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

Background

As the diagnosis of non-alcoholic steatohepatitis (NASH) has become more accurate, the number of patients with cryptogenic cirrhosis (CC) has decreased. Despite this, there are patients in the liver transplant registry who are currently listed as having CC. Therefore, knowledge of the natural course of CC after liver transplantation (LT) is important. However, the natural course of CC after LT is unknown. We aimed to clarify the natural course post LT for CC and to infer the etiology of CC.

Methods

Eighteen patients who underwent LT for CC were included. To rule out the possibility of including NASH patients with CC patients, those with obesity (body mass index [BMI] ≥ 25 kg/m^2) and type-2 diabetes mellitus or liver steatosis found on pretransplantation images, were excluded. Liver biopsy was performed 1 year after LT, and annually thereafter.

Results

Liver steatosis and steatohepatitis were identified in 61% and 39% of the patients after LT, respectively with a median time to onset of 12 and 27 months, respectively. No other pathological findings could identify the etiology of CC. The BMIs after LT were significantly higher in the steatosis group than in the non-steatosis group (28.5 vs. 22.4 kg/m2; P = 0.002). The mean muscle attenuation (MA) at the time of LT was significantly higher and the postoperative hospitalization period was shorter in the steatosis group than in the non-steatosis group (MA: 33.3 vs. 25.8 Hounsfield unit, P = 0.03; hospitalization: 50 vs. 102 days, P = 0.02). Recipients were significantly younger in the steatohepatitis subgroup than in the simple steatosis subgroup (55.0 vs. 63.5 years, P = 0.04).

Conclusions

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. 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.

1. Background

Liver transplantation (LT) is the treatment of choice for patients with end-stage liver disease. Over the course of 50 years after the first LT was performed by Starzl et al. [1], more than 1,000,000 LTs 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. Long-term survival has been achieved after LT, and more 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 post-transplant 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, persons with NASH were mistakenly diagnosed 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 reported that CC likely results from the progression of unrecognized NASH in a large proportion of cases [11]. This was because the characteristics of patients with CC were similar to those of patients with NASH in that there was a 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 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 when a large database of over 14,000 patients was analyzed; diagnosed CC cases should not be

considered the same as NASH cirrhosis [13]. This suggests that most patients currently listed as having CC have a poorly clarified liver disease. Despite this, about 4% of the patients in the liver transplant registry at the USA are currently listed as having CC [14]. This suggests that there is still a certain number of true CC cases; therefore, knowledge of the natural course of CC after LT is important. However, many previous studies have considered CC and NASH to be synonymous, and the natural course of true CC is unknown. In this study, we reveal the natural course after LT for patients with CC and verify whether it is possible to infer the etiology of CC based on pre-transplant clinical and histological data and post-transplant follow-up.

2. Methods

2.1. Study Design

A total of 280 patients underwent LT at the 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 cases with histological features of fatty liver changes, who consumed excessive alcohol (> 21 standard drinks per week in men and > 14 standard drinks per week in women), who were exposed to toxins and drugs known to cause of hepatic injury, 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 including NASH patients with CC patients, patients with obesity (body mass index [BMI] ≥ 25 kg/m^2) and type 2 diabetes mellitus or with liver steatosis on pretransplant imaging were excluded from this study. Finally, among the 23 patients analyzed, 18 were included in this retrospective study as they were monitored for more than 1 year after LT. The relevant recipient and donor characteristics are summarized in Table 1.

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	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 drugs	TAC (n = 13), CyA (n = 2), PSL (n = 3), MMF (n = 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. PNPLA3, patatin-like phospholipase domain-containing protein 3; BMI: body mass index, LT: liver transplantation, TAC: tacrolimus, CyA: cyclosporine, PSL: prednisolone, MMF: mycophenolate mofetil.

