Table 3. 移植後再発PBCに関する報告

	報告年	N	観察期間（中央値，月）	肝生検	再発率（%）	再発までの期間
Liermann Garcia[35]	2001	400	56	protocol	17	36
Sanchez[39]	2003	156	72	protocol	11	50
Sylvestre[40]	2003	100	44	protocol	17	56
Neuberger[41]	2004	485	79	protocol	23	
Jacob[42]	2006	100	118	protocol	14	61
Charatcharoenwitthaya[43]	2007	154		protocol	34	
Morioka[44]	2007	50	29	ad hoc	18	36
Montano-Loza[45]	2010	108	83	ad hoc	26	70
Manousou[46]	2010	103	108	protocol	26	44
Kaneko[47]	2012	81	74	ad hoc	1	61

と報告されている[10]．この結果は，ウイルス性肝硬変の移植後5年生存率72%，急性肝不全の59%，肝細胞癌の58%と比較して明らかに良い成績で，代謝性疾患などと並んで移植成績の最も良い疾患のひとつであることがわかる．OPTNの統計でも1997年から2004年に移植を受けたPBCを含む胆汁うっ滞性疾患の成績は1年生存率89.8%，5年生存率79.7%と極めて良好である[32]．UNOS(United Network for Organ Sharing)データベースを使った疾患別の移植成績の検討でもPBCの移植成績が最も良好である[33]．PBC患者の各年齢での死亡数を解析した結果では，女性PBC患者の死亡数には1980年代は50歳代後半と70歳代の二峰性のピークが認められたが，1990年代になると若年のピークがなくなり，健常人と同じように加齢にともなう死亡数の上昇のみが認められるようになる[34]．このようなPBC患者の若年での死亡数の減少の一因は，肝移植の普及と成績向上にともなうものと考えられている．なお，PBC患者の移植後死亡原因としては，6カ月以内の早期死亡は主に敗血症，多臓器不全などが原因となり，6カ月以降の晩期死亡は主に敗血症，悪性腫瘍の発生，腎不全，慢性拒絶などが報告されている[35]．

一方，わが国での移植成績は，全体で5年，10年生存率はそれぞれ76.5%，55.6%と報告され[15]，単施設では3年，5年生存率がそれぞれ88%，80%と報告されている[36]．欧米では脳死ドナーを用いた肝移植が主流であるのに対し，わが国では肝移植の98%以上が生体ドナーを用いて行われるという背景の違いはあるが，これらの結果をみるとPBCの肝移植に関しては脳死ドナーと生体ドナーで移植成績に差はないと考えられる．

PBCをはじめとする胆汁うっ滞性肝疾患は，皮膚掻痒感や倦怠感といった症状が他の疾患より高頻度で認められ，これらの症状によるQOLの低下が移植適応とされる場合もある．このようなQOLに対する肝移植の治療成績に関し，Grossらによる肝移植を受けたPBCもしくはPSC患者157人を対象とした検討が報告されている[37]．この報告では術前51%の症例で耐えがたい疲労感，不眠あるいは皮膚掻痒感が認められていたが，移植1年後にはその割合は25%まで減少している．ただし，皮膚掻痒感は最も改善効果が高いが，慢性的な疲労感は移植後も比較的残る場合が多いとされている．また，「ほぼ正常な日常生活を送ることができる」と感じている患者の割合は，移植前の29%から移植後は61%に増加している．このように肝移植は生命予後ばかりでなく，皮膚掻痒感や倦怠感といった症状，あるいは患者のQOLをも改善させるが，術前要因でこのようなQOLの改善効果を予測する因子は見出されていない．

IV PBCの移植後再発

肝移植後におこるPBCのグラフト肝への再発

は不明な部分が多い．なぜなら，報告により再発の診断基準が一定していないからである．移植前の状態であれば，①アルカリフォスファターゼ（ALP）値の上昇に代表される胆汁うっ滞を示す生化学検査所見，②抗ミトコンドリア抗体（AMA）陽性，③慢性非化膿性破壊性胆管炎（chronic non-suppurative destructive cholangitis；CNSDC）に代表される組織学的所見，という3項目の診断基準が一般的に用いられる[17]．しかし，血清ALP値の上昇は慢性拒絶やサイトメガロウイルス感染，薬剤性胆汁うっ滞などの移植後のさまざまな病態で認められる．AMAに関しても，特にM2抗体は，組織学的な再発がなくても移植後に陽性で経過することが報告されている[38]．このような理由から，組織学的な診断が移植後にPBCの再発を診断する唯一の方法となっている．

これまでの移植後再発PBCに関する主な報告をTable 3[35][39]~[47]に示す．PBC再発頻度はおおむね10%から30%，再発までの期間は3年から6年と一定の傾向はない．しかし，組織学的なPBCの再発は必ずしも臨床症状や検査値異常をともなわないため[35]，肝生検が症状出現時のみに行われたのか（ad hoc），プロトコール肝生検を行ったかで結果は異なると考えられる．再発関連因子としてレシピエントの年齢[35][42]，性別[43]，免疫抑制剤の種類[41]~[43]，ドナーとレシピエントのHLA適合[41]などが報告されているが，いまだに確立されたものはない．

グラフト肝への再発は，現時点では患者の予後に与える影響は少ないと考えられている．Liermann Garciaらは，68例の移植後再発PBC患者のうち，肝硬変に至るか再移植を要した例は2例のみであったと報告している[35]．しかし今後，移植後観察期間が長期になった場合の影響はいまだに明らかではないと思われる．また，移植後再発PBCに対する治療としてはUDCAを用いた報告が多いが[35][39][43]，生化学検査の改善以外の組織学的進展や予後への影響はやはり明らかではない．

おわりに

UDCAの登場により末期肝硬変に至るPBC患者が減少していることは実臨床上でも実感される．しかし，一部の症例は内科的治療に抵抗し，このような患者が肝硬変に至ると現時点では移植以外に治療法がないことも事実である．肝移植の黎明期から適応疾患のひとつとされてきたPBCに対する移植成績はほぼ確立したものになりつつあるが，移植後再発PBCの病態，治療，予後など新しい課題が生まれつつある．

本論文内容に関連する著者の利益相反
：なし

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Original Article

Chronologic changes of explanted liver volume and the use of ursodeoxycholic acid in patients with end-stage primary biliary cirrhosis

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Aim: The clinical presentation of Primary biliary cirrhosis (PBC) at the time of liver transplantation (LT) may have changed, due to the long-term use of ursodeoxycholic acid (UDCA). The aim of this retrospective study was to investigate whether the clinical characteristics of LT recipients with PBC have changed over the years.

Methods: Of all 421 adults undergoing LT from 1997 to 2012 at our center, we included 85 recipients with PBC into the present study. The 85 recipients were divided into three groups according to the year LT was performed: group 1 (1997–2001, $n = 29$), group 2 (2002–2005, $n = 29$) and group 3 (2006–2012, $n = 27$).

