

B. 研究方法

1) 平成12年9月から、熊本大学小児外科・移植外科で生体肝移植を受けたHCV肝硬変患者について、移植後HCV肝炎の再発の実態をウイルス学的及び病理組織学的に検討するとともに、再発症例に対する肝炎治療の成績を分析した。

2) HCV関連肝硬変患者の生体肝移植後肝炎再発防止を目指した新しい免疫抑制療法の開発として、従来タクロリムスとステロイド剤による免疫抑制療法を行う群(A群)と、ステロイド剤を一切使用せずミコフェノール酸モフェチル(MMF)とタクロリムスを使う新しい免疫抑制療法を行う群(B群)の2群における前向き無作為比較試験を開始した。この臨床試験に関する倫理面への配慮については、本学の倫理委員会の審議を経てその指針を受けている。

C. 研究結果

1) 当科にてこれまでC型肝炎、肝硬変にて肝移植を受けた症例は35例であるが、術前血中HCVRNAが陰性の4例を除いた31例中全例において、移植後HCVRNA量は移植前値を大きく越える上昇を認めた。また、6例が肝炎再発によると思われる急激なビリルビンの上昇を来した。2例は肝炎に対する治療を行う機会を逸し、死亡した。もう1例はインターフェロンなどの肝炎治療を行ったが、その進行を止めることが出来ず肝不全にて失った。残りの3例はインターフェロン+リバビ

リン療法に反応し、救命できた。これらは、HCV肝炎再発による、CH(cholestatic hepatitis)ともいふべき病態であろうと考えられる。

移植後生存例28例のうち肝炎再発に対してインターフェロン+リバビリン療法を施行した症例は現在まで14例あり、そのうちHCVRNAが陰性化した症例が7例あるが、癌再発を来した1例を除き6例は今もインターフェロン療法を継続しており、いずれもSVR(sustained viral response)を確認するまでには至っていない。

2) 平成16年9月から本無作為比較試験に参加しており、今年度、新たに登録したのはステロイド使用群が1例、ステロイド非使用MMF投与群が1例であった。残念ながら、1例は免疫抑制剤の変更で脱落し、もう1例のMMF投与症例は難治性の下痢のため、MMFを中止せざるをえなかった。以前からの登録症例も含め、現在再発の有無に対するステロイドの影響について検討中である。

D. 考察

本年度も移植後FCHともいふべき、肝炎再発に起因すると思われる急激なビリルビンの上昇を来した症例を2例認め、治療に難渋したことから、HCV肝炎に対する肝移植後には、早期より再発に対しての厳密な監視が必要であると考えられた。移植後の抗ウイルス療法の開始については、組織学的所見のみならず、RNA量やト

ランスアミナーゼ値の変動を考慮して行ったが、治療開始前の肝の病理組織確認を行うことを前提として、全例に早期から予防的に投与する方法はとらなかった。一方、免疫抑制療法下における抗ウイルス療法の効果はそれほど良好とは言えず、インターフェロン+リバビリン療法を施行した生存14例中、HCVRNAが陰性化したのは7例(50%)であり、これまでSVRを確認できた症例はまだない、汎血球減少症などのために治療を後退、中断せざるを得ない症例もあった。残念ながら、今までのところ、本臨床試験への参加症例は6例と少なく、脱落症例もあり、その効果を判定するには至っていないが、京都大学や他施設での症例を加えた分析結果に期待するものである。

E. 結論

多施設共同の無作為比較試験として立ち上げられた本研究に本年度も参加した。肝移植後のC型肝炎の再発の克服は依然として大きな課題であった。

G. 研究発表

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ORIGINAL ARTICLE

Effect of tacrolimus and partial hepatectomy on transthyretin metabolism in ratsManuel E. Zeledon R.,¹ Yukio Ando,² Katsuhiko Asonuma,¹ Masaaki Nakamura,² Xuguo Sun,² Mitsuharu Ueda,² Junko Fujii³ and Yukihiko Inomata¹¹ Department of Pediatric Surgery and Transplantation, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan² Department of Diagnostic Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan³ Department of Pharmacy, Kumamoto University, Kumamoto, Japan**Keywords**

familial amyloidotic polyneuropathy, hepatectomy, mRNA, tacrolimus, transthyretin.

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Summary

Liver transplantation, which serves as treatment of familial amyloidotic polyneuropathy (FAP), and domino liver transplantation, which utilizes resected livers from patients with FAP for treatment of liver diseases, may induce changes in transthyretin (TTR), a pathogenic FAP-related protein. To evaluate this possibility, we performed a 70% hepatectomy or administered tacrolimus to Dark Agouti (DA) rats for 7 days and then measured changes in liver TTR mRNA levels and changes in serum TTR concentrations. After hepatectomy, TTR mRNA levels decreased by 77%; at day 3, they returned to preoperative levels. Except for slightly elevated serum TTR concentrations 12 h after operation, serum TTR levels remained unchanged. Thus, partial hepatectomy did not influence serum TTR concentrations. After tacrolimus administration, TTR mRNA declined by 56% 12 h after the experiment started; however, after day 3, a rebound phenomenon occurred until day 7. Tacrolimus may facilitate serum TTR degradation, although production of TTR in the liver also increased. This finding – that TTR, the source of FAP-inducing amyloid, did not increase after transplantation – may help post-transplantation treatment of patients who have FAP and other liver diseases.

Introduction

Various proteins, including transthyretin (TTR), have been identified as amyloidogenic proteins related to familial amyloidotic polyneuropathy (FAP); of these proteins, amyloidogenic transthyretin (ATTR) is the most common throughout the world [1–3]. TTR-related FAP is a hereditary amyloidosis in which mutated amyloidogenic proteins accumulate in organs and tissues such as peripheral nerves, heart, kidney, gastrointestinal tract, and ocular tissues [4].

Familial amyloidotic polyneuropathy can cause a myriad of symptoms and signs including polyneuropathy, cardiac and renal dysfunction, gastrointestinal abnormalities, and ocular disorders. Although certain new therapeutic options exist, treatment of FAP is still limited [5]. At present, because TTR is predominantly synthesized by the liver,

liver transplantation is the only established therapy capable of halting production of ATTR and symptoms of FAP [6].

The positive outcome of such transplantations has stimulated research and use of more complex procedures, such as sequential liver transplantation (or domino liver transplantation), in which a resected liver from a patient with FAP is transplanted into a patient with a severe liver disorder or cancer [7]. By the end of March 2005, approximately 908 liver transplantations for FAP and 355 domino liver transplantations have been performed worldwide (FAP World Transplant Register; retrieved from <http://www.fapwtr.org/index.htm>, June 2005). Also, because of the shortage of donor livers, living donor liver transplantation (LDLT), which utilizes partial liver grafts, has often been performed.

Because serum TTR has proved to be a reliable indicator of nutritional status [8,9], serum TTR concentrations

have served for monitoring patients with malnutrition or cancer. It is also well known that serum TTR concentrations decrease during inflammation and infection [10]. Nevertheless, TTR behavior in the circulation has not been thoroughly evaluated after liver transplantation in domino graft recipients as well as in FAP patients.

The behavior of TTR under various liver transplant-related situations, such as after hepatectomy and during administration of immunosuppressants such as tacrolimus, may aid understanding of TTR metabolism. With the increasing number of liver transplantations being performed for FAP patients, the growing number of sequential liver transplant recipients, and the increasing need for LDLT, the impact of tacrolimus use and liver regeneration on TTR concentrations and amyloid formation has become an important subject worthy of extensive study.

Therefore, to better understand TTR metabolism during and after liver transplantation, we examined the effect of tacrolimus administration or hepatectomy on TTR mRNA in the liver and on TTR serum concentrations in rats.

