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【 ORIGINAL ARTICLE 】

Most Cases of Cryptogenic Cirrhosis May Be Nonobese Nonalcoholic Steatohepatitis - Risk Factors of Liver Steatosis After Liver Transplantation for Cryptogenic Cirrhosis: A Retrospective Study

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Abstract:

Objective The course of cryptogenic cirrhosis (CC) after liver transplantation (LT) is unknown. We therefore clarified the natural course post-LT for CC and investigated the etiology of CC.

Methods Eighteen patients who underwent LT for CC were included. To rule out the possibility of nonalcoholic steatohepatitis (NASH) in patients with CC, those with a history of obesity or liver steatosis found pretransplantation were excluded. A liver biopsy was performed one year after LT and annually thereafter.

Results Liver steatosis and steatohepatitis were identified in 61% and 39% of patients after LT, respectively, with a median time to the onset of 12 and 27 months, respectively. There were no other pathological findings such as liver allograft rejection, autoimmune hepatitis, or primary biliary cholangitis. The body mass index after LT (28.5 vs. 22.4 kg/m^2; p=0.002) and mean muscle attenuation at the time of LT were significantly higher (33.3 vs. 25.8 Hounsfield units, p=0.03) and the postoperative hospitalization period shorter (50 vs. 102 days; p=0.02) in the steatosis group than in the non-steatosis group. Recipients were significantly younger in the steatohepatitis subgroup than in the simple steatosis subgroup (55.0 vs. 63.5 years old; p=0.04).

Conclusion Despite excluding CC patients with a history of obesity, we observed that patients with CC had a high prevalence of steatosis after LT than those without CC. Young patients with a favorable postoperative course were noted to have a high risk of NASH after LT for CC. Patients with CC may represent cases of non-obese NASH.

Key words: liver transplantation, nonalcoholic steatohepatitis, cryptogenic cirrhosis, liver steatosis

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Introduction

Liver transplantation (LT) is the treatment of choice for patients with end-stage liver disease. In the more than 50 years after the first LT was performed by Starzl et al. (1), over 1 million LT procedures have been performed worldwide. Patients who undergo LT have a survival rate of approximately 80-90% in the first year following the procedure. This is due to the progressive evolution of surgical techniques, immunosuppressive regimens, and treatments for rejection and infection. A long-term survival has been achieved after LT, and increasing attention is being paid to post-transplantation management. For example, liver steatosis is known to recur after LT for nonalcoholic steatohepatitis (NASH) (2, 3), potentially attenuating the post-transplant

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patient and graft survival.

Cryptogenic cirrhosis (CC) is a diagnosis of exclusion when no other known etiology is identifiable (4). Some investigators have reported that the main possible etiologies of CC are burn-out NASH, silent autoimmune hepatitis, occult virus, or occult alcoholism (5-9). About two decades ago, patients with NASH were misdiagnosed with CC when progressive liver injury and fibrosis occurred. Steatosis typically disappears after the development of cirrhosis, which makes identification of the etiology of cirrhosis difficult (10). In fact, it has been suggested that CC may result from the progression of unrecognized NASH in a large proportion of cases (11). This is because the characteristics of patients with CC were shown to be similar to those of patients with NASH with regard to the high prevalence of metabolic syndrome (12). However, over the past two decades, physicians have become more confident in making a firm diagnosis of NASH cirrhosis based on the medical history, risk factors, and the absence of other etiological factors. Therefore, the number of patients with CC has decreased.

Thuluvath et al. recently demonstrated that the characteristics of CC and NASH were significantly different in their analysis of a large database of over 14,000 patients, concluding that CC diagnoses should not be considered the same as NASH cirrhosis (13). This suggests that most patients with CC have poorly explained liver disease.

Despite these previous findings, however, about 4% of patients in the liver transplant registry in the USA are currently listed as having CC (14). This suggests that there remains a certain number of true CC cases; therefore, an understanding of the natural course of CC after LT is critical. However, several previous studies have considered CC and NASH to be synonymous, so the natural course of true CC is unknown.