Results: There were no significant differences in sex, recipient age, Model for End-Stage Liver Disease score, updated Mayo risk score for PBC, or liver-related complications except for esophageal varices among the three groups. Patients in group 1 were complicated with esophageal varices less frequently than those in the other two groups. In older cases, the ratio of explanted liver volume to standard liver volume (ELV/SLV) was significantly higher, and the duration of pre-LT UDCA treatment was significantly shorter. The duration of UDCA treatment was significantly correlated with ELV/SLV.

Conclusion: Recent LT patients were characterized by more frequent portal hypertension and more severe liver atrophy, with longer UDCA therapy prior to LT, which might have prevented the somewhat rapid progression of liver failure characterized by hepatomegaly with insignificant fibrosis or portal hypertension.

Key words: explanted liver, hepatomegaly, liver transplantation, living donor liver transplantation, primary biliary cirrhosis

INTRODUCTION

PRIMARY BILIARY CIRRHOSIS (PBC) is a chronic and cholestatic liver disease characterized by inflammatory destruction of the intrahepatic bile ducts that is thought to be autoimmune mediated.[1,2] The mechanism underlying the development of PBC remains controversial.[3] Cholestatic liver cirrhosis leading to hepatic failure is the most severe clinical manifestation of PBC, resulting in death or requiring liver transplantation (LT).[2,4] Potential subtypes of PBC disease progression, which are hepatic failure type and portal hypertension type, were recently proposed, and are represented by the presence or absence of autoantibodies, such as anti-gp210 and anticentromere.[5–7]

First introduced into clinical practice in the 1990s, the efficacy of ursodeoxycholic acid (UDCA) for suppressing the disease progression of PBC is well established. Although the mechanisms of action of UDCA are not clarified, the prognosis of PBC has improved since the introduction of UDCA, which decreases the need for LT.[8,9] Some patients with progressive PBC, however, still require LT despite adequate treatment with UDCA.[10] We hypothesized that there would be some different or

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stepwise trends in the pretransplant characteristics of recipients with PBC according to when LT was performed, presumably because UDCA might have been administrated to PBC patients at an earlier stage in more recent LT recipients.

The main scope of this retrospective, single-center study was to examine the above hypothesis by comparing the detailed characteristics of explanted livers at LT as well as the clinical characteristics among patients who underwent LT for PBC at our center.

METHODS

Subject

USING A PROSPECTIVELY collected database in our institution, we retrospectively reviewed all consecutive patients who underwent LT between January 1996 and March 2013. Among them, we included patients who received LT for end-stage PBC, and they were divided into three groups according to when the LT was performed: group 1 (1997–2001), group 2 (2002–2005) and group 3 (2006–2012). Patients with overlapping non-immune-related liver disease such as viral or alcoholic hepatitis were excluded.

Retrospective review of the records of all LT recipients at the University of Tokyo was approved by the University of Tokyo Institutional Review Board. Often, patients were referred from other hospitals or clinics. The treatment strategy for PBC prior to LT, such as the use of UDCA or bezafibrate, was continued after referral to our center unless unnecessary. These treatment strategies were recorded as pre-LT therapy.

All candidates for LT in our center received upper gastrointestinal endoscopy to evaluate the presence and/or significance of esophagogastric varices immediately prior to living donor LT (LDLT), or before or after being listed as a deceased donor LT (DDLT) candidate. The presence of ascites and hepatocellular carcinoma (HCC) was evaluated by contrast enhanced computed tomography or magnetic resonance imaging immediately (within 1 month) pre-LT. The Model for End-Stage Liver Disease (MELD) score,[11] updated Mayo risk score for PBC[12] and M2 antimitochondrial antibody titer were also recorded from the laboratory data pretransplantation.

Histopathological evaluation of explanted liver

The size of the explanted liver was evaluated by calculating the ratio of the explanted liver volume (ELV) to standard liver volume (SLV) (ELV/SLV). The calculation formula for SLV (mL) was $706.2 \times$ body surface area (m^2) + 2.4.[13] Explanted liver specimens were reviewed by one of the experienced pathologists at our center and scored according to Scheuer's classification (stage 1–4),[14] and explants in stage 1–3 were labeled as "non-cirrhotic". Diagnostic criteria of PBC–autoimmune hepatitis (AIH) overlap syndrome was based on the clinical guideline published by the European Association for the Study of the Liver,[15] namely, at least two of the three following criteria for PBC and AIH, respectively, should be present: PBC ([i] alkaline phosphatase, >2 upper limit of normal [ULN] or γ-glutamyltransferase, >5 ULN; [ii] antimitochondrial antibody, >1:40; and [iii] liver biopsy specimen showing florid bile duct lesions) and AIH ([i] alanine aminotransferase, >5 ULN; [ii] immunoglobulin G, >2 ULN or a positive test for anti-smooth muscle antibodies; and [iii] liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis). Other findings, such as the presence of HCC, were also noted.

Statistical analysis

We used SPSS version 17.0 statistical software (SPSS, Chicago, IL, USA) to analyze the relevant data. Differences between groups were analyzed by the Mann–Whitney *U*-test or Kruskal–Wallis test for continuous variables and the χ^2-test for categorical variables. $P < 0.05$ was considered significant.

RESULTS

Patient characteristics

OF ALL THE 421 adult LT recipients from January 1996 to December 2012 at our center, we included 86 recipients with PBC in the present study. One patient who had overlapping HCV infection was excluded. The remaining 85 patients included in the present study were divided into three groups according to when the LT was performed, as noted above: group 1 (1997–2001, $n = 29$), group 2 (2002–2005, $n = 29$) and group 3 (2006–2012, $n = 27$). Three patients in group 3 underwent DDLT, whereas the remaining 82 patients underwent LDLT. Four patients had overlapping AIH (one in group 1, one in group 2, two in group 3, $P = 0.20$).

The details of the characteristics of all three groups are shown in Table 1; older cases were complicated with esophageal varices less frequently (70% in group 1 vs greater than 90% in the other two groups, $P = 0.019$),

Table 1 Patient characteristics at LT ($n = 85$)

	Group 1 ($n = 29$)	Group 2 ($n = 29$)	Group 3 ($n = 27$)	*P*-value
Age (years)	53 (35–64)	50 (37–66)	56 (32–64)	0.19
Female sex	23 (79)	26 (90)	26 (96)	0.14
MELD score	20 (9–32)	19 (11–39)	17 (9–36)	0.69
Updated Mayo risk score	9.3 (6.1–11.8)	9.3 (5.3–12.9)	9.5 (7.2–13.1)	0.21
Total bilirubin (mg/dL)	14 (2–30)	10 (2–39)	8 (2–36)	0.55
INR	1.3 (1.1–2.4)	1.5 (1.1–3.2)	1.2 (1.2–3.0)	0.27
Ascites	17 (59)	16 (55)	20 (74)	0.15
HE	5 (17)	4 (14)	5 (19)	0.84
HCC	2 (7)	0 (0)	1 (4)	0.34
Esophageal varices	20 (70)	26 (90)	26 (96)	0.019

Unless otherwise indicated, data are given as the number (%) or the median (range).
HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; INR, International Normalized Ratio; LT, liver transplantation; MELD, Model for End-Stage Liver Disease.

although no significant difference in other factors, including MELD score or updated Mayo risk score, which represent the severity of illness, was detected.