Materials and methods

Animals

Ninety adult male Dark Agouti (DA) rats, each weighing between 190 and 270 g, were purchased from the SLC Co. (Hamamatsu, Japan). The rats were kept in the Kumamoto University Animal Center and given free access to water and rodent chow. All experiments were performed according to the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985). Procedures were carried out with the animals under ether inhalation anesthesia.

Experimental procedure

Rats were divided into four groups. The rats in the first group ($n = 25$) underwent a 70% hepatectomy using the Higgins–Anderson technique [11]. Those in the second group ($n = 25$) were injected with tacrolimus at a dose of 2 mg/kg i.m. in the leg, with each dose being injected in the alternate leg. The third ($n = 15$) and fourth ($n = 20$) groups of rats were used as controls for the first and second groups. A sham operation was performed on the third group, and an i.m. injection of normal saline was used as a placebo in the fourth group. In the four groups, the rats used at the 0 time point were the same ($n = 5$).

Tacrolimus or normal saline was injected at 0.5, 1, 1.5, 2, 3, 4, 5, and 6 days after the initial dose. All rats were exsanguinated at five time points: 0.5, 1, 2, 3, and 7 days. After the rats were killed, blood and liver tissue samples

were obtained. Liver tissues were immediately frozen in liquid nitrogen and stored in a -80°C refrigerator.

Measurement of blood tacrolimus concentrations (trough levels) and serum analysis

Tacrolimus (Prograf[®]; Astelas, Osaka, Japan) was generously provided by the Astelas Co. Trough levels of whole blood tacrolimus concentrations were measured by means of the microparticulate enzyme immunoassay (MEIA) using the IMx analyzer from Abbott Laboratories (Abbott Park, IL, USA).

Serum samples from rats given tacrolimus (1.0 or 2.0 mg/kg) were analyzed at the Department of Laboratory Medicine at Kumamoto University Hospital. Serum samples from each time point were evaluated for aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and blood urea nitrogen (BUN). Serum TTR concentrations were measured by using the enzyme-linked immunosorbent assay (ELISA) technique as described previously [12] and an anti-rat-TTR immunoglobulin, previously prepared by Tajiri *et al.* [13].

Quantitative reverse transcription-polymerase chain reaction. (RT-PCR)

Total RNA was isolated from the liver of the rats by means of PURESRIPT RNA Isolation Kit (Gentra, Minneapolis, MN, USA) and TURBO DNase treatment and reagents (Ambion, Austin, TX, USA). External standards, consisting of serial dilutions of rat TTR cDNA (107, 105, and 103 copies), were constructed by using RT-PCR. To evaluate rat TTR mRNA copies, upstream and downstream primer sequences were 5'-TATTTGCG TCTGAAGCTGG-3' and 5'-CCTTCC-GTGAA-CTTCTCA TCT-3', respectively. The hybridization probe sequences were 5'-TGTGGCCGTGAAAGTGT-3'-Flu and LC Red 640-5'-CAAAAGGACTGCAGACGGAAGCTGGGAGCCGT TTGCCTCTGGG-3'-P. The primers, hybridization probes, and rat GAPDH external standards (4×10^4 , 4×10^3 , and 4×10^2 copies) for evaluating rat GAPDH mRNA copies were obtained from Nihon Gene Research Laboratories (Sendai, Japan). The reaction mixture consisted of 3.25 mM Mn(OAc)₂, 0.3 μM each primer, 0.2 μM hybridization probes, 7.5 μl of RNA LightCycler RNA Master Hybridization probes mixture (Roche Molecular Biochemicals, Tokyo, Japan), 10-ng cDNA samples or external standards, and MilliQ water up to a final volume of 20 μl . The crossing point values of these standards were used to generate an external standard curve to allow accurate quantification. The ratio of rat TTR mRNA copies to rat GAPDH mRNA copies was estimated.

Statistical analysis

Statistical evaluation was performed with the JMP statistical analysis software (SAS Institute, Cary, NC, USA) by means of the paired *t*-test. A *P*-value of <0.05 was considered to be statistically significant.

Results

Effect of hepatectomy on TTR metabolism

Serum samples from rats having had a 70% hepatectomy showed that TTR concentrations remained stable except for a small but significant elevation (*P* < 0.05) at 12 h after the procedure. Serum TTR concentrations did not fall below the initial value at any time point (Fig. 1a). However, the TTR mRNA level immediately decreased and was lowest – 23% of the initial value – at 1 day after the surgery (*P* < 0.05). After this point, the TTR mRNA level began to increase, reached the original value at day 3, and remained unchanged until day 7 (Fig. 1b). The sham operation caused significantly reduced serum TTR concentrations from day 1 until day 7 after the operation (*P* < 0.05) (Fig. 2).

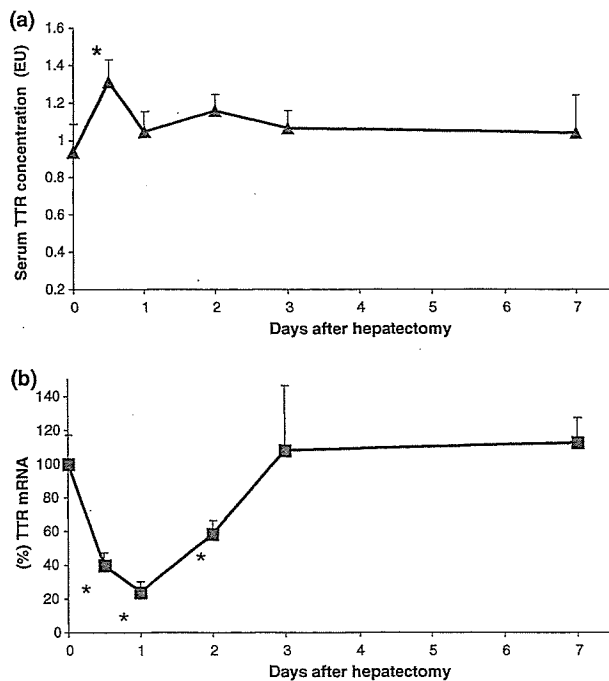


Figure 1 Effect of 70% hepatectomy on TTR metabolism. Dark Agouti (DA) rats (*n* = 30) underwent a 70% hepatectomy, and serum TTR concentrations (a) and TTR mRNA levels in the liver (*n* = 18) (b) were measured as described in the text. **P* < 0.05 for each time point versus the 0 time point.

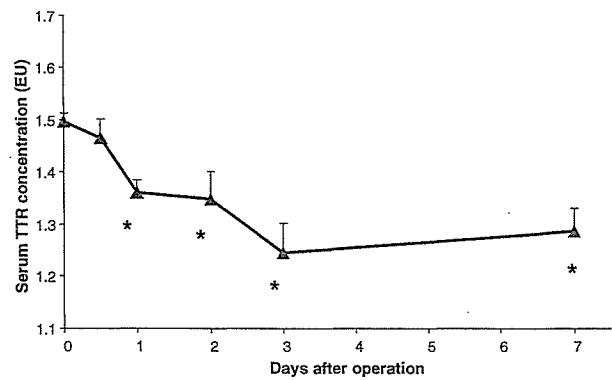


Figure 2 Effect of sham operation on serum transthyretin (TTR) concentrations. DA rats (*n* = 25) underwent a sham operation, after which the serum TTR concentration was measured by using ELISA. **P* < 0.05 for each time point versus the 0 time point.

Effect of tacrolimus administration on TTR metabolism

In a preliminary experiment, we administered different doses of tacrolimus (1.0 or 2.0 mg/kg) to rats. We first used 1 mg/kg, but trough levels (levels in whole blood) increased slowly. In another study, a 2 mg/kg dose had been used with only slight signs of toxicity [14]; therefore, we chose 2 mg/kg as a nontoxic high dose. Trough levels of tacrolimus after the dose of 2.0 mg/kg showed a rapid increase; at day 2, levels had reached >20 ng/ml, and they remained between 20 and 30 ng/ml until day 7 (Fig. 3).