The 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 BMI and not waist circumference—BMI ≥ 25 kg/m^2 was considered according to the World Health Organization (WHO) definition of obesity for Asian populations [19]; dyslipidemia was defined by low serum HDL cholesterol (< 40 mg/dl) or high serum triglyceride level (≥ 150 mg/dl); hypertension by systolic or diastolic blood pressure of 130/85 mmHg or higher; hyperglycemia by 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).

2.2. 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 and when it was needed or 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 on the basis of the FLIP algorithm (the 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%.

2.3. 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 (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, −190 to −30 HU for the subcutaneous adipose tissue, and −150 to −50 HU for the visceral adipose tissue [21]. 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 also calculated the mean muscle attenuation using the same computed tomography images to assess the skeletal muscle quality. According to reports, low muscle attenuation indicates an increased intramuscular fat content that contributes to muscle weakness independent of the age-associated loss in muscle mass [22, 23].

2.4. Statistical analysis

On the basis of the histopathological diagnosis after LT, the patients were divided into the steatosis group (n = 11) and the 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 analysis was performed using the Fisher's exact test. A P-value of < 0.05 was considered statistically significant. Data analysis was performed using SPSS, version 22.0 (SPSS, Chicago, IL, USA).

3. Results

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

The patients' characteristics are summarized in Table 1. There were nine males (50%) and nine females (50%). The median age at LT was 62.5 years (interquartile range [IQR]: 58–65 years). 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 months (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 seven 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. Reviewing the specimens of recipients' excised livers at LT, we found histopathological findings of only severe liver fibrosis and no 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. Hematoxylin/eosin (HE)-stained liver specimens of post-LT are shown in Fig. 1. In this case (Case 6 in Table 2), there was no steatosis in the graft at LT, but one 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.

Table 2
Summary of the characteristics of the 18 patients after liver transplantation

Case	Sex	Age (years)	PNPLA3 rs738409	BMI at LT (kg/m^2)	BMI at biopsy (kg/m^2)	Post-LT liver	NAS	Time to NAFL (months)	Time to NASH (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] NAFL was defined as a steatotic hepatocyte presence ≥ 5 %. [3] 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.

3.2. 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 according to the two study groups. Regarding the recipient characteristics, the BMI at the time of 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 the 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 many cases of liver steatosis after LT, we also 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 muscle attenuation at the time of LT was significantly higher and the postoperative hospitalization period was significantly shorter in the steatosis group than in the non-steatosis group (MA: 33.3 HU vs. 25.8 HU, P = 0.03 and hospitalization period: 50 days vs. 102 days, P = 0.02). Regarding donor characteristics, no factor (including donor steatosis) differed significantly between the recipients from the steatosis and non-steatosis groups.

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	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	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 stays (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. 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.

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). A decrease in these BMIs after the third year were attributed to the effect of interventions for liver steatosis.

3.3. 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, P = 0.04). There were no significant intergroup differences in the donor characteristics and the recipient BMI, muscle attenuation, and postoperative hospitalization period.

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,	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	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 stays(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. 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.

4. Discussion

This retrospective study is the first to examine the clinical course of CC, while excluding NASH patients with a history of obesity as far as possible, after LT in Japan. In the course of post-LT, there were no histopathological findings that could identify the primary disease such as autoimmune hepatitis, primary biliary cholangitis and so on. There were also 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. This explains that there is a possibility that most cases with CC are actually cases with 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 maintained muscle quality at the time of LT, and short postoperative hospitalization period were identified as the novel risk factors for steatosis development after LT. In particular, young age was a risk factor for

steatohepatitis among patients with post-LT steatosis. Our findings can help predict patients at a high risk of liver steatosis development on the basis of the postoperative course, and are 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, 24–26]. In the general population of Western countries, the prevalence of NAFLD is reported to be 19.0–31.3%; thus, there is no difference between its prevalence after LT and in the general population [27] [28]. 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, 29–31]. 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 the risk factors for liver steatosis after LT [13, 14, 32–34]. Similar to in these reports, in this study, 61% and 39% of the 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 months 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 [26, 35]. 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 with NASH, the incidence of post-LT NAFLD was still high in patients with CC. This indicates that a majority of the patients with CC may actually have non-obese NASH. In Western countries, patients with CC comprise 4% of the patients with liver cirrhosis [13]. However, the number of LTs 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 [36]. These data also support our conclusion that the patients with CC in our study might 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 [37]. 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 an important risk factor for the development and progression of non-obese NAFLD [38]. 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 [39]. Thus, it seems right to presume that CC should actually be diagnosed as non-obese NASH. PNPLA3 genotype GG is considered to be one of the factors of post-LT steatosis in patients with CC.