Treatment for PBC pre-LT

The duration of UDCA treatment until LT was significantly shorter in older cases (Fig. 1a). The dose of UDCA per bodyweight (BW) was not significantly different among the three groups (Fig. 1b), whereas the cumulative dose of UDCA per BW until LT was significantly higher in recent cases (Fig. 1c). The use of bezafibrate tended to be more frequent in recent cases, but did not reach statistical significance (19% in group 1, 26% in group 2 and 33% in group 3, $P = 0.6$).

Association among liver volume, histopathological stage and UDCA treatment by years

Based on calculations of ELV/SLV, there was a significant difference in the size of the explants between groups; significantly larger explants were extirpated in older cases (Fig. 2). The rate of non-cirrhotic explants was also significantly greater in older cases (35% in group 1, 7% in group 2 and 12% in group 3, $P = 0.03$).

Considering the association between volume and histopathological stage of the explanted liver, the volume of cirrhotic explants (median ELV/SLV, 1.04 [range, 0.45–2.43]) was significantly smaller than those of non-cirrhotic explants (median ELV/SLV, 1.50 [range, 0.70–2.03]) ($P = 0.01$).

The duration of UDCA treatment prior to LT correlated significantly with ELV/SLV ($r^2 = 0.151$, $P = 0.001$, Fig 3a), whereas the cumulative UDCA dose per BW until LT was less closely associated with ELV/SLV ($r^2 = 0.09$, $P = 0.008$, Fig. 3b).

The explants of the patients with overlapping AIH ($n = 4$) were all cirrhotic, whereas 18% of the patients without overlapping AIH ($n = 81$) had non-cirrhotic explants. There was no statistical difference in ELV/SLV, however, between patients with or without AIH (median, 1.09 [range, 0.66–1.30]; and median, 1.08 [range, 0.45–2.43], respectively, $P = 0.56$).

DISCUSSION

IN THIS RETROSPECTIVE study, we present our experience that the clinical manifestations of PBC at the time of transplantation have drastically changed over the years. UDCA was initiated at an earlier stage in patients who underwent LT in more recent years. In parallel with this transition, explanted livers have become smaller, more severely shrunken, with more fibrosis and accompanied more frequently by esophageal varices. Based on these findings, we propose the following hypothesis: (i) early introduction of UDCA therapy could prevent rapid development of cholestatic liver failure with hepatomegaly, which was the dominant clinical manifestation of LT recipients with PBC in the 1990s or earlier 2000s, that is, prior to the introduction of UDCA; and (ii) even with adequate treatment with UDCA with or without bezafibrate, however, some patients develop liver cirrhosis, which eventually leads to life-threatening portal hypertension and liver atrophy (cirrhosis) and the requirement for LT in later 2000s.

An observation that may support our notion is that, in the published work before the era in which UDCA treat-

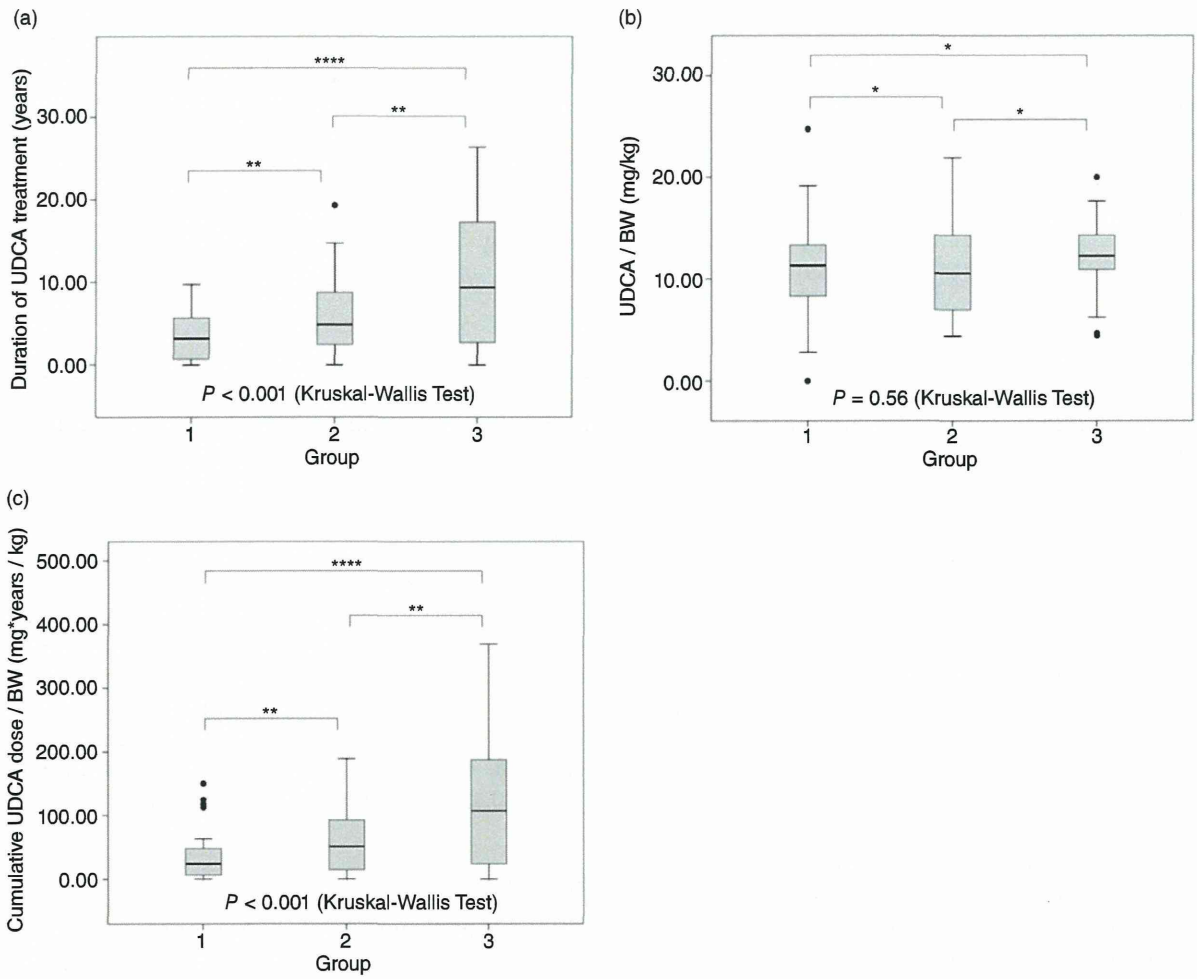

Figure 1 Box plots of (a) duration of ursodeoxycholic acid (UDCA) treatment (years), (b) UDCA dose per bodyweight (mg/kg) and (c) cumulative UDCA dose/bodyweight (mg × years/kg), for each group. The top and bottom of the boxes represent the first and third quartiles, respectively. The boxes enclose the interquartile range, with the median value denoted by the horizontal line (*P = n.s., **$P < 0.05$, ***$P < 0.01$, ****$P < 0.001$).

ment became a common practice, hepatomegaly (as well as portal fibrosis and cirrhosis) in PBC patients was accepted as a factor predicting a worse prognosis.[16,17] Hepatomegaly, however, is seldom mentioned as a prognostic factor of PBC in more recent published work.[18,19] Our current study revealed a stronger correlation between ELV/SLV with the duration of UDCA treatment than with the cumulative UDCA treatment per BW. This may indicate that the introduction of UDCA at an earlier stage is indeed beneficial and an important factor for preventing rapid progression of the disease.