Serum samples of rats that had received tacrolimus (2 mg/kg) revealed no significant elevation in serum AST and ALT values (Fig. 4a). Serum creatinine and BUN did show the increased levels after 7 days of treatment (Fig. 4b), as has been reported in previous experiments [14]; because of the short duration of the experiment, however, renal function did not deteriorate significantly.

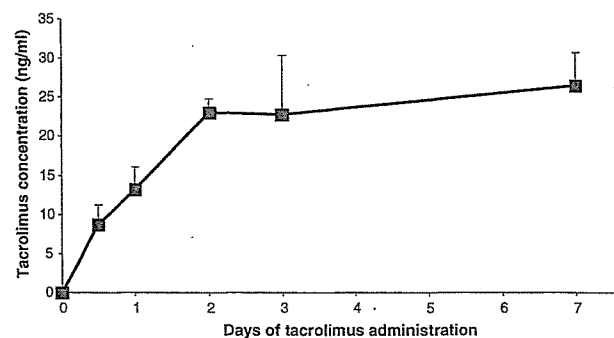


Figure 3 Trough levels of tacrolimus after the administration of 2 mg/kg. DA rats (*n* = 30) were given a 2 mg/kg dose of tacrolimus. As described in the text, trough levels were measured by using the microparticulate enzyme immunoassay (MEIA) technique.

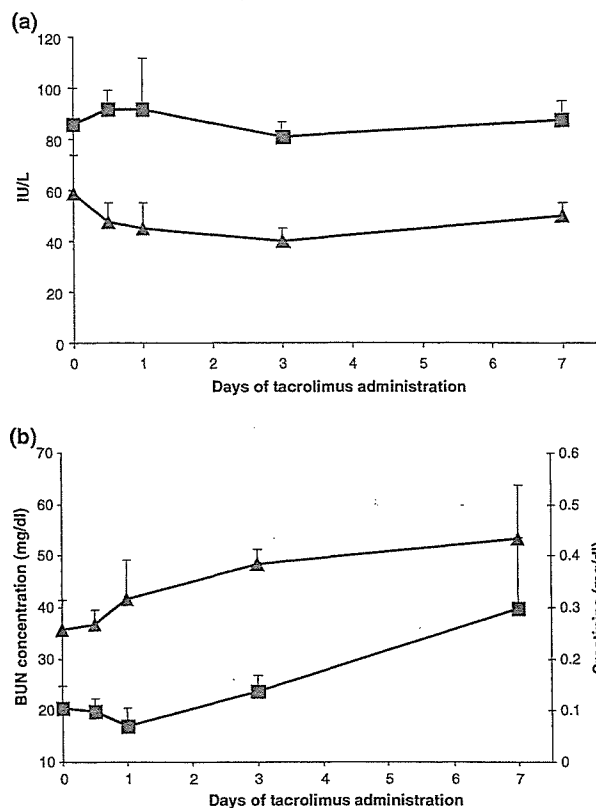


Figure 4 Aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine, and blood urea nitrogen (BUN) levels after tacrolimus administration. Serum samples from rats that had been treated with tacrolimus at 2 mg/kg ($n = 15$) were evaluated as described in the text. (a) AST (closed squares) and ALT (closed triangles). (b) Creatinine (closed triangles) and BUN (closed squares).

Serum TTR concentrations of rats treated with tacrolimus (2 mg/kg) were stable for 2 days (Fig. 5a), when trough levels were <20–25 ng/ml. However, on day 3, serum TTR concentrations decreased slightly but significantly and remained at a somewhat similar level until day 7. The TTR mRNA value decreased immediately after the first tacrolimus administration to 40% of the initial level and remained low until day 2 (Fig. 5b). Between days 2 and day 3, TTR mRNA levels increased quickly to 170% and remained unchanged until day 7, although this change was not statistically significant. Samples from rats that had received saline injections showed no variation in serum TTR concentrations over time; TTR mRNA levels also showed no changes and remained stable until day 7 (data not shown).

Discussion

Liver transplantation for management of FAP and sequential liver transplantation using a resected liver

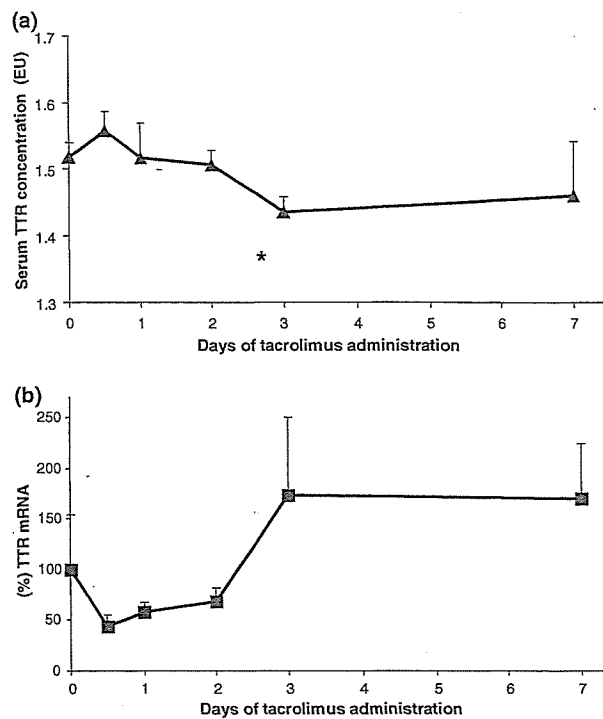


Figure 5 Effect of tacrolimus administration on TTR metabolism. After tacrolimus administration to DA rats ($n = 30$), serum TTR concentrations (a) and TTR mRNA levels in the liver ($n = 18$) (b) were measured as described in the text. * $P < 0.05$ for each time point versus the 0 time point.

from FAP patients are frequently performed. Nevertheless, these methods have resulted in several problems: for example, clinical manifestations of FAP, which may ultimately be related to the amyloidogenic protein TTR, have progressed in several patients even after liver transplantation [15,16]. Also, in one case, a recipient of an FAP liver started to manifest symptoms of FAP 8 years after the surgery [17]. Moreover, partial liver transplants obtained from living donors have often been used because of the shortage of donor livers. Thus, the effects of surgical procedures and immunosuppressants on TTR metabolism should be elucidated. In this report, we describe changes in TTR metabolism in rats after hepatectomy and after administration of the immunosuppressant tacrolimus, as evaluated by measuring TTR mRNA levels in the liver and serum TTR concentrations.

After a 70% hepatectomy, TTR mRNA levels decreased significantly for 2 days even though they returned to pre-operative values at day 3 after the surgery. Studies on liver regeneration have found that in rats, after 70% hepatectomy, the liver regenerates quickly and attains its original size by 7–10 days [18]; nevertheless, during the

initial days, the liver mass is clearly reduced. Therefore, the net TTR production was significantly decreased, and as a result, serum TTR concentrations should have decreased. However, serum TTR concentrations did not decrease as expected. It should be noted that the liver is one of the major catabolic sites [19], as well as the major production site, of TTR. A comparably higher reduction in TTR catabolism by the liver may be the reason for the higher-than-expected serum TTR concentrations.

With regard to tacrolimus administration, a dose of 2 mg/kg caused an initial decrease in TTR mRNA levels in the liver, but only for 2 days after the first dose. Thereafter, levels increased to 170% of the original value at day 3 and remained elevated until day 7. At the same time, serum TTR concentrations decreased (significantly at day 3) and remained low until day 7.

Clinically, immunosuppressive therapy for liver transplantation mainly consists of tacrolimus or cyclosporine. Previous studies have found that after liver transplantation in FAP patients, serum TTR concentration may be modified. A study by Stangou *et al.* [20] found that the serum TTR levels of FAP patients receiving liver transplantation mostly decreased or remained constant in tacrolimus-treated patients. Other studies have also documented that FAP patients who have had transplants and recipients of FAP livers have shown lower serum wild-type TTR and mutated TTR levels than those in control subjects [21]. Serum TTR concentrations in rats after tacrolimus administration were quite consistent with those found in these patients. With regard to the TTR mRNA finding in the liver, protein mRNA levels are well known to be regulated by serum protein concentrations. We believe that the rebound phenomenon for the TTR mRNA finding, as shown in Fig. 5, may be the result of the reduced serum TTR concentrations.

The reason why serum TTR levels decreased after tacrolimus administration is not yet clear. Even though the main action of tacrolimus is by inhibition of interleukin 2, a powerful cytokine in the immune system response [22], tacrolimus also acts on interleukin 1-interleukin 6 pathways [23,24], which are important in the TTR production system. This phenomenon may be beneficial for recipients of an FAP liver because the level of TTR, the pathogenic protein of FAP, may decrease during tacrolimus administration. The first case of amyloid deposition documented in a patient 8 years after receiving an FAP liver was published recently [17]. In a personal communication (to Y. Ando), the authors reported that this patient was using tacrolimus as the main immunosuppressive therapy.

In summary, a large partial hepatectomy did not produce decreased or increased serum TTR concentrations. In contrast, tacrolimus administration did cause decreased

serum TTR concentrations. In recipients of FAP livers, the variant ATTR produced by the donor liver appears in the blood circulation, and this ATTR may cause symptomatic FAP in the future, as demonstrated in one case [17]. Therefore, the effect of tacrolimus on the TTR serum concentration may help delay the onset of FAP. Investigations with a longer-time frame are required to obtain more precise information on the behavior of TTR in these two transplant-related situations.

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Automated Hepatic Volumetry for Living Related Liver Transplantation At Multisection CT¹

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Purpose:

To prospectively compare in vivo hepatic automated volumetry with manual volumetry and measured liver volume.

Materials and Methods:

The study was conducted in accordance with the guidelines of the Institutional Review Board of Kumamoto University (Japan). Patient informed consent was obtained. Preoperative multisection computed tomography (CT) was performed in 35 consecutive patients (21 men, 14 women; mean age, 42.8 years; range, 28–72 years) with hepatic disease awaiting living related liver transplantation. The CT scans covered the entire liver at a section thickness of 2.5 mm. Liver volume was estimated by using both the automated and the manual methods. Actual liver weight was obtained for all patients and was converted to hepatic volume on the basis of a predetermined relationship between actual liver weight and volume. Processing time required for both methods was also recorded. Two-tailed paired *t* test, correlation coefficient, and Bland-Altman tests were used for statistical analyses.

Results:

Mean liver weight was $881.7 \text{ g} \pm 249.8$ (standard deviation), and mean measured liver volume was $956.00 \text{ cm}^3 \pm 280.10$. Volumetry performed with the automated and manual methods provided liver volumes of $982.99 \text{ cm}^3 \pm 301.98$ and $937.10 \text{ cm}^3 \pm 301.31$, respectively. There was good correlation between measured and estimated volumes obtained with the automated method ($r = 0.792$, $P < .01$). The manual and automated methods required $32.8 \text{ minutes} \pm 6.9$ and $4.4 \text{ minutes} \pm 1.9$, respectively.

Conclusion:

The automated method reduced the time required for volumetry of the liver and provided acceptable measurements.

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The size of the liver is considered to be an important prognostic factor in patients with cirrhosis (1) or fulminant hepatic failure (2,3), and imaging techniques have been used for obtaining quantitative measurements of liver volume. In patients scheduled for liver surgery for primary hepatic tumor (4,5), metastatic lesions (6), and transplantation (7), the liver volume must be known preoperatively. The liver volume is one of the most important factors in the selection of appropriate donors, especially in a patient undergoing living related liver transplantation (LRLT). Volumetry of the hepatic graft and remnant is mandatory for LRLT and is usually performed with cross-sectional computed tomography (CT) or magnetic resonance (MR) imaging. These methods yield reliable organ volume measurements when appropriate scanning protocols are used (7–9). Volumetry of the liver on CT images is usually performed by manual tracing of the liver boundary and summation of the liver area on each section. However, manual methods require considerable user involvement in the segmentation of the liver on each section, which is a time-consuming process.

To our knowledge, the reliability of in vivo automated volumetric measurements of the liver has not been compared with the actual volume of resected livers. We have developed a fully automated hepatic volumetric method by using multi-section CT. Thus, the purpose of our study was to prospectively compare the in vivo hepatic automated volumetry we developed with manual volumetry and measured liver volume.

Materials and Methods

Patient Population

This study was conducted in accordance with the guidelines of the Institutional Re-

Advances in Knowledge

- Automated CT volumetry of the liver yields acceptable measurements when compared with data obtained from the resected liver.
- The automated method is quicker than the manual method.

view Board of Kumamoto University. Prior written informed consent was obtained from all patients.

Between November 1999 and September 2004, 35 consecutive patients (mean age, 42.8 years; range, 28–72 years) who underwent LRLT at our facility were included in this study. There were 14 women (mean age, 39.6 years; range, 28–56 years) and 21 men (mean age, 48.8 years; range, 28–72 years). The underlying liver diseases were liver cirrhosis ($n = 21$), familial amyloidotic polyneuropathy ($n = 9$), and fulminant hepatic failure ($n = 5$). All patients underwent a preoperative three-phase enhanced abdominal CT study within 3 months before LRLT. All recipients' livers excised at the time of liver transplantation and drained of all blood were weighed directly after the gallbladder, portal structures, attachment ligaments, and other extraneous tissues had been dissected free.

CT Protocol

CT was performed with a four-section scanner (LightSpeed QXi; GE Medical Systems, Milwaukee, Wis) in 20 patients and a 16-section scanner (IDT16, Philips Medical Systems, Cleveland, Ohio) in the remaining 15 patients. For patients with liver cirrhosis and fulminant hepatic failure, we used a multisection CT protocol to acquire three image sets of the liver (arterial, hepatic parenchymal, and equilibrium phases). Patients with familial amyloidotic polyneuropathy underwent scanning in the arterial, portal venous, and hepatic parenchymal phases to obtain three-dimensional (3D) images of the hepatic artery and the portal and hepatic veins. Because a patient with familial amyloidotic polyneuropathy is usually considered a domino (second) donor for a second recipient (10), the additional portal venous images were obtained to generate a 3D image of the portal vein. The scans for these phases were acquired at 25–35 seconds (arterial), 45–65 seconds (portal venous), 80–100 seconds (hepatic parenchymal), and at more than 200 seconds (equilibrium) after the administration of 100 mL of nonionic contrast material iopamidol (Iopamiron 370; Nihon Schering, Osaka, Japan) at a rate

of 3.0 mL/sec through a 20-gauge catheter placed in an antecubital vein. Hepatic parenchymal phase data were used in this study.

With the four-section scanner, the scanning parameters for the portal venous phase were 120 kVp, 200–250 mAs, 0.8-second rotation time, four sections at 2.5-mm beam collimation, 15-mm table feed per rotation, 1.5 beam pitch, 36-cm field of view, and 512×512 -pixel matrix. With the 16-section scanner, the scanning parameters were 120 kVp, 200–300 mAs, 0.75-second rotation time, 16 sections at 2.5-mm beam collimation, 21.1-mm table feed per rotation, 0.659 beam pitch, 36-cm field of view, and 512×512 -pixel matrix. A section thickness of 5 mm (without overlaps) and 2.5 mm (with 1.25-mm overlaps) was used for manual and automated volumetry, respectively.

In Vitro Experiment

Prior to the in vitro study, a power analysis was performed to determine the study size. Because the sample size was calculated as 6.62 with the assumption of a significance level of .05, a power of .8, and a correlation coefficient of 0.9, we designed the sample size as 7.

To determine the relationship between liver weight and liver volume, we obtained the volume of the resected livers

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Abbreviations:

CI = confidence interval

LRLT = living related liver transplantation

3D = three-dimensional

Author contributions:

Guarantors of integrity of entire study, Y.N., Y.Y., K.D.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, Y.N., Q.L., Y.H., S. Kusunoki; clinical studies, Y.N., R.I., S. Kusunoki, H.O., Y.I.; experimental studies, Y.N., Q.L., S. Katsuragawa, R.I.; statistical analysis, Y.N., S. Katsuragawa; and manuscript editing, Y.N., Q.L., S. Katsuragawa, K.A., Y.Y., K.D.

S. Katsuragawa and K.D. are shareholders in R2 Technology, Los Altos, Calif.

from another seven transplant recipients (three men and four women; age range, 32–61 years; mean, 45.3 years). The underlying liver diseases of those recipients were liver cirrhosis ($n = 5$) and hepatocellular carcinoma ($n = 2$). One radiologist (S. Kusunoki) with 5 years of abdominal CT experience directly measured the actual volumes of the excised livers by means of water displacement in a water bath filled with distilled water at 25°C. Immediately after volume measurement, the excised livers were placed on a platform scale, and the exact liver weights were measured. The excised livers were drained of all blood before water displacement and weight measurements. Finally, a regression line was obtained for the relationship between the weight and the volume of the liver by using the measured data of the seven livers.

Manual Method of Liver Volumetry

For imaging prior to LRLT, one radiologist (Y.N.) with 10 years of abdominal CT experience manually traced the contours of all liver sections on a Digital Imaging and Communications in Medicine viewer (spatial resolution of 1600 × 1200 pixels, RadiForce R22; Nanao, Ishikawa, Japan) by using an electronic cursor and recorded the time required for these measurements. The manufacturer's software automatically calculated the number of pixels included within the traced contours on each section and provided the cross-sectional area of the liver on a section-by-section basis. The circumscribed areas were then multiplied by the CT section thickness, yielding an approximate volume for each liver section, and the volumes of all sections were summed to give the total volume of the liver.

Automated Method of Liver Volumetry

CAD technologies developed in the Kurt Rossmann Laboratories, Department of Radiology, the University of Chicago have been licensed to companies including R2 Technology, Deus Technologies, Riverain Medical Group, Mitsubishi Space Software Median Technologies, GE, and Toshiba.

A radiologic technologist (R.I.) with 10 years of CT experience recorded the time used for the measurement of liver

volume. The computerized method of liver volumetry used in this study was based on automated liver segmentation on multisection CT images. The overall scheme for automated volumetry is illustrated in Figure 1. There is no human input throughout the process. Our segmentation technique initially estimated the mean CT number of the liver by means of an analysis of CT numbers in a 3D volume of interest with a 32 × 32-pixel in-section matrix and an automatically determined height. The location of the in-section 32 × 32-pixel region was determined empirically at a location where the liver was likely to be included. The height of the 3D volume of interest was also automatically determined so that 32 × 32-pixel regions of the volume of interest were completely located inside liver. Next, the pixels in the range of the estimated mean CT number −30 and +30 HU were retained to form initial liver regions. A Sobel operator was then used for detecting the edge inside the initial liver regions to separate the liver from the other adjacent organs, such as the pancreas and spleen, that may have similar CT numbers as the liver. Many regions that included those organs were removed by means of feature analysis of CT numbers and locations. A 3D connected-component labeling technique was then used for selecting the 3D object that had the maximum volume among all objects, which further eliminated false liver regions. The selected 3D object was refined by using a restricted region-growing technique. Finally, the liver volume was automatically calculated by summation of the products of the section thickness and area

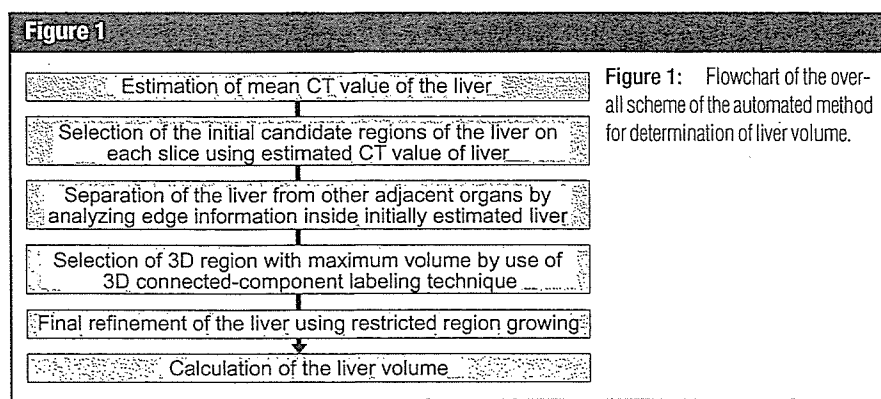
of the segmented liver in each section (Fig 2).

Determination of Measured Liver Volume

Determination of the measured liver volume was performed by a radiologist (Y.N.). The 35 patients underwent LRLT 2 days to 3 months (mean, 72.5 days; median, 75 days) after undergoing a preoperative CT examination. After complete resection, the entire liver was weighed and its volume was determined on the basis of the predetermined relationship between liver weight and volume. The entire liver volumes estimated by using the manual and automated methods were compared with the measured liver volume. The error (Er) between the estimated volume (V_e) and the measured volume (V_m) was defined as $Er = (V_e - V_m)/V_m$.

Statistical Analysis

A regression line between the estimated volume and the manual volume of the liver was generated and the correlation coefficient (r) was calculated. In addition, a two-tailed paired t test was used to compare the automated and manual methods in terms of the time required to estimate the liver volumes. The average errors for the two methods were determined. The 95% confidence interval (CI) of the error was calculated for both methods. Differences between the average errors of the manual and of the automated methods were evaluated with the two-tailed paired Student t test. In addition, Bland-Altman analysis was used to determine the agreement between these methods (11,12). All statistical analyses were performed with a



software program (MedCalc Software; MedCalc, Mariakerke, Belgium). A *P* value of less than .05 was considered to indicate a significant difference.

Results

In Vitro Experiment

The weight and volume of the resected livers were measured in vitro to predetermine the relationship between the weight and volume (Fig 3). The mean actual weight and volume were $660.0 \text{ g} \pm 145.6$ (\pm standard deviation) (range, 500–900 g) and $670.0 \text{ cm}^3 \pm 174.1$ (range, 480–970 cm^3), respectively. There was a statistically significant positive correlation ($r = 0.957$, $P < .01$) between actual liver volume and weight. The regression line was determined by a forced through the origin equation as $y = 1.06x$, where x and y indicate the measured weight and converted volume, respectively (Fig 3). This regression line was used for converting liver weight to liver volume. We used the converted volume as the reference standard. The power of analysis indicated greater than .99 in the sample size of seven, a significance level of .05, and a correlation coefficient of 0.957.

Surgical Results

After LRLT, the liver weight ranged from 450 to 1595 g (mean, $881.7 \text{ g} \pm 249.8$); it was $985.0 \text{ g} \pm 166.2$ (range, 680–1150 g) in nine patients with familial amyloidotic polyneuropathy, $919.5 \text{ g} \pm 242.8$ (range,

650–1595 g) in 21 patients with liver cirrhosis, and $550.0 \text{ g} \pm 77.4$ (range, 450–640 g) in five patients with fulminant hepatic failure. The measured volume converted from the actual weight ranged from 511.3 to 1690.7 cm^3 (mean, $956.0 \text{ cm}^3 \pm 280.1$).

Manual Liver Volume Estimation

The mean liver volume estimated with the manual method was $937.10 \text{ cm}^3 \pm 301.3$ (range, 386.2–1709.5 cm^3) (Fig 4). The relationship between the volume estimated with the manual method and the reference standard showed a strong correlation ($y = 1.01x$, $r = 0.899$, $P < .001$). The average error between the volume estimated with the manual method and the reference standard was -0.024 ± 0.157 (95% CI: -0.080 , 0.032). The time required for calculating the liver volume with the manual method was 19.0–46.5 minutes (mean, 32.8 minutes ± 6.9).

Automated Liver Volume Estimation

The liver volume estimated with the automated method was $982.99 \text{ cm}^3 \pm 301.98$ (range, 390.4–1700.1 cm^3). There was a good correlation between the volume estimated with the automated method and the measured liver volume ($y = 1.04x$, $r = 0.782$, $P < .001$) (Fig 5). The average error between the volume obtained with the automated method and the measured liver volume was 0.033 ± 0.220 (95% CI: -0.047 , 0.114). The 95% CI of the automated method was slightly larger than that of the manual method, but there was

no significant difference between the two methods ($P = .407$). The correlation between the two methods (Fig 6) was excellent ($y = 1.03x$, $r = 0.883$, $P < .001$). The mean time required for computerized data acquisition was 4.4 minutes ± 1.9 (range, 3.0–7.0 minutes). Results are summarized in the Table.

Measurement Agreement

The mean difference between the manual method and the measured liver volume was 2.5 cm^3 (95% CI: -42.8 cm^3 , 47.9 cm^3). The limits of agreement were -256.3 and 261.3 cm^3 . The mean difference between the automated method and the measured liver volume was 51.3 cm^3 (95% CI: -11.9 cm^3 , 114.5 cm^3). The limits of agreement were -309.3 and 412.0 cm^3 . The mean difference between the automated and the manual methods was 48.8 cm^3 (95% CI: -0.1 cm^3 , 97.7 cm^3). The limits of agreement were -230.3 and 327.9 cm^3 .

Discussion

To simplify the determination of liver volume in donors scheduled for LRLT, we developed a fully automated method. We found that there was a good correlation between the actual measurements and the liver volume estimated with the automated method. The average error of the automated method (0.033 ± 0.220) was slightly larger than that of the manual method (-0.024 ± 0.157). However, the difference between the average errors of

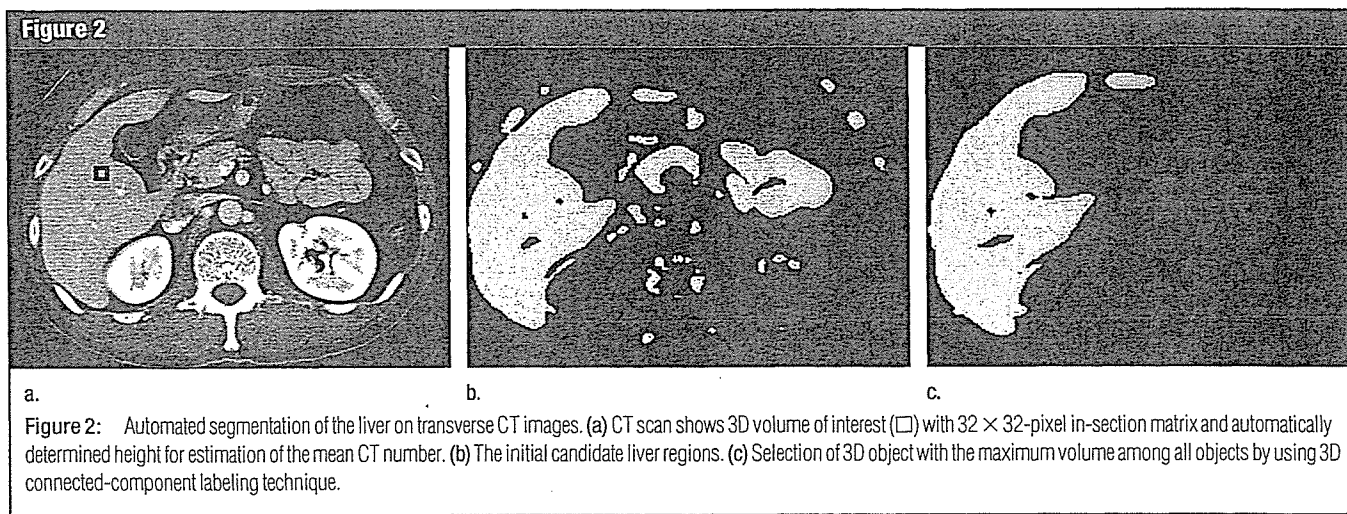
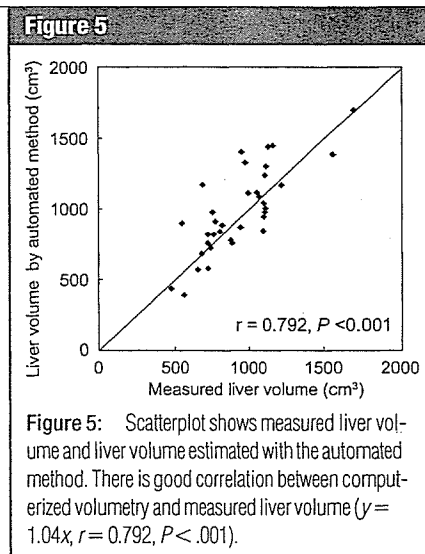
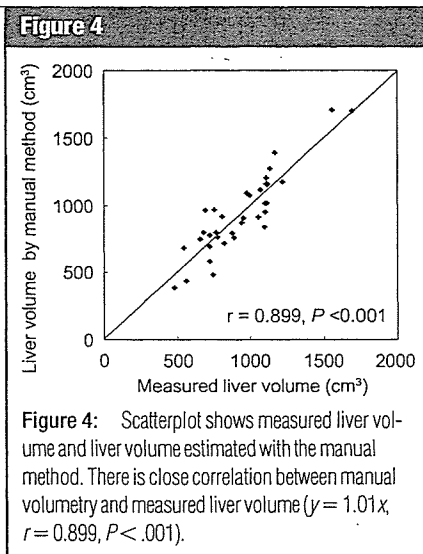
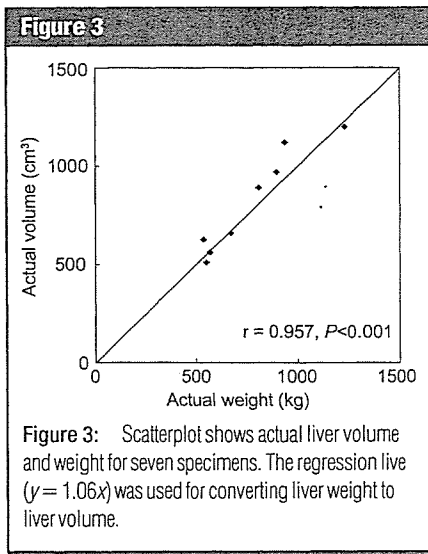
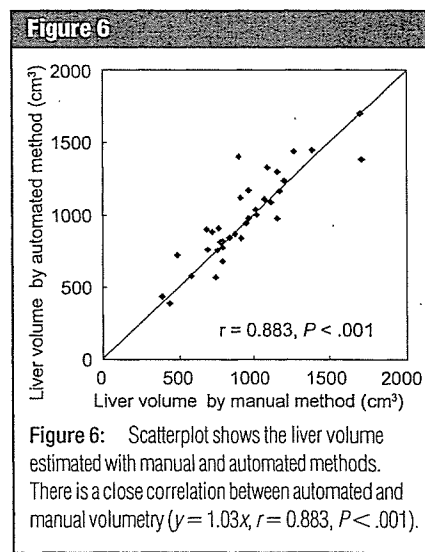


Figure 2: Automated segmentation of the liver on transverse CT images. (a) CT scan shows 3D volume of interest (\square) with 32×32 -pixel in-section matrix and automatically determined height for estimation of the mean CT number. (b) The initial candidate liver regions. (c) Selection of 3D object with the maximum volume among all objects by using 3D connected-component labeling technique.



Comparison of Manual and Automated Methods with Respect to Volume, Relative Error, and Time Required for Data Acquisition			
Variable	Actual Measurement	Manual Method	Automated Method
Liver volume (cm ³)	956.00 ± 280.10	937.10 ± 301.31	982.99 ± 301.98
Average error	Not applicable	-0.024 ± 0.157*	0.033 ± 0.220*
95% CI	Not applicable	-0.080, 0.032	-0.047, 0.114
Measurement time (min)	Not applicable	32.8 ± 6.9†	4.4 ± 1.9†

Note—Values are mean ± standard deviation.
 * No significant difference between manual and automated methods ($P = .407$).
 † Significant difference between manual and automated methods ($P < .05$).



the automated and manual methods was not statistically significant ($P = .407$). Although the best agreement was confirmed between the manual method and the measured liver volume, the limits of agreement (-256.3 and 261.3 cm³) were not small. In the automated and manual method agreement, the difference tended to give a higher reading by 48.8 cm³; however, the limits of agreement (-230.3 and 327.9 cm³) were within the acceptable range.

One benefit of computerized volumetry is the speed with which the liver volume can be estimated. The section thickness and the amount of data represent the limiting factors in manually performed volumetry; the effect of partial volume renders thinner sections preferable to thicker sections. Because multisec-

tion CT provides useful 3D images with thinner sections, multisection CT data sets are larger than those obtained with nonhelical or single-detector helical CT, which renders manual tracing cumbersome and time consuming. The large data sets produced with multisection CT call for automated segmentation methods. In our study, the automated method was 7.5 times faster than the manual method. Our automated CT volumetry method can be used in the clinical setting, for example, in the follow-up of patients who had a partial hepatectomy or fulminant hepatitis.

Volume measurements obtained manually are relatively accurate (7,13-16), and attempts have been made to determine liver volume by using semiautomated (17) and automated (18) meth-

ods. However, previous studies did not compare the liver volume estimated with these methods with the actually measured liver volume. Hermoye et al (9), who compared the measurements obtained by using semiautomated method on MR sections with the graft liver volume, assumed that the density of the liver is 1.0 g/cm³. In our preliminary study, we determined the actual liver volume based on water displacement in a water bath. We found an excellent correlation between liver volume and weight, although the density of the liver was not equal to that of water. Because the acquisition of exact volume is cum-

bersome in LRLT, we obtained the measured liver volume by converting the weight to the volume by using the regression line predetermined in the preliminary study.

Previously reported segmental volume measurements were performed in LRLT donor livers (7,19,20). These studies compared the segmental volume of the liver in vivo and its weight. However, because the actual cutting line of the graft is determined by temporary clumping of the hepatic vessels, it may differ from the preoperative estimation, and use of CT volumetric data from graft studies appears inappropriate. In our comparative study, we measured livers resected from transplant recipients; this made possible a direct comparison between values obtained on the actual entire liver in vitro and values obtained with CT volumetry in vivo, and good correlation was demonstrated.

Other investigations (9,21) have used MR images to obtain volume measurements. MR imaging studies of patients scheduled for LRLT reveal the arterial, portal venous, hepatic parenchymal, and biliary anatomy. However, because the spatial resolution is lower on MR images than it is on CT scans, CT—including CT angiography—appears necessary, especially for evaluation of donors. CT volumetry usually uses the same data set as CT angiography; however, if MR volumetry is to be used, the donors must undergo an additional preoperative MR study.

There were limitations to our study. First, although our automated method carefully extracted candidate areas on each CT section, some measurement errors did occur. Because the areas were extracted on the basis of their CT number, the errors may be attributable to a partial volume effect at the liver edge, to adhesion of the adjacent tissue with attenuation similar to that of the liver parenchyma, and/or to exclusion of intrahepatic regions that have CT numbers different from those of the surrounding parenchyma (eg, cysts or tumors). Second, for our liver volume measurements, we used livers of patients who had a long-standing hepatic disease. Therefore, their livers may have been severely damaged and deformed. These patients frequently had markedly developed collateral vessels

and a large amount of ascites. We found that automated tracing of the contours of severely damaged livers was more difficult than tracing in the healthy donor liver, and in such patients relative errors tended to be larger. Finally, our technique can be applied to only a total liver volume determination. However, segmental liver volume, which is donated to a recipient, is not available. At the moment, automated segmental volumetry is difficult because the cutting-line determination is extremely complicated. We consider segmental volumetry with automated measurement as our next challenge.

In conclusion, our comparative study revealed that automated CT volumetric assessment of the liver volume in vivo yielded acceptable measurements when compared with data obtained from the resected liver. We found the automated method to be quicker than the manual method, and we suggest that automated hepatic CT volumetry may be useful for determination of total liver volume in the donor candidate scheduled to undergo LRLT and for monitoring of postoperative liver regeneration.

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Long-Term Outcome of Adult-to-Adult Living Donor Liver Transplantation for Post-Kasai Biliary Atresia

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Our objective was to analyze problems in the perioperative management and long-term outcome of living donor liver transplantation (LDLT) for biliary atresia (BA). Many reports have described the effectiveness of liver transplantation (LT) for BA, particularly in pediatric cases, but little information is available regarding LT in adults (≥ 16 years old). Between June 1990 and December 2004, 464 patients with BA underwent LDLT at Kyoto University Hospital, of whom 47 (10.1%) were older than 16 years. In this study, we compared the outcomes between adult (≥ 16 years old) and pediatric (< 16 years old) patients. The incidence of post-transplant intestinal perforation, intra-abdominal bleeding necessitating repeat laparotomy and biliary leakage was significantly higher ($p < 0.0001$, < 0.001 and < 0.001 , respectively) in adults. Overall cumulative 1-, 5- and 10-year survival rates in pediatric patients were significantly higher ($p < 0.005$) than in adults. Two independent prognostic determinants of survival were identified: a MELD score over 20 and post-transplant complications requiring repeat laparotomy. Outcome of LDLT in adult BA patients was poorer than in pediatric patients. It seems likely that LT will be the radical treatment of choice for BA and that LDLT should be considered proactively at the earliest possible stage.

Key words: Adult, biliary atresia, liver transplantation, living donor

Abbreviations: BA, biliary atresia; GRWR, graft-to-recipient weight ratio; LDLT, living donor liver transplantation; LT, liver transplantation

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Introduction

Biliary atresia (BA), a congenital obliterative cholangiopathy of unknown etiology, has a reported incidence of about one in 14 000–19 500 live births and is a major cause of obstructive jaundice in neonates. Untreated infants succumb to either liver cirrhosis or liver failure or both within a year or two of birth (1). Introduction of the Kasai operation (hepatic portoenterostomy) in the late 1950s contributed to a dramatic improvement in the long-term survival in patients with BA and this procedure is now accepted as the standard surgical technique (2). Although extended survival of more than 20 years has been obtained in some cases, outcome is not uniformly satisfactory (3,4). One crucial determinant of prognosis is the prevention of cholangitis. Success with the Kasai operation depends on early diagnosis and surgical procedure, and particularly on the precise evaluation of clinical findings, adequate operative technique and the prevention of post-operative cholangitis. Prognosis varies according to BA classification (5), with the worst prognosis associated with complete extrahepatic BA. Actuarial 10-year survival rates following the Kasai operation without liver transplantation (LT) are 83% when atresia is limited to the common bile duct; 56% when atresia occurs with a cyst in the liver hilum communicating with dystrophic intrahepatic ducts; 36% when present with a patent gallbladder, cystic duct or common bile duct; and only 21% with complete extrahepatic BA (6).

Success with the Kasai operation is also closely associated with patient age. Ohi reported that this surgery should be performed in the early stage of the disease, preferentially in patients before 8 weeks of age (7), while Altman et al. identified age at surgery, surgical decade and anatomy of the atretic bile ducts as independent prognostic factors of a poor outcome for post-Kasai procedure (8).

Indications for LT in patients with post-Kasai BA include liver cirrhosis, liver failure, gastrointestinal bleeding due to portal hypertension, growth retardation, progressive intrapulmonary shunting, hepatopulmonary syndrome and repeated cholangitis, either singly or in combination (9). Prior to the introduction of LT, various palliative procedures were employed to correct post-Kasai complications, including a repeated Kasai operation, esophageal transection, portosystemic shunt and splenectomy, but the era of LT has brought dramatic improvements in the prognosis in these patients and numerous reports have described the

effectiveness of LT for pediatric cases of BA (10). In contrast, data on LT for adult patients with BA are limited due to a lack of accumulated experience.

The results of living donor liver transplantation (LDLT) are comparable to those with deceased donor LT, including reduced-LT or split-LT (11). Experience and technical improvements acquired using left lobe donation have facilitated the ability to use right lobe grafts in adult-to-adult LDLT to overcome problems encountered with small-for-size grafts (12). Here, we report the long-term outcome of LDLT in 47 consecutive adult patients (≥ 16 years old) with BA performed at Kyoto University, Japan.

Patients and Methods

Study population

Between June 1990 and December 2004, 1056 patients underwent LDLT at Kyoto University Hospital. In 464 (43.9%) of these patients, the procedure was performed for BA; they were 417 pediatric and 47 adult patients. Table 1 shows the characteristics of the recipients and donors.

Recipient characteristics

The 47 adult patients consisted of 19 males (40.4%) and 28 females (59.6%), with a median age of 19.6 years (range, 16.0–33.9 years). The median age at first hepatic portoenterostomy was 65 days (range, 26–144 days). The type of portoenterostomy performed was the Kasai operation in 36 cases, portoenterostomy with jejunal conduit in 4, portohepaticoduodenostomy in 2, and hepatic portoduodenostomy, cholecystoduodenostomy, choledochoduodenostomy, portocholecystostomy and cholecystojejunostomy in 1 each. The classification of BA was type I in 8 cases, II in 1, III in 33 and unknown in 5. Prior to referral to Kyoto University Hospital for LDLT, 47 patients underwent 129 sessions of abdominal surgery (median, 2 sessions), including repeated Kasai operation in 20 cases, esophageal transection in 13, stomal closure in 11, splenectomy in 11, repair of bowel obstruction in 4, splenic artery ligation and devascularization in 2, distal spleno-renal shunt in 2, hepaticolithotomy in 2, proximal gastrectomy in 1, left lateral segmentectomy in 1 and additional surgeries to correct complications. Median body weight at LDLT was 52.0 kg (range,

30.0–66.5 kg). At the time of LDLT, six patients had acute liver failure following acute cholangitis, which necessitated ICU management. These patients received mechanical ventilation, plasmapheresis and continuous hemodiafiltration for ventilated or organ-perfusion support until LDLT. The median model for end-stage liver disease (MELD) score was 16.9 (range, 8–37) (13), and median follow-up was 58.5 months (range, 14–136 months).

Donor characteristics and surgical techniques

Living donors were 17 fathers, 27 mothers, 2 brothers and 1 sister, with a median age of 47.0 years (range, 19–61 years) and a median body weight of 58.0 kg (range, 40.8–90.0 kg). Blood type combinations were identical in 38 cases (80.9%), compatible in 5 (10.6%) and incompatible in 4 (8.5%). Regarding surgical technique, 16 patients (34%) received a left lobe graft and 31 (66%) a right lobe graft from the living donor. Forty-five patients underwent LDLT by the standard procedure (14), while two underwent auxiliary LDLT to prevent small-for-size graft (15). The rationale for auxiliary partial orthotopic living donor liver transplantation (APOLT) in small-for-size grafting is that the remnant native liver is expected to support the function of the implanted graft during the early post-operative period. Graft function improves in proportion to volume growth, and can be expected to meet the hepatic functional demands of the recipient once sufficient graft growth has been obtained.

Characteristics of pediatric cases

A comparison was made between adult patients with BA ($n = 47$) and pediatric patients with BA (defined as < 16 years old) (16) ($n = 417$) recipients who received LDLT in the same study period. The 417 pediatric patients consisted of 144 males (34.5%) and 273 females (65.5%), with a median age at LDLT of 1.33 years (range, 0.25–15.9 years) and a median body weight of 8.6 kg (range, 3.7–62.4 kg). The median number of previous abdominal surgeries before LDLT was 2 (range, 0–5 times), and the median age at first hepatic portoenterostomy was 64 days (range, 12–301 days). At the time of LDLT, 21 (5%) patients required ICU management. Living donors were 173 fathers, 240 mothers, 1 brother and 3 grandmothers, with a median age of 32 years (range, 18–65 years) and a median body weight of 56.4 kg (range, 39.6–93.9 kg). Blood type combinations were identical in 284 cases (68.1%), compatible in 74 (17.8%) and incompatible in 59 (14.1%). Regarding surgical technique, 332 patients (79.6%) received a left lateral segment graft from the living donor, 63 (15.1%) a left lobe graft, 16 (3.9%) a mono-segment graft and 6 (1.4%) a right lobe graft. A total of 413 patients underwent LDLT by the standard procedure and 4 underwent APOLT to prevent small-for-size graft. Among operative variables, the median duration of the operation was 647.5 min (range, 324–1800 min); median blood loss was 106.1 g/kg (range, 6.4–2007.0 g/kg); median cold and warm ischemic time was 104 min (range, 18–943 min) and 44 min (range, 20–145 min), respectively; and actual median graft-to-recipient weight ratio (GRWR) was 2.65% (range, 0.54–5.94%). Median follow-up was 87 months (range, 1–177 months).

Immunosuppression and ABO-incompatible protocol

The immunosuppression protocol consisted of tacrolimus and low-dose steroids (17). Tacrolimus was begun one day prior to transplantation at a dose of 0.1 mg/kg/day divided into two doses, except for cases with hepatic encephalopathy and severe infection. The target post-transplantation whole blood trough concentration of tacrolimus was 10–12 ng/mL during the first 2 weeks and around 10 ng/mL thereafter. Steroids were started on graft reperfusion at a dose of 10 mg/kg, and then gradually reduced from 1 mg/kg/day to 0.3 mg/kg/day over the first month. For patients receiving ABO-incompatible grafts, plasma exchange or double filtration plasmapheresis was performed to reduce anti-ABO antibody titers before transplantation. Prostaglandin E1, azathioprine and additional steroids were administered post-operatively via the hepatic artery or portal vein (18).

Table 1: Recipient and donor characteristics of a adult-to-adult living donor liver transplantation for biliary atresia

Recