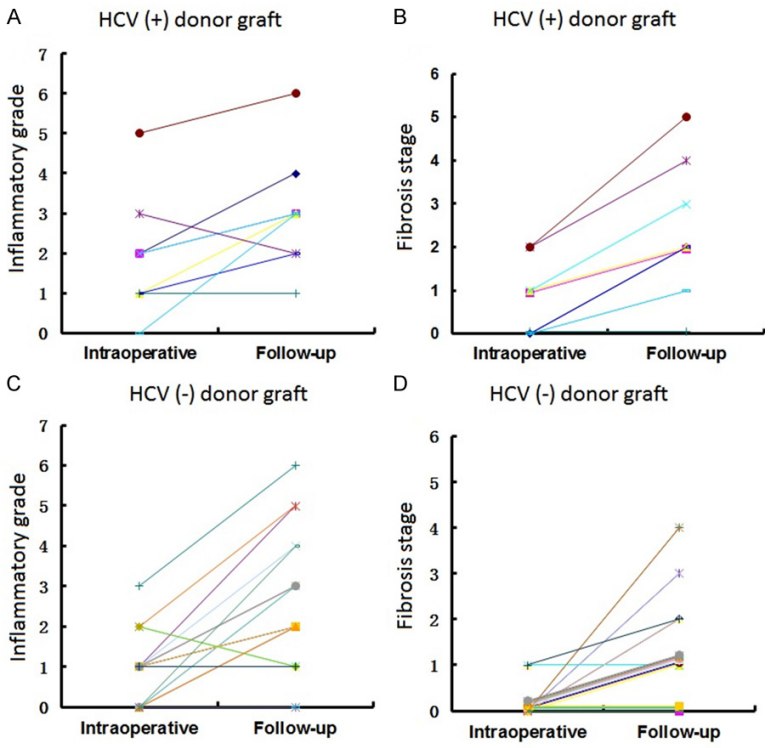

Figure 4. Changes of inflammatory grade and fibrosis stage between intraoperative and follow-up in patients receiving HCV-positive or HCV-negative grafts.

Table 3. Assessment of liver function at year 1 post-surgery

	HCV+ n=9	HCV- n=31	*P*
ALT (U/L), median (IQR)	43 (9)	28 (29)	0.04
AST (U/L), median (IQR)	37 (9)	23 (13)	0.02
ALB (g/L), mean ± SD	45.2±10.3	45.6±4.0	0.49
TBIL (mmol/L), median (IQR)	15.75 (15.98)	17.80 (9.78)	0.71
INR, median (IQR)	1.03 (0.09)	0.99 (0.08)	0.26

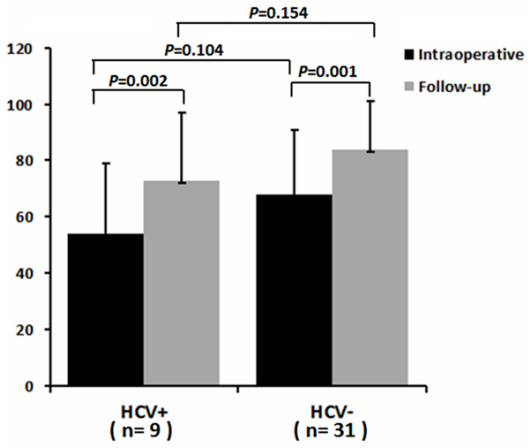

Figure 5. Comparison of creatinine levels during operation and follow-up between HCV-positive and HCV-negative graft recipients.

Discussion

Majority of patients underwent liver transplantation had underlying liver disease at advanced stage or HCC caused by HCV [9]. Liver transplantation is a potentially curative treatment for them. However, the waiting time for the transplantation is often very long because of the limited liver donor pool. Facing this situation, the use of graft from anti-HCV positive donors has been proposed. Reports have shown that the use of graft from HCV-positive donor would not affect the prognosis of recipients after liver transplantation [1-4]. There was limited information, however, on whether HCV-positive graft would affect the liver histology or other organs of the recipients.

Our study confirmed that the survival time of patients was not affected by the HCV status of the graft donor, suggesting the feasibility of using HCV-positive graft as a source for liver transplantation for HCV-positive recipients. However, it is important not to use HCV-positive graft at advanced inflammatory grade or fibrosis stage (> Stage 2), as primary graft non-function may happen after transplantation. Assessment of fibrosis stage of the donor is therefore important before transplantation. Conventional imaging may have limited capability to diagnose fibrosis, and pathological assessment is recommended. In clinical settings in which the pathological assessment is not possible, Fibroscan might be considered. Fibroscan allows for a rapid measurement of liver stiffness that provides a higher accuracy in identifying fibrosis than conventional imaging.

The recipients had progression in liver inflammation grade or fibrosis stage regardless of the HCV status of the donors, although higher stage of liver inflammation and fibrosis were found in HCV-positive graft recipients

at follow-up. The observations were consistent with other studies [5, 6]. The changes in inflammation or fibrosis were similar though between the HCV+ and HCV-groups after correcting for baseline differences. Some studies reported that HCV end-stage patients receiving healthy graft could also experience significant liver fibrosis. Other risk factors, such as elderly donors, were suggested contributing to the progression of fibrosis [10, 11]. Steatotic grafts with high MELD score were also reported to shorten survival time of recipients [12]. Therefore, the graft from HCV-positive donor with fibrosis-related factors, such as the elderly donor, fatty liver, should be prevented.

This risk of extrahepatic damage should also be considered when using HCV-positive graft. Studies have shown that patients experienced a higher chance of chronic renal injury when receiving graft from HIV/HCV co-infection donors than from HIV donors [13]. The present study found that patients had higher levels of creatinine after transplantation in both HCV+ and HCV-groups. The causes of such observations remain elusive. Whether there is an association between HCV and nephropathy await further investigation.

HCV recurrence is a major factor that contributes to the progression of liver disease after transplantation. Today, direct acting antiviral (DAA) agents are highly effective therapies for HCV. They are well-tolerated with minimal side effects. There have been reports showing the use of DAA, such as Sofosbuvir alone or in combination with ribavirin/daclastasvir, could effectively reduce the HCV recurrent rate [14-16]. Whether the use of DAA for patients receiving HCV-positive graft could reduce liver inflammation/fibrosis, and reduce extrahepatic complications, worth further investigation.

The limitations of the current study were the relatively small sample size and short follow-up time. Longer follow-up time will need to observe if HCV-positive graft may induce renal damage or other extrahepatic complications. In conclusion, it is possible to use graft from HCV-positive donors for liver transplantation for HCV-related end-stage liver disease. It is important not to use the graft with advanced fibrosis to prevent primary graft non-function or poor function.

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Disclosure of conflict of interest

None.

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