Our findings are also consistent with several recent studies indicating that the prognosis of PBC improves with the introduction of UDCA, especially in those with an early treatment response.[18,20–23] Further, interestingly, presumably due to the general acceptance of UDCA for the management of PBC after this evidence was reported, the need for LT for PBC has dramatically decreased,[8,9] although authors understand that the benefit of UDCA seems controversial as there are publications doubting the influence of UDCA on patients' survival or time to LT.[24–26] In addition, LT still remains a last resort for those developing end-stage liver disease due to PBC despite adequate treatment with UDCA,[27] and there are additional areas for further dedicated studies to identify such patients.[28]

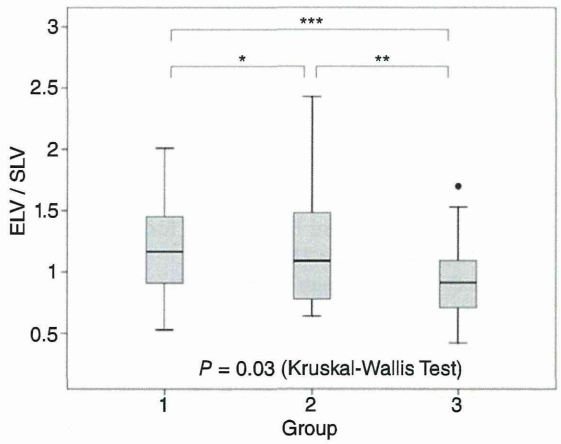

Figure 2 Box plots of the ratio of explanted liver volume to standard liver volume (ELV/SLV) for each group. The top and bottom of the boxes represent the first and third quartiles, respectively. The boxes enclose the interquartile range, with the median value denoted by the horizontal line (*P = n.s., **$P < 0.05$, ***$P < 0.01$).

Based on the histological findings of PBC, two major mechanisms are involved in the progression of PBC.[29] The first is bile duct destruction, which leads to chronic cholestasis and the development of cirrhosis with a biliary pattern. The second is interface hepatitis, which also leads to cirrhosis, the pattern of which resembles cirrhosis following patterns of chronic viral hepatitis. More recently, Nakamura *et al.* reported that the pattern of disease progression in PBC patients may be strongly associated with specific autoantibodies, such as anti-gp210 or anticentromere.[5,6] They proposed two different types of clinical manifestations of disease progression in PBC patients; one is a "hepatic failure type" progression, which is represented by positive anti-gp210 antibodies, and the other is a "portal hypertension type" progression, which is represented by positive anticentromere antibodies. As the group proposed that the "hepatic failure type" progression is characterized by rapid development of cholestatic liver failure,[30] this type could reach significant bilirubinemia and high International Normalized Ratio (i.e. high MELD score) enough to consider LT ahead of the establishment of cirrhosis, which may be correspondent with the characteristics of our older cases. In addition, several groups have proposed that some single nucleotide polymorphisms are related to the development of PBC.[28,31] Analyzing such genetic backgrounds in relation with the potential benefit of UDCA, as indicated in our study, may be beneficial.

In conclusion, recent LT patients were characterized by frequent portal hypertension, significant liver atrophy and fibrosis, and longer UDCA therapy prior to LT. According to these findings, we hypothesized that the early introduction of UDCA prevented comparatively rapid development of liver failure characterized by non-cirrhotic hepatomegaly and insignificant portal hypertension; however, it remains a challenge to identify and treat patients who will slowly develop liver

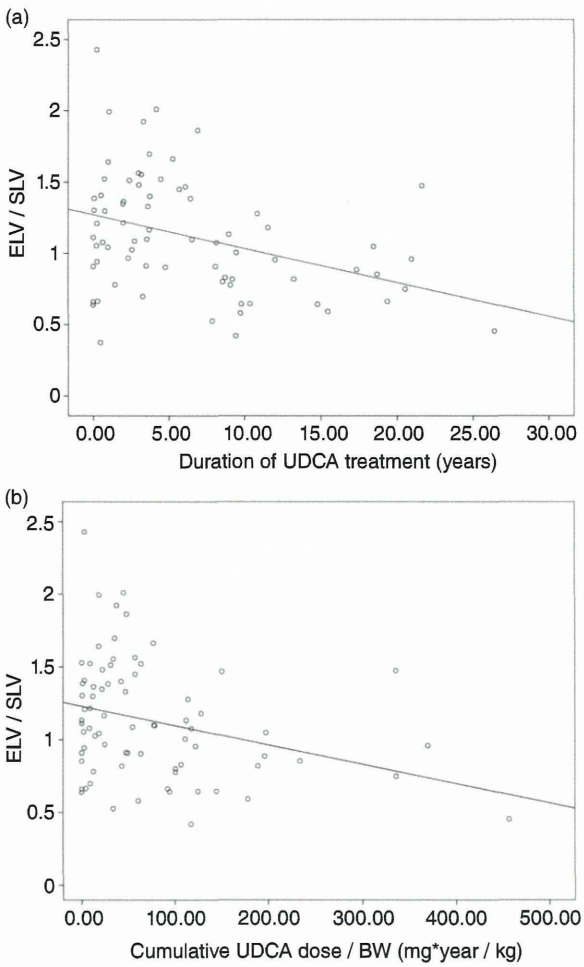

Figure 3 Relationship between the ratio of explanted liver volume to standard liver volume (ELV/SLV) and (a) duration of ursodeoxycholic acid (UDCA) treatment (years) and (b) cumulative UDCA dose/bodyweight (mg × years/kg). The duration of UDCA treatment correlated significantly with ELV/SLV ($r^2 = 0.151$, $P = 0.001$), whereas the cumulative UDCA dose until LT was less strongly correlated with ELV/SLV ($r^2 = 0.09$, $P = 0.008$).

failure requiring LT. Further studies are strongly warranted to examine our theory in a larger and prospective patient cohort, as well as the immunological or genetic corroborations.

ACKNOWLEDGMENTS

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Original Article

Living donor liver transplantation for non-alcoholic steatohepatitis: A single center experience

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Aim: The number of patients referred for liver transplantation (LT) with non-alcoholic steatohepatitis (NASH) continues to increase, but information about living donor liver transplantation (LDLT) for NASH is scarce. We conducted this study to document the details of LDLT for NASH in a Japanese LT center.

Methods: Among all LDLT recipients in our institution from March 1996 to March 2013 ($n = 425$), we identified seven patients that underwent LDLT for NASH.

Results: Of all the seven recipients, most of the patients (86%) were obese. The median follow-up period post-LDLT was 5.3 years. All were alive at the last follow-up. Recurrent NASH was detected in one patient (14%), and no recurrent hepatic steatosis was detected among the remaining six recipients on prospectively performed ultrasonography. No significant comorbidities were observed following donor surgery among the respective living donors during the follow-up period. We also retrospectively reviewed 22 patients with NASH-related end-stage liver disease (ESLD) who were evaluated but rejected for LDLT during the same period. The reasons for rejection for LDLT were presumably associated with the nature of NAFLD/NASH in either potential recipients or donors.

Conclusion: The post-transplant outcome of LDLT for NASH-related ESLD in our institution was feasible, although the sample size was small. Further studies in a larger patient cohort are warranted to investigate the long-term outcome of LDLT for NASH, both for recipients and living donors.

Key words: living donor liver transplantation, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease

INTRODUCTION

NON-ALCOHOLIC FATTY LIVER disease (NAFLD) has recently become one of the most common etiologies of liver disease worldwide, although it depends on the geographic area: Asian countries (16.9%) are estimated to have a lower prevalence than the Middle East (34.7%) or Europe/North America (23.2%).[1] The recent report by Kojima *et al.* supports the estimation that the proportion of NAFLD in the Japanese population is also increasing.[2]

Non-alcoholic steatohepatitis (NASH), a progressive form of NAFLD,[3] is a risk factor for the development of liver cirrhosis and/or hepatocellular carcinoma (HCC) that may require liver transplantation (LT).[4–7] The proportion of patients undergoing LT due to NASH-related liver disease has recently drastically increased in the United States, and the outcome of deceased donor liver transplantation (DDLT) for NASH is estimated to be comparable to that of other etiologies.[8,9]

Information regarding living donor liver transplantation (LDLT), however, remains limited. Several recent studies reported that genetic polymorphisms could be related to the establishment of NASH;[10–15] therefore, NASH is potentially heritable. Thus, the safety of liver

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resection in relative living donors of recipients with NASH and the long-term outcome of recipients receiving such grafts, in particular, require further clarification.

The present study was performed to describe the details of patients with NASH-related end stage liver disease (ESLD) who were evaluated for LDLT in our institution: we documented the characteristics and outcomes of patients transplanted for NASH-related ESLD, including both recipients and living donors, as well as the patients rejected for LDLT.

METHODS

Patients

USING A PROSPECTIVELY collected database in our institution, we retrospectively reviewed all consecutive patients who underwent LDLT from April 1996 to March 2013. The retrospective review of records of all liver transplant recipients at the University of Tokyo was approved by the University of Tokyo Institutional Review Board.

Pre-transplant diagnosis of NASH was based on the histopathologic findings pre-LT. In addition, extensive serologic testing and exclusion of significant alcohol consumption (>10 g/day) and hepatotoxic medications were performed to exclude non-NASH liver disease. Moreover, we reviewed all recipients that were diagnosed with cryptogenic cirrhosis (CC), among whom patients meeting the following definition were re-classified as having NASH-related cirrhosis and included in the present study: patients with explant histopathology compatible with NASH based on a retrospective and blind review by an experienced pathologist, in addition to obesity (defined as a body mass index [BMI] greater than 25 kg/m^2 according to the criteria of the Japan Society for the Study of Obesity[16]), diabetes mellitus (DM, defined as HbA1c $\geq$ 6.5 and/or medication for DM), hyperlipidemia (defined as serum triglycerides greater than 150 mg/dL, low-density lipoprotein cholesterol greater than 140 mg/dL, high-density lipoprotein cholesterol less than 40 mg/dL and/or medication for hyperlipidemia), and/or hypertension (defined as blood pressure greater than 130/85 mmHg and/or medication for hypertension). The histopathologic features of NASH include steatosis, and hepatocyte injury, such as ballooning, lobular inflammation, and/or pericellular/perisinusoidal fibrosis.[3] All patients were followed until April 2013. We also retrospectively reviewed all patients referred for ESLD due to NASH who were evaluated but rejected for LDLT in our pre-LT assessment clinic during the same period.

We instructed obese recipients to control their diet and increase physical activity pre- and post-transplant. Our selection criteria for living donors and surgical techniques for LDLT are described elsewhere.[17,18]

Laboratory tests

Serum alanine aminotransferase (ALT; in IU/L), alkaline phosphatase (ALP; IU/L), and creatinine (mg/dL) at the latest follow-up were evaluated. Estimated glomerular filtration rate (eGFR; mL/min per 1.73 m^2) was calculated using the Japanese 2009 version of the equation developed by the Modification of Diet in Renal Disease (MDRD) study group.[19]

Immunosuppression

As previously reported, the immunosuppression regimen post-LDLT comprised steroid induction with tacrolimus or cyclosporin A.[20] The doses of each drug were gradually tapered for 6 months after LDLT. Methylprednisolone was tapered from 3 mg/kg on the first postoperative day to 0.05 mg/kg at the sixth postoperative month, and a maintenance dose of 2 to 4 mg of methylprednisolone was continued in all patients, both NASH and non-NASH recipients.

Evaluation of graft injury

Protocol biopsy is not performed at our center. Liver biopsy is indicated for patients with elevated liver function test results, after excluding biliary tract complications and infection. Histopathologic assessment of recurrent NASH was documented based on the NASH Clinical Research Network (NASH CRN) scoring system.[21] Abdominal imaging such as by computed tomography, magnetic resonance imaging, or ultrasonography was performed at least annually post-LDLT. After inclusion of the current study population, all LDLT recipients for NASH prospectively underwent abdominal ultrasonography focusing mainly on hepatic steatosis, as well as transient elastography (TE) by Fibroscan (Echosens, Paris, France) at the last follow-up. The liver stiffness measurement (LSM) was considered valid only when at least eight acquisitions were successful with a success rate of at least 60% and the ratio of the interquartile range to the median value was larger than 30%. LSM operators were blinded to the clinical data.

Statistical analysis

We used the SPSS 17.0 statistical software (SPSS, Chicago, IL, USA) to analyze the relevant data.

Table 1 Characteristics and surgical factors of the seven recipients

Case	Sex	Age	Pre-transplant variables							
			Initial diagnosis	BMI	MELD	C-P Score	HCC	DM	HL	HTN	ICU stay
1	M	67	CC	33	14	8	Yes	No	No	No	No
2	M	56	CC	28	13	9	Yes	No	Yes	No	No
3	F	40	CC	23	27	10	No	Yes	No	No	Yes
4	F	55	CC	26	21	13	No	Yes	No	No	Yes
5	F	61	NASH	30	24	13	No	No	Yes	No	Yes
6	F	61	CC	27	19	12	No	No	No	No	No
7	F	62	CC	26	17	12	Yes	Yes	No	No	No

Case	Pre-transplant variables					
	Ascites	Splenomegaly	HE	EGV	Creatinine (mg/dL)	eGFR (mL/min per 1.73 m^2)
1	No	Yes	No	Yes	0.9	61
2	Yes	Post splenectomy	No	Yes	0.73	64
3	Yes	Yes	Grade 2	Yes	0.70	68
4	Yes	Yes	Grade 2	Yes	0.61	74.4
5	Yes	Yes	Grade 1	Yes	0.92	48.6
6	No	Yes	Grade 1	Yes	0.91	41.5
7	Yes	Yes	Grade 1	Yes	0.90	49

CC, cryptogenic cirrhosis; C-P, Child-Pugh; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; EGV, esophagogastric varices; HCC, hepatocellular carcinoma; HE, hepatic encephalopathy; HL, hyperlipidemia; HTN, hypertension; MELD, Model for End-Stage Liver Disease; NASH, non-alcoholic steatohepatitis.

Differences between groups were analyzed by the Mann–Whitney *U*-test for continuous variables and the χ^2 test for categorical variables. *P*-values <0.05 were considered significant.

RESULTS

Characteristics and surgical factors of the recipients

OF ALL 425 recipients transplanted from April 1996 to March 2013, we identified seven patients (1.6%) that underwent LDLT for NASH: one was diagnosed with NASH pre-transplant based on liver biopsy, which was performed 18 months prior to LDLT, and 6 of 18 who were considered to have CC at LT met the diagnostic criteria for NASH-related cirrhosis, as described in the Methods section (none of these six recipients had not received liver biopsy pre-LT). The histopathologic findings of all seven explants were compatible with NASH-related cirrhosis.

Of these seven patients, six were obese (BMI >25). Three patients required intensive care pre-transplant. HCC was detected pre-transplant (and confirmed in explants as well) in three patients; two within the Milan criteria and one within the Tokyo Criteria,[22] but beyond the Milan criteria. Other details of the seven patients are included in Table 1.

Recipients with NASH experienced significantly more total blood loss than non-NASH recipients: median 6040 (range, 3960–53 135) mL vs 4950 (range, 630–81 450) mL ($P = 0.02$). Nevertheless, recipients with NASH experienced the similar warm ischemic time (median 60 [range, 32–85] min), cold ischemic time (median 107 [range, 76–203] min), surgical duration (median 808 [range, 698–1245] min), or total hospital stay post-LT (median 45 [range, 23–98] days), compared to non-NASH recipients (*P*-values are 0.47, 0.36, 0.78 and 0.90, respectively).

Post-transplant outcomes

The median follow-up period for the seven patients was 5.3 (1.3–11.0) years. Acute cellular rejection was observed only in case #1 at 47 days post-transplant, and was treated successfully with increased immunosuppression and steroid recycling. None of the HCCs detected pre-transplant recurred.

The detailed characteristics of the seven patients at the end of the follow-up are summarized in Table 2; all

Table 2 Last follow-up post-living donor liver transplantation (LDLT) of seven patients

Case	BMI	DM	HL	HTN	Creatinine (mg/dL)	eGFR (mL/min/1.73 m^2)	ALT (IU/L)	ALP (IU/L)
1	28	No	No	Yes	0.90	62.3	13	144
2	23	Yes	No	Yes	1.42	40.0	23	69
3	17	No	No	No	1.02	46.4	8	18
4	23	Yes	Yes	Yes	0.73	62.5	65	226
5	24	No	Yes	Yes	1.24	34.2	25	512
6	28	No	No	Yes	0.99	44.1	13	190
7	24	Yes	No	Yes	0.77	58.1	26	291

IS	LKC in ultrasound	Fibroscan (kPa)	Recurrent NASH by liver biopsy	Outcome/follow-up period (years)
FK + MMF	no	6.9	NA	Alive/11.0
CyA + CS	no	2.6	NA	Alive/9.8
CyA + CS	no	6.1	NA	Alive/6.5
FK + CS	yes	5.2	NA	Alive/5.3
FK + MMF + CS	no	4.4	Yes	Alive/4.3
CyA + MMF + CS	no	3.1	NA	Alive/2.5
FK + MMF	no	8.8	NA	Alive/1.3

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HL, hyperlipidemia; HTN, hypertension; IS, immunosuppression; LKC, liver-kidney contrast; NA, not available; NASH, non-alcoholic steatohepatitis.

seven were alive. Median BMI values of the seven recipients at the last follow-up were 23.7 (range, 17.3–28.5) which showed median increase rate of 12.5 (range, 3.8–28.1)% compared to their minimum BMI values since LDLT (median 22.2 [range, 15.6–25.2]). Of the seven patients, three (43%) had DM, two (29%) had hyperlipidemia, and six (86%) had hypertension. Corticosteroids were gradually tapered and maintained in all cases; in most cases, 2 mg or 4 mg of methylprednisolone was administrated orally at the last visit. Median serum creatinine and eGFR levels were 0.99 (range, 0.73–1.42) mg/dL and 46.4 (34.2–62.5) mL/min per 1.73 m^2, respectively, except in case #1 who underwent living donor kidney transplantation for calcineurin inhibitor toxicity 6.7 years post-LDLT. The renal function in case #1 at the last follow-up was preserved (creatinine: 0.9 mg/dL, eGFR62.3 mL/min per 1.73 m^2).

Recurrent NASH post-LDLT

Except for one patient (case #4), none showed liver-kidney (LK) contrast in the prospectively performed ultrasonography; median LSM of those six patients by TE was 4.4 (range, 2.6–8.8) kPa. Ultrasonography performed in case #4 5.3 years after LDLT showed an LK contrast with an LSM of 5.2 kPa by TE, and this patient has had a consistently abnormal ALT (above 36 IU/L in our institution) for approximately 3 years post-LDLT except during the peri-operative period. This recipient subsequently underwent a liver biopsy, which revealed recurrent NASH with macrovesicular hepatic steatosis (40%), Mallory's hyaline, ballooning degeneration, predominantly neutrophilic inflammation, and perisinusoidal fibrosis. Based on the NASH CRN scoring system, the NAFLD activity score was 5 (steatosis = 2, lobular inflammation = 1 and hepatocellular ballooning = 2) and fibrosis stage was 1B. This patient developed DM and hypertension soon after LDLT was performed and hyperlipidemia 38 months post-LDLT, although she was never obese (BMI >25) since immediately after the LDLT. The clinical course of case #4 is shown in Figure 1.

Donor characteristics

The details of the seven respective donors are shown in Table 3. None of them were obese at the time of live donation. Three patients underwent liver biopsy before the donation according to our criteria of liver biopsy for potential living donors,[23] and none of them showed significant steatosis or any other liver injury/fibrosis. All seven donors tolerated and recovered from the liver resection without significant comorbidities. After a median follow-up period of 1.8 (range, 0.3–9.9) years,

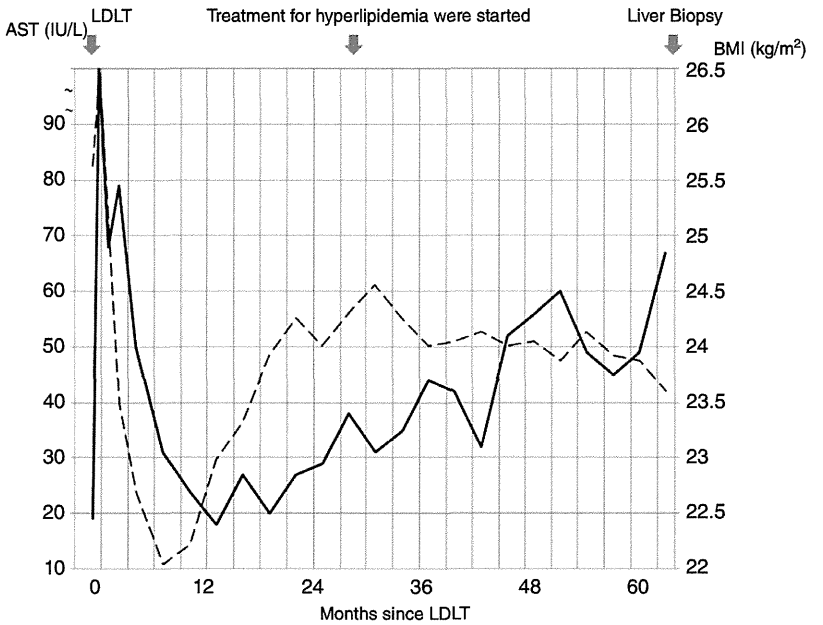

Figure 1 Clinical course of a recipient who experienced recurrent nonalcoholic steatohepatitis (NASH) (case #4). This patient never had a body mass index (BMI) >25 since immediately after the living donor liver transplantation (LDLT), although she developed diabetes mellitus and hypertension soon post-LDLT and hyperlipidemia 38 months after LDLT. Serum aspartate aminotransferase (AST) was consistently above the normal range (36 IU/L in our institution) for approximately 3 years post-LDLT except during the perioperative period. A liver biopsy was performed 5.4 years post-LDLT, which revealed recurrent NASH (NAS = 5, fibrosis stage = 1b). —, AST (IU/L); – –, BMI (kg/m^2).

two donors (cases #2 and 3) developed hyperlipidemia that has been successfully managed by diet control and increased physical activity, but none of the others suffered significant health problems.

Patients evaluated but rejected for LDLT

In total, 22 patients with ESLD due to NASH were evaluated but rejected for LDLT from April 1996 to March 2013. The characteristics of those 22 patients are summarized in Table 4. Mean BMI was 30 (range, 24–44). Of these 22, 11 (48%) had DM, three (13%) had hyperlipidemia, and five (22%) had hypertension. The reasons for rejection included donor issues ($n = 12$: four were obese with significant LK contrast in the ultrasonography, one was under treatment for DM, two were beyond the volumetric criteria of our institution, five withdrew their willingness to donate, recipient issues ($n = 7$: two died during evaluation; two were older than 65 years, which is beyond the criteria of our institution; one had an extrahepatic malignancy; one had HCC beyond the Tokyo criteria; one had too early stage of liver disease to transplant); and others ($n = 3$, due to cancellation of the evaluation clinic and lost to follow-up). Of the 22 patients rejected for LDLT, four patients were listed for DDLT, but died while awaiting transplantation.

Table 3 Characteristics of each of the seven donors

Case	Age	Sex	BMI	Donor relation	Number of HLA mismatch†	Preoperative liver biopsy
Case 1	34	Male	24	Son	3	NA
Case 2	54	Female	22	Spouse	4	NA
Case 3	36	Male	24	Spouse	4	Steatosis: 2–3%
Case 4	58	Male	21	Spouse	3	NA
Case 5	36	Female	18	Daughter	3	NA
Case 6	28	Male	22	Son	2	Steatosis: <1%
Case 7	64	Male	23	Spouse	2	Steatosis: <1%

†HLA-A, -B and -DR loci were used to calculate total mismatch score of 0–6.
BMI, body mass index; HLA, human leukocyte antigen; LDL, living-donor liver transplantation; NA, not available.

Table 4 Characteristics of the patients with NASH referred but rejected for liver transplantation (LT) evaluation ($n = 22$)

		n (%)
Age (median)		57 (32–70)
Male Sex		13 (56)
BMI (median)		30 (24–44)
MELD (median) score		17 (9–23)
Child–Pugh score		11 (8–13)
HCC		2 (9)
DM		11 (48)
HL		3 (13)
HTN		5 (22)
Reason for rejection	Donor issue	12 (55)
	Recipient issue	7 (32)
	Other reasons	3 (14)

BMI, body mass index; DM, diabetes mellitus; HCC, hepatocellular carcinoma; HL, hyperlipidemia; HTN, hypertension; MELD, Model for End-Stage Liver Disease.

DISCUSSION

SEVEN PATIENTS UNDERWENT LDLT for ESLD related to NASH in our institution from April 1996 to March 2013. The detailed characteristics of these seven recipients reveal an excellent survival rate; 100% during the median follow-up period of 5.3 years. None of the seven recipients showed liver dysfunction at the end of the follow-up, although one patient developed recurrent NASH with mild fibrosis.

Non-alcoholic steatohepatitis as an indication for LT is dramatically increasing in parallel with the increasing proportion of obesity and metabolic syndrome worldwide,[8,24] consistent with the theory that central obesity and insulin resistance are the most important risk factors for NASH or NAFLD.[25] The outcome post-LT has been regarded to be poorer than other indications due to technical difficulties of the transplant surgery itself or the higher rate of postoperative complications.[26] Recent large retrospective studies, however, indicated that post-transplant survival of recipients with NASH is comparable to that of patients with all other liver diseases.[8,9]

The number of patients with NAFLD/NASH in Japan has increased over the last 20 to 30 years.[2,27] A similar trend was observed in our patient cohort; no LT was performed on patients with NASH among 27 LDLT recipients before 2000. Patients with NASH accounted for two (0.7%) of 285 LDLT recipients between 2000 and 2006, and five (4.5%) among 111 LDLT recipients from 2007 to April 2013.

The reported rate of recurrent NASH following LT (mostly DDLT) ranges from 8% to 33%.[9,28–32] Of these studies, Malik *et al.* reported that 13 patients received LDLT from 1997 to 2008 in their institution, although they did not document the specific outcome focusing on LDLT.[31] Here we showed that recurrent NASH following LDLT was observed in one recipient (14%) proven by liver biopsy. As none of the other recipients showed any signs of recurrent NASH/NAFLD, such as abnormal liver function tests or LK contrast in the ultrasonography, we have not performed further investigations, especially liver biopsy, which might be harmful considering the risk-benefit ratio.[33] The diagnostic criteria for recurrent NASH are uncertain, and vary among previously published reports. Indeed, Agopian *et al.*[34] defined recurrent NASH based on histopathologic as well as radiologic findings.[9] TE was recently introduced to the LT setting, predominantly to assess recurrent hepatitis C post-transplant. Rigamonti *et al.*[35] showed that TE adequately identified the presence or absence of liver injury by nonviral graft diseases, including steatohepatitis. Using receiver operating characteristic curve analysis, they identified two cutoffs of LSM for the diagnosis of graft damage: 5.3 kPa with 100% sensitivity and 7.4 kPa with 100% specificity. In the present study, of all seven recipients, six patients, including case #4 with biopsy-proven recurrent NASH, had LSM < 7.4. The LSM in four of those six was <5.3. On the other hand, TE in case #7 showed a somewhat higher LSM (8.8 kPa), but we found no other signs of graft injury, such as abnormal serum ALT/ALP or LK contrast on ultrasonography, which supports our current view not to consider liver biopsy in this patient. We do, however, realize the potential importance of paying continuous attention to the clinical course of such a patient/graft, as well as the transition of LSM, which might reflect the progression of graft injury.

Bhagat *et al.*[30] reported that recipients receiving LT for NASH did not develop more de-novo DM but did develop more de-novo hypertension than recipients undergoing LT for alcoholic liver disease (16% vs. 29% [$P = 0.22$], 35% vs. 61% [$P = 0.04$], respectively). Likewise, in our present study, de-novo DM post-transplant was detected only in one patient and was not insulin-dependent, under a maintenance dose of 2 to 4 mg of methylprednisolone used in all recipients. On the other hand, de-novo hypertension occurred in six of seven recipients in our patient cohort. One patient (case #1) underwent living donor kidney transplantation after LDLT, and five of the remaining six recipients showed impaired renal function with an eGFR <60 mL/min per 1.73 m^2 following LDLT, indicating that 86% (6/7) recipients developed chronic kidney disease with

stage ≥ 3,[19] although three patients had eGFR < 60 mL/min per 1.73 m^2 at LDLT. Renal dysfunction in these cases might be associated with NASH, but it is always a challenge to determine the exact cause of renal dysfunction post-transplant, due to several factors such as the general use of calcineurin inhibitors. It is also important to keep an eye on obesity post-LDLT as all the seven recipients in our patient cohort have gained weight since LDLT, although five (71%) of the seven recipients were not obese (BMI > 25) at the last follow-up.

Several recent reports suggest that genetic polymorphisms are related to the development of NASH.[10-14] Kawaguchi *et al.* reported in 2012 that the progression of NASH in the Japanese population was strongly associated with the genetic polymorphisms of the human PNPLA3 gene.[15] According to those fascinating findings, it could be hypothesized that living related donors would develop NASH in the future or that recurrent NASH post-LDLT from related donors would be higher than DDLT or LDLT from unrelated donors such as spouses. In the present study, however, the patient who developed recurrent NASH received the graft from her husband, although the number of the patients included is too small to lead to robust conclusions. This patient had a consistently abnormal ALT level for almost 3 years post-LDLT, but the elevation was somewhat marginal (below 60) and fluctuated such that we did not perform liver biopsy until it became higher (>60). Thus, the actual time point when the recurrent NASH occurred is unknown. We also did not strongly suspect this patient had recurrent NASH as her BMI was below 25. This fact suggests that recurrent NASH should be suspected when the ALT is consistently elevated, despite the absence of obesity, and liver biopsy should be considered.

In our institution, none of the seven donors included had obesity or marked hepatic steatosis at the donor surgeries, and the operations were performed safely without significant surgical or postoperative complications, although two donors developed hyperlipidemia during the follow-up period. In addition to a larger study to answer this important question, a prospective study to investigate the long-term outcome of living donors related to recipients undergoing LDLT for NASH is strongly required.

In our patient cohort, more than 70% of the potential recipients with NASH failed to undergo LDLT due to donor issues; of these, 40% due to obesity with significant LK contrast in the ultrasonography and 40% due to a graft size mismatch. It might have been difficult to find a suitable donor from the graft size perspective as most patients with NASH are significantly obese. Thirty-two percent (7/22) of the patients were rejected for LDLT due to recipient issues, especially too late or too sick to transplant, which might reflect the difficulty in diagnosing and screening for NASH at its earlier stages, partly because NASH was not, until recently, well recognized among physicians.

The present study is a summary of our experience of LDLT for NASH-related ESLD. In conclusion, the outcome of LDLT for NASH was excellent without patient or graft loss, although the sample size was small. Further studies are warranted to investigate the outcome of LDLT for NASH in a larger patient cohort. It is also important to follow up living donors who provide grafts to recipients with NASH to observe the occurrence of obesity, metabolic syndrome, and/or NAFLD prospectively.

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De Novo Malignancies After Adult-to-Adult Living-Donor Liver Transplantation With a Malignancy Surveillance Program: Comparison With a Japanese Population-Based Study

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Background. Organ transplant recipients have an increased incidence of malignancy. Race differences in a variety of malignancies are observed among the general population, but de novo malignancies after adult-to-adult living-donor liver transplantation (LDLT) have not been compared with those from a Japanese population-based study.
Methods. The subjects were 360 adult LDLT recipients who survived more than 1 year after transplantation. An annual medical checkup and screening examinations were performed as follows: abdominal computed tomography or magnetic resonance imaging, upper gastrointestinal endoscopy, and total colonoscopy and immunochemical fecal occult blood test every 1 to 2 years. Complete blood count, liver function tests, and several tumor markers were checked every 1 to 3 months after LDLT.
Results. Mean follow-up period was 7.5±3.4 years. During the follow-up period, 27 de novo malignancies were diagnosed in 26 recipients. Colorectal cancer was the most commonly detected malignancy. The overall mortality of the recipients with de novo malignancies was similar to the findings of the Japanese general population-based study (standardized mortality ratio=0.9). Overall, the incidence of cancer was significantly higher in transplant recipients than in the Japanese general population (standardized incidence ratio=1.8). The 5-year estimated survival rate of recipients with de novo malignancies was 81% and those of recipients without malignancies was 93% ($P<0.0001$).
Conclusions. Colorectal malignancies predominated in Japanese liver transplant recipients. Although de novo malignancies correlated with a poor prognosis, the standardized mortality ratio was 0.9 compared with that of subjects of a Japanese population-based study.

Keywords: Living donor, Liver transplantation, De novo malignancy, Japanese.

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Since the Shinshu Group reported the first successful adult-to-adult living-donor liver transplantation (LDLT) in 1993 (*1*), the number of LDLT procedures for adult patients has increased in eastern Asia. Adult-to-adult LDLT is now an established treatment option for end-stage liver disease and the number of long-term survivors after LDLT continues to increase. Transplant recipients, however, exhibit a high incidence of malignancy (*2, 3*). The incidence of de novo malignancies in liver transplant recipients is approximately 10% at 10 years (*4*), and the risk of de novo malignancies is threefold to sevenfold higher than that in the normal population (*5*). Race differences in a variety of malignancies are observed among the general population (*6*), but de novo malignancies after adult-to-adult LDLT compared with a Japanese population-based study (*7*) have not been investigated. Additionally, malignancy surveillance programs after liver transplantation and the prognosis after a de novo malignancy diagnosis have not been fully examined.

In the present study, we aimed to describe de novo malignancies after adult-to-adult LDLT and compare their mortality and incidence with the findings of a Japanese population-based malignancy study.

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