In this study, we monitored the post-LT course of patients with CC and verified the possibility of assessing the etiology of CC based on pre-transplant clinical and histological data and post-transplant follow-up findings.

Materials and Methods

Study design

A total of 280 patients underwent LT at Nagasaki University Hospital (Nagasaki, Japan) between 1997 and 2018. Of these, 23 patients were treated with LT for CC. The diagnosis of CC required the exclusion of other potential causes of liver disease in accordance with the usual criteria (15-17). The criteria for the diagnosis of CC were the exclusion of patients with histological features of fatty liver changes; those who consumed excessive alcohol (>21 standard drinks per week in men, >14 standard drinks per week in women) and who were exposed to toxins and drugs known to cause hepatic injury; and those with HCV antibody positivity, with hepatitis B surface antigen positivity, or with autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, or genetic liver disease such as Wilson's disease. Furthermore, to rule out the possibility of NASH in patients with CC, those with a history of peak weight with a body mass index (BMI) ≥25 kg/m^2 or with a history of liver steatosis on pretransplant imaging were excluded from this study. Ultimately, among the 23 patients initially analyzed, 18 were included in this retrospective study, as they had been monitored for over 1 year after LT.

Metabolic syndrome was defined as the presence of obesity in combination with any two of the following abnormalities: dyslipidemia, hypertension, and hyperglycemia (18). The main metabolic features were defined as follows: obesity was defined based on the BMI and not waist circumference, with a BMI ≥25 kg/m^2 considered according to the World Health Organization (Geneva) (WHO) definition of obesity for Asian populations (19); dyslipidemia was defined by a low serum high-density lipoprotein (HDL) cholesterol (<40 mg/dL) or high serum triglyceride level (≥150 mg/dL); hypertension was defined by a systolic or diastolic blood pressure ≥130/85 mmHg; and hyperglycemia was defined by a high fasting blood sugar level (≥110 mg/dL).

Written informed consent was obtained from all patients. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Nagasaki University Hospital ethics committee (#19021803).

The assessment of liver histopathology and steatosis

Recipients underwent a liver biopsy one year after LT and on a yearly basis, in principle. The biopsy was performed in some recipients as needed or when abnormal liver enzyme levels were detected. The liver histopathology was assessed in all cases to identify those wherein the primary disease was apparent after LT. The presence of steatohepatitis was defined based on the FLIP algorithm (necessary combination of three histological features: steatosis, ballooning/clarification of hepatocytes, and lobular inflammation) (20). Steatosis was defined as a steatotic hepatocyte presence of ≥5%. Furthermore, the nonalcoholic fatty liver disease (NAFLD) activity score (NAS) is shown as a reference. The NAS can range from 0 to 8 and is calculated by the sum of the scores of steatosis (0-3), lobular inflammation (0-3), and hepatocyte ballooning (0-2). An NAS of ≥5 strongly correlates with a diagnosis of NASH (21).

Computed tomography analyses of body composition variables

We analyzed cross-sectional, unenhanced computed tomography images of the third lumbar vertebra using the Slice-O-Matic software program (version 5.0; TomoVision, Montreal, Canada) to determine the skeletal muscle and abdominal adipose tissue areas. Muscle areas included the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis muscles. Tissue Hounsfield unit (HU) thresholds were employed as follows: -29 to 150 HU for the skeletal muscle,

Table 1. Characteristics of the 18 Recipients (Who Received Liver Transplantation for Cryptogenic Cirrhosis) and Their Donors.