As for gender differences in the present study, there were no gender differences 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 [40]. However, our study is based on LT recipients; therefore, there is a selection bias and hence, 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, 41].

Regarding the progression after LT, patients in the steatosis group in this study showed a significant increase in the BMIs 1 and 2 years after LT. The median times to the diagnosis of simple steatosis and steatohepatitis after LT were 12 months and 27 months, respectively, and relatively early. Although CC and NASH are hypothesized to be belonging to the same spectrum of liver diseases associated with the metabolic syndrome, CC seems to be more aggressive [12]. The study of non-obese NAFLDs has also reported that fibrosis progression was faster in patients with non-obese NAFLD than in patients with NAFLD with a higher BMI [42]. This might indicate that the period to the onset of post-LT steatosis, especially the progression time to steatohepatitis, is short in patients with CC. Therefore, an early initiation of treatment for steatosis after LT is required.

Regarding donor characteristics, although Miyaaki et al. [35] 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 case of patients with CC, the influence of the recipient factors was strong, and 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 [43–46]. The quality or quantity of the patient's muscles is an important factor in liver disease. In the present study, a higher muscle attenuation was significantly associated with the prevalence of post-LT steatosis. High muscle attenuation indicates a low

intramuscular fat deposition, which thereby indicates that the quality of muscle is maintained. The muscle attenuation, which indicates a poor prognosis for hepatocellular carcinoma, is reported to be ≤ 39.3 HU in women [45]. Therefore, the muscle attenuation in the steatosis group, though 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 high muscle attenuation were discharged early after LT. This is consistent with previous reports that have shown that patients with higher muscle attenuation have a better postoperative course and prognosis after LT or resection of hepatocellular carcinoma [44, 45]. Moreover, in the post-LT steatosis group, younger patients were more likely to progress to NASH. 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. Such cases with a favorable postoperative course are more likely to have NAFLD because they are no longer restricted in their lives due to their good physical condition after LT.

The limitations of our study need to be acknowledged. 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 needed.

5. Conclusions

In conclusion, patients undergoing LT for CC had a high prevalence of liver steatosis development within 1–2 years. This suggests that many cases of CC represent non-obese NASH. PNPLA3 rs738409 genotype GG and a postoperative weight gain were associated with post-LT liver steatosis. In particular, young age, non-low MA, and postoperative early discharge are the risk factors for 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.

Abbreviations

BMI, body mass index; CC, cryptogenic cirrhosis; CyA, cyclosporine; FBS, fasting blood sugar; HDL, high-density lipoprotein cholesterol; HU, Hounsfield unit; LT, liver transplantation; MA, mean muscle attenuation; MMF, mycophenolate mofetil; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing protein 3; PSL, prednisolone; SMI, skeletal muscle index; TAC tacrolimus, TG, triglyceride; VATI:visceral adipose tissue index.

Declarations

Ethics approval and consent to participate: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Nagasaki University Hospital (#19021803, 2/19/2019). Written informed consent was obtained from all subjects involved in the study.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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M.F. participated in the study design, statistical analysis, data interpretation, article preparation, literature search. H.M. participated in the study design, data interpretation. R.S., M.H., S.M., T.H., A.S., M.H. and S.E. participated in the data collection. K.N. participated in the data interpretation.

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Figures

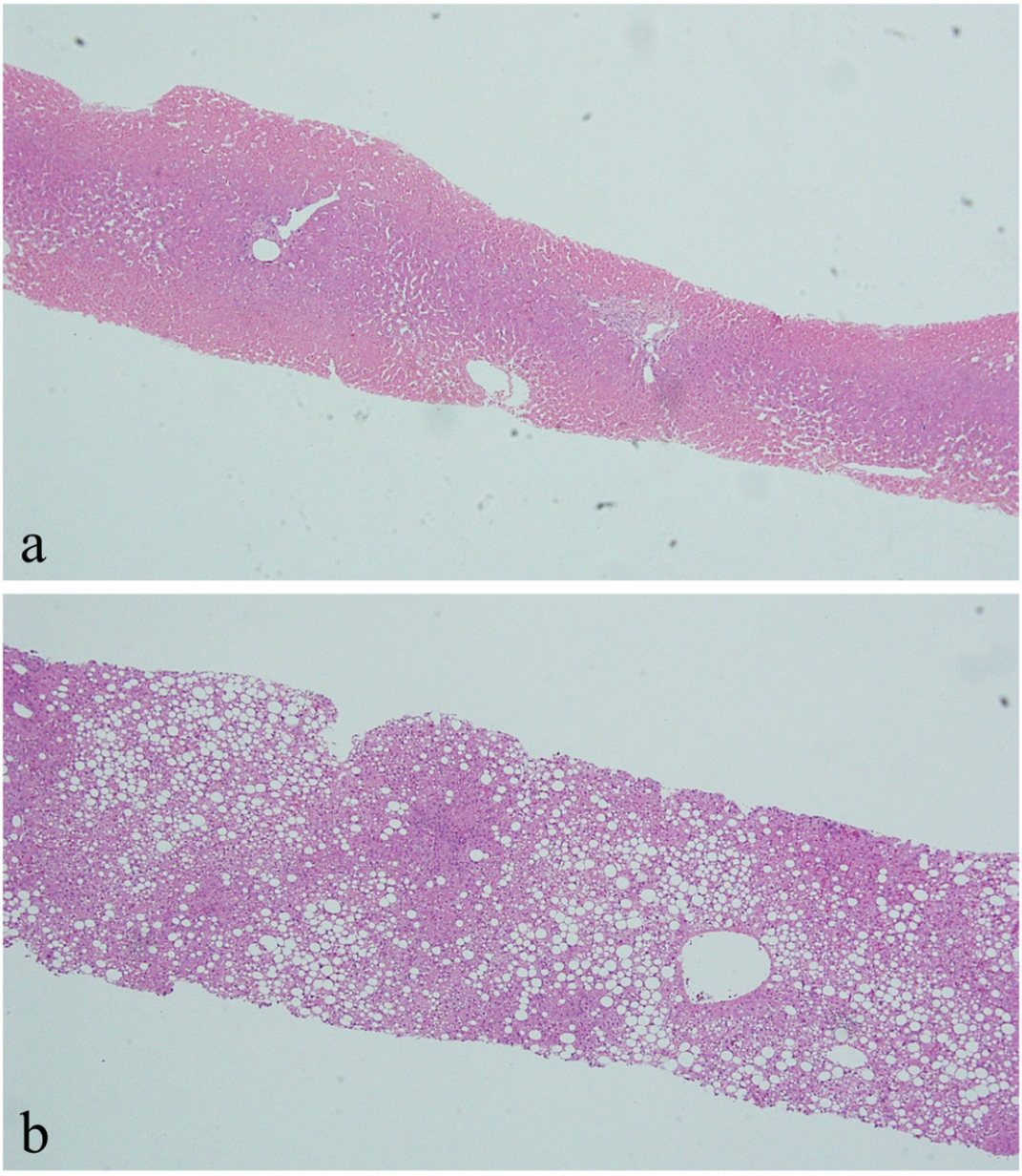

Figure 1

Histological examinations of liver biopsy after liver transplantations. A case of liver specimen in our patients is shown. a: Hematoxylin/eosin (HE) stained liver specimen collected at liver transplantation, x40 magnification. b: HE stained liver specimen one year later after liver transplantation, x40 magnification.

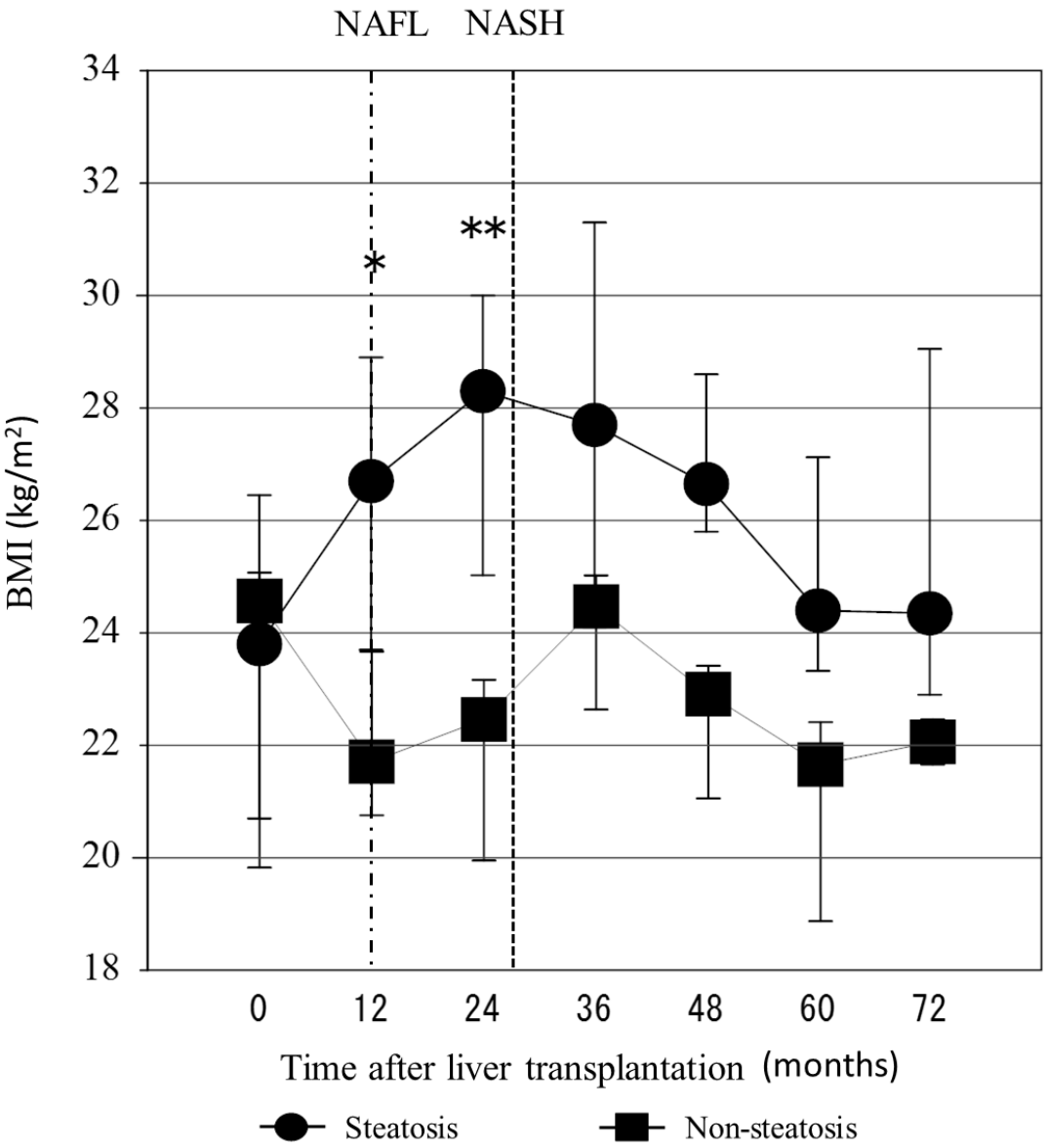

Figure 2

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