Recipient characteristics (n=18)	
Age (years)	62.5 (58-65)
Sex: male/female	9 (50%)/9 (50%)
PNPLA3 rs738409 (GG/CG/CC)	11 (61.1%)/4 (22.2%)/3 (16.7%)
BMI (kg/m^2) at LT[1]	23.8 (20.5-25.4)
Diabetes mellitus at LT	3 (16.7%)
BMI (kg/m^2) at liver biopsy[2]	24.5 (22.6-28.8)
Metabolic syndrome at liver biopsy	5 (27.8%)
Follow up period (months)	63.4 (44.8-94.2)
Number of biopsies	3 (2-4)
Immunosuppression drug	TAC 13, CyA 2, PSL 3, MMF 14
Donor characteristics (n=18)	
Living donor/deceased donor	16 (88.9%)/2 (11.1%)
Age (years)	35.5 (32-44)
Sex: male/female	11 (61.1%)/7 (38.9%)
BMI (kg/m^2)	22.6 (21.1-24.5)
Steatosis (≥5%)	6 (33.3%)

Data are shown as medians (interquartile ranges) or numbers (percentages). [1]BMI at LT included the effect of ascites. [2]BMI at liver biopsy represents BMI when steatosis was detected for the first time. PNPLA3: patatin-like phospholipase domain-containing protein 3, BMI: body mass index, LT: liver transplantation, TAC: tacrolimus, CyA: cyclosporine, PSL: prednisolone, MMF: mycophenolate mofetil

-190 to -30 HU for the subcutaneous adipose tissue, and -150 to -50 HU for the visceral adipose tissue (22). As in previous studies, these body composition variables were normalized for height in meters squared and were expressed as cm^2/m^2. We termed the parameters for the skeletal muscle and visceral adipose tissue as the skeletal muscle index and visceral adipose tissue index, respectively. We further calculated the mean muscle attenuation (MA) using the same computed tomography images to assess the skeletal muscle quality. According to reports, low MA indicates an increased intramuscular fat content that contributes to muscle weakness, independent of the age-associated loss of muscle mass (23, 24).

Statistical analyses

Based on the histopathological diagnosis after LT, the patients were divided into the steatosis group (n=11) and non-steatosis group (n=7). Continuous variables were dichotomized based on the median values, and the significance of differences in these variables among the study groups was calculated using the Mann-Whitney *U* test. Categorical data analyses were performed using Fisher's exact test. A p value of <0.05 was considered statistically significant. Data analyses were performed using the SPSS software program, version 22.0 (SPSS, Chicago, USA).

Results

Characteristics of the patient population and the liver histopathology of post-LT

The patients' characteristics are summarized in Table 1. There were 9 men (50%) and 9 women (50%). The median age at LT was 62.5 [interquartile range (IQR), 58-65] years old. Sixteen patients (89%) underwent living-donor LT. Overall, 61% of the patients had the patatin-like phospholipase domain-containing protein 3 (PNPLA3) rs738409 GG genotype, 22% had the CG genotype, and 17% had the CC genotype. Over a median follow-up period of 63.4 (IQR, 44.8-94.2) months after LT, no cases of liver allograft rejection were observed. Liver steatosis was identified in 11 cases (61%), with steatohepatitis identified in 7 of these cases; however, there were no other pathological findings (such as autoimmune hepatitis or primary biliary cholangitis) that could be used to identify the etiology of liver cirrhosis from the liver biopsies after LT. On reviewing the specimens of recipients' excised livers at LT, we found histopathological findings of only severe liver fibrosis and no evidence of steatosis. The median times to the onset of steatosis and steatohepatitis after LT were 12 and 27 months, respectively.

A summary of the post-LT characteristics of the 18 patients is shown in Table 2. An example of hematoxylin and eosin (H & E)-stained liver specimens of post-LT is shown in Fig. 1. In this case (Case 6 in Table 2), there was no stea-

Table 2. Summary of the Characteristics of the 18 Patients after Liver Transplantation.

Case	Sex	Age (years)	PNPLA3 rs738409	BMI at LT[1] (kg/m^2)	BMI at biopsy[2] (kg/m^2)	Post-LT liver	NAS	Time to NAFL[3] (months)	Time to NASH[4] (months)	Follow-up period (months)	Metabolic syndrome at biopsy
1	F	63	CC	22.4	28.5	NASH	4		29	157	Yes
2	M	55	CG	23.8	32.0	NASH	3	37	73	153	Yes
3	M	49	GG	25.4	28.8	NASH	4		12	136	
4	M	58	GG	20.5	23.7	NASH	4		34	95	
5	F	54	GG	27.5	29.2	NASH	6		12	64	
6	F	64	GG	21.3	27.8	NASH	5		11	45	Yes
7	M	40	GG	31.1	41.1	NASH	5	13	27	39	Yes
8	M	62	CC	20.3	23.1	NAFL	1	37		78	
9	F	66	GG	23.9	26.9	NAFL	3	25		63	
10	F	65	GG	17.1	23.0	NAFL	2	11		28	Yes
11	M	60	GG	26.8	28.9	NAFL	2	7		26	
12	M	63	CC	24.5	21.0	Normal	0			89	
13	M	61	CG	19.2	22.6	Normal	0			94	
14	F	63	CG	21.5	15.6	Normal	0			65	
15	F	66	GG	27.0	22.3	Normal	0			51	
16	M	65	CG	17.0	23.1	Normal	0			53	
17	F	68	GG	25.0	22.4	Normal	0			47	
18	F	62	GG	24.9	25.2	Normal	0			28	

[1]BMI at LT included the effect of ascites. [2]BMI at liver biopsy represents BMI when steatosis was detected for the first time. [3]NAFL was defined as a steatotic hepatocyte presence ≥5 %. [4]NASH was defined on the basis of the FLIP algorithm. PNPLA3: patatin-like phospholipase domain-containing protein 3, BMI: body mass index, LT: liver transplantation, NAFL: nonalcoholic fatty liver, NASH: nonalcoholic steatohepatitis, NAS: NAFLD activity score

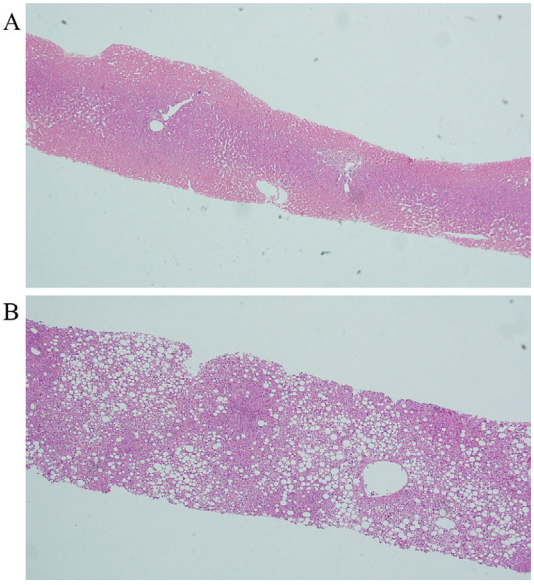

Figure 1. Histological examinations of liver biopsy specimens after liver transplantations. A sample liver specimen from our patients is shown. (a) Hematoxylin and Eosin (H&E) staining liver specimen collected at liver transplantation, ×40 magnification. (b) H&E staining liver specimen one year after liver transplantation, ×40 magnification.

tosis in the graft at LT; however, 1 year later, there was 60% fat deposition, mild lobular inflammation, and ballooning. This case was diagnosed with NASH based on the FLIP algorithm, and the NAFLD activity score was 5.

The comparison of the recipient and donor characteristics according to the presence or absence of steatosis after LT

Table 3 shows the recipient and donor characteristics of the two study groups. Regarding the recipient characteristics, the BMI at the time of the biopsy was significantly higher in the steatosis group than in the non-steatosis group (28.5 kg/m^2 vs. 22.4 kg/m^2; p=0.002). The prevalence of metabolic syndrome after LT tended to be higher in the steatosis group than in the non-steatosis group (54% vs. 0%; p=0.10). Since there were several cases of liver steatosis after LT, we paid attention to body composition in our analysis. We analyzed the laboratory data and body composition before LT and the postoperative course to predict any risk factors of post-LT steatosis. The MA at the time of LT was significantly higher and the postoperative hospitalization period significantly shorter in the steatosis group than in the non-steatosis group (33.3 HU vs. 25.8 HU, p=0.03; and 50 days vs. 102 days, p =0.02). Regarding donor characteristics, no factors (including donor steatosis) differed significantly between the recipients in the steatosis and non-steatosis groups.

The progress of the recipients' BMIs after LT is shown in Fig. 2. The BMIs 1 and 2 years after LT were significantly higher in the steatosis group than in the non-steatosis group (p=0.01 and p=0.009, respectively). The decrease in these BMIs after the third year were attributed to the effect of interventions for liver steatosis.

Table 3. Recipient and Donor Characteristics According to the Presence or Absence of Steatosis.

	Steatosis n=11	Non-steatosis n=7	p value
Recipient characteristics			
Age (year)	60.0 (54.2-63.7)	63.0 (62.2-65.7)	0.06
Sex: male/female	5 (45.5%)/6 (54.5%)	4 (57.1%)/3 (42.9%)	1.00
PNPLA3 rs738409 (GG/CG/CC)	8 (72.7%)/1 (9.1%)/2 (18.2%)	3 (42.9%)/3 (42.9%)/1 (14.2%)	0.33
BMI (kg/m^2) at LT[1]	23.7 (20.7-26.4)	24.5 (19.8-25.0)	0.31
Diabetes mellitus at LT	2 (18%)	1 (14%)	1.00
Body composition variable at LT			
SMI (cm^2/m^2) at LT	45.5 (37.4-59.2)	41.1 (37.3-48.3)	0.25
MA (HU) at LT	33.3 (29.8-38.2)	25.8 (25.1-27.0)	0.03
VATI (cm^2/m^2) at LT	43.7 (24.5-52.2)	56.1 (38.9-74.6)	0.17
BMI (kg/m^2) at liver biopsy[2]	28.5 (24.5-29.1)	22.4 (21.3-23.0)	0.002
TG (mg/dL) at liver biopsy	144 (80-196)	65 (53-94)	0.05
HDL (mg/dL) at liver biopsy	47 (39-55)	50 (46-59)	0.61
FBS (mg/dL) at liver biopsy	110 (95-130)	95 (90-111)	0.13
Metabolic syndrome at liver biopsy	5 (54%)	0	0.10
Follow up period (month)	63.5 (40-125)	52.5 (48-83)	0.68
Number of biopsies	3 (2.3-4.8)	3 (2.3-4.0)	0.61
Postoperative hospital stay (days)	50 (42-57)	102 (59-156)	0.02
Donor characteristics			
Age (years)	35 (33-42)	39 (31-52)	0.92
Sex: male/female	4 (36.4%)/7 (63.6%)	3 (42.9%)/4 (57.1%)	1.00
BMI (kg/m^2)	22.0 (21.1-23.3)	23.7 (21.5-25.7)	0.60
Steatosis (≥5%)	3 (27%)	3 (43%)	0.62

Data are shown as medians (interquartile ranges) or numbers (percentages). [1]BMI at LT included the effect of ascites. [2]BMI at liver biopsy represents BMI when steatosis was detected for the first time. PNPLA3: patatin-like phospholipase domain-containing protein 3, BMI: body mass index, LT: liver transplantation, SMI: skeletal muscle index, MA: mean muscle attenuation, VATI: visceral adipose tissue index, TG: triglyceride, HDL: high-density lipoprotein cholesterol, FBS: fasting blood sugar

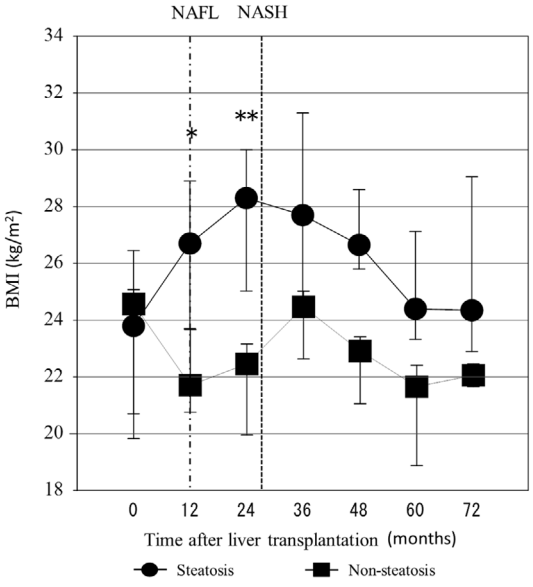

Figure 2. The course of the recipients' body mass indices (BMIs) after liver transplantation. *, p<0.05, **, p<0.01. These dotted lines show the median time of the onset of NAFL and NASH.

Risk factors of steatohepatitis after LT

We further divided the steatosis group into the simple steatosis and steatohepatitis subgroups and analyzed the patient characteristics accordingly (Table 4) The recipient age was significantly lower in the steatohepatitis subgroup than in the simple steatosis subgroup (55.0 vs. 63.5 years old, p= 0.04). However, there were no significant intergroup differences in the donor characteristics or recipient BMI, MA, or postoperative hospitalization period.

Discussion

This retrospective study is the first to examine the clinical course of CC, while excluding NASH patients with a history of obesity as much as possible, after LT in Japan. Post-LT, there were no histopathological findings that could identify the primary disease, such as autoimmune hepatitis, primary biliary cholangitis, etc. In addition, there were no findings of liver allograft rejection. Interestingly, despite excluding patients with a history of obesity from our cohort of patients with CC, we observed that patients with CC had a high prevalence of steatosis after LT, such as patients with NASH. Furthermore, we found that the genetic background of patients with CC was similar to that of non-obese NASH

Table 4. Recipient and Donor Characteristics According to Simple Steatosis or Steatohepatitis.

	Simple steatosis n=4	Steatohepatitis n=7	p value
Recipient characteristics			
Age (years)	63.5 (60.0-66.0)	55.0 (50.3-61.8)	0.04
Sex: male/female	2 (50%)/2 (50%)	4 (57.1%)/3 (42.9%)	1.00
PNPLA3 (GG/CG/CC)	3 (75%)/0/1 (25%)	5 (71.4%)/1 (14.3%)/1 (14.3%)	0.69
BMI (kg/m^2) at LT[1]	22.1 (17.1-26.8)	23.8 (21.5-26.9)	0.41
Body composition variable at LT			
SMI (cm^2/m^2) at LT	40.8 (30.0-64.3)	51.6 (41.6-59.2)	0.60
MA (HU) at LT	31.0 (29.3-38.2)	35.4 (32.0-41.1)	0.47
VATI (cm^2/m^2) at LT	46.0 (23.4-75.6)	35.4 (24.5-52.2)	0.13
BMI (kg/m^2) at liver biopsy[2]	25.0 (23.0-28.9)	28.8 (27.9-31.3)	0.10
TG (mg/dL) at liver biopsy	103 (60-216)	151 (99-196)	0.41
HDL (mg/dL) at liver biopsy	53 (43-62)	45 (38-51)	0.31
FBS (mg/dL) at liver biopsy	115 (95-142)	110 (95-128)	0.92
Metabolic syndrome at liver biopsy	1 (25%)	4 (57%)	0.54
Postoperative hospital stay (day)	51 (47-58)	49 (38-62)	0.55
Donor characteristics			
Age (years)	35.5 (32.5-41.5)	35.0 (33.0-48.8)	0.92
Sex: male/female	2 (50%)/2 (50%)	5 (71.4%)/2 (28.6%)	0.57
BMI (kg/m^2)	22.8 (21.6-26.8)	21.9 (20.8-22.8)	0.41
Steatosis (≥5%)	1 (25%)	2 (29%)	1.00

Data are shown as medians (interquartile ranges) or numbers (percentages). [1]BMI at LT included the effect of ascites. [2]BMI at liver biopsy represents BMI when steatosis was detected for the first time. PNPLA3: patatin-like phospholipase domain-containing protein 3, BMI: body mass index, LT: liver transplantation, SMI: skeletal muscle index, MA: mean muscle attenuation, VATI: visceral adipose tissue index, TG: triglyceride, HDL: high-density lipoprotein cholesterol, FBS: fasting blood sugar

with regard to PNPLA3. This suggests that most cases with CC are actually cases of non-obese NASH. We also observed that an increase in the BMI one year after LT for CC was associated with steatosis after LT. Furthermore, a young age, relatively well-maintained muscle quality at the time of LT, and short postoperative hospitalization period were identified as novel risk factors for steatosis development after LT. In particular, a young age was a risk factor for steatohepatitis among patients with post-LT steatosis. Our findings can help predict patients at high risk for liver steatosis development based on the postoperative course and are expected to be useful for the post-LT management of CC.

Some studies have reported that the incidence of nonalcoholic fatty liver disease (NAFLD) after LT ranges between 18% and 40% (15, 25-27). In the general population of Western countries, the prevalence of NAFLD is reported to be 19.0-31.3%; thus, there is no marked difference between its prevalence after LT and in the general population (28, 29). However, among LT patients with NASH or CC, the prevalence of post-LT NAFLD is reported to be 33-63%, which is higher than the prevalence in patients with LTs for other etiologies and in the general population (2, 3, 15, 30-32). Obesity, pre- and post-LT diabetes mellitus, hyperlipidemia, arterial hypertension, tacrolimus-based regimen, pretransplant liver graft steatosis, and the PNPLA3 genotype GG of the recipient are risk factors for liver steatosis after LT (13, 14, 33-35). Similar to these previous reports, in the present study, 61% and 39% of patients developed liver steatosis and steatohepatitis, respectively, after undergoing LT for CC. The median times to the diagnosis of simple steatosis and steatohepatitis after LT were 12 and 27 months, respectively. In our previous studies, among 100 patients with LT in our institution, 33% developed steatosis and 9% developed steatohepatitis after LT, and the average time to steatosis development after LT was 3.81±2.46 years (27, 36). Compared to this, the incidences of simple steatosis and steatohepatitis after LT for CC were significantly higher (p=0.03 and p=0.003, respectively), and the times to steatosis development were shorter in our study. Even though we separated and excluded patients with a history of obesity from the patients with CC to rule out patients suspected of having NASH, the incidence of post-LT NAFLD was still high in patients with CC. This indicates that a majority of patients with CC may actually have non-obese NASH. In Western countries, patients with CC comprise 4% of patients with liver cirrhosis (13). However, the number of LT procedures performed for patients with CC at our institution increased from 4% (3/78) between 1997 and 2007 to 10% (20/202) between 2008 and 2018. In Asian countries, the prevalence of non-obese NAFLD was reported to be twice of that in the Western countries (37). These data further support our conclusion that patients with CC in our

study may have non-obese NASH.

Regarding the characteristics of our patients with CC, the prevalence of the PNPLA3 rs738409 genotype GG was 61%, which is higher than that in the general population in Japan (38). This genetic factor is associated with an increase in the liver fat and hepatic inflammation. Previous studies have reported that the rs738409 GG genotype was a significant risk factor for the development and progression of non-obese NAFLD (39). Furthermore, the proportion of subjects with the rs738409 GG genotype was higher among those with non-obese NAFLD than among those with obese NAFLD (47.8% vs 36.5%), and the GG genotype was identified as an independent predictor of NAFLD in the non-obese cohort (40). Thus, the genetic background of patients with CC is similar to that of non-obese NASH. This is another reason to believe that patients with CC may actually have non-obese NASH. PNPLA3 genotype GG is considered to be a factor associated with post-LT steatosis in patients with CC.

Regarding gender differences in the present study, there were no gender differences noted in the entire cohort - all patients with CC - or in the group who developed liver steatosis after LT, which was assumed to be non-obese NASH. A systematic review reported that women have a higher risk of NASH and advanced fibrosis than men (41). However, our study is based on LT recipients; therefore, there is a selection bias, so we cannot evaluate the gender difference. Furthermore, according to previous reports, there is no evidence of gender differences in CC or non-obese NASH (13, 42).

Regarding the progression after LT, patients in the steatosis group in the present study showed a significant increase in their BMI one and two years after LT. The median times to the diagnosis of simple steatosis and steatohepatitis after LT were 12 and 27 months, respectively, and relatively early. Although CC and NASH are hypothesized to lie on the same spectrum of liver diseases associated with metabolic syndrome, CC seems to be the more aggressive of the two (12). In addition, a study of non-obese NAFLDs reported that fibrosis progression was faster in patients with non-obese NAFLD than in those with NAFLD with a higher BMI (43). This suggests that the time to the onset of post-LT steatosis, especially the progression time to steatohepatitis, may be short in patients with CC. Therefore, early initiation of treatment for steatosis after LT is required.

Assuming that patients with CC have non-obese NASH, there seems to be a discrepancy in the development of NAFL/NASH with weight gain after LT. However, weight gain is common after LT. This can be explained by the fact that the catabolic state due to liver cirrhosis improves after LT, and the use of immunosuppressive drugs exacerbates insulin resistance, making the patient more susceptible to developing MS (44, 45). The BMI reportedly increases in 60-70% of patients after LT, and even in patients who were not obese before transplantation, over 20% become obese within 1 year and 30% within 3 years (46). Thus, there are conditions that facilitate weight gain after LT, and we consider patients with CC to also be likely to gain weight due to improved nutrition and immunosuppressive drugs. However, even in cases with a similar post-LT condition, the higher incidence of NAFL/NASH in patients with CC than in patients with other etiologies besides NASH indicates that patients with CC are predisposed to NASH/NAFL. This may be due to a difference in the genetic background between patients with CC and those with other etiologies. Thus, CC patients have a genetic background that is predisposed NASH.

Regarding donor characteristics, although Miyaaki et al. (36) reported that donor steatosis is a risk factor for liver steatosis after LT, in our study, donor factors did not have a significant effect. In the patients with CC, the influence of the recipient factors was strong, while that of the donor factors may have been relatively small.

Previous studies have reported that the muscle composition is associated with NAFLD, hepatocellular carcinoma, and the prognosis after LT (47-50). The quality or quantity of a patient's muscles is a significant factor in liver disease. In the present study, a higher MA was significantly associated with the prevalence of post-LT steatosis. A high MA indicates low intramuscular fat deposition, therefore indicating that the quality of muscle is maintained. The MA associated with poor prognosis for hepatocellular carcinoma is reported to be ≤39.3 HU in women (49). The MA in the steatosis group, while low at 33.3 HU, was higher than that in the non-steatosis group. This finding suggests that the quality of muscle at the time of LT is an indicator of post-LT steatosis. Interestingly, patients who developed post-LT steatosis and those who had a high MA were discharged early after LT. This is consistent with previous reports showing that patients with a higher MA have a better postoperative course and prognosis after LT or resection of hepatocellular carcinoma than those with a lower MA (48, 49). Furthermore, in the post-LT steatosis group, younger patients were more likely to progress to NASH than older ones. The results of this study suggest that post-LT NASH is more likely to occur in young patients with a relatively well-maintained muscle quality and a favorable postoperative course than in others. Such cases with a favorable postoperative course are more likely to progress to NAFLD than other cases because they are no longer restricted in their lives due to their good physical condition after LT.

Several limitations associated with the present study warrant mention. Our study was retrospective; therefore, annual liver biopsy specimens were not available for some patients, and this may have caused a selection bias. Our study sample was taken from a single center and was small in size. Therefore, our conclusions and the interpretation of results are limited, and to validate our conclusions, a study with a larger sample size is required.

In conclusion, patients undergoing LT for CC, excluding those with a history of obesity, had a high prevalence of liver steatosis development within one to two years, similar

to after LT in patients with NASH. In addition, the genetic background of patients with CC was similar to that of those with non-obese NASH. This suggests that several cases of CC were actually non-obese NASH. Postoperative weight gain was associated with post-LT liver steatosis. In particular, a young age, non-low MA, and postoperative early discharge were associated with a high risk of NASH after LT for CC. Our findings indicate that the postoperative body weight should be carefully monitored, and strict weight control is required immediately after LT for CC, especially in patients with a favorable postoperative course.

The authors state that they have no Conflict of Interest (COI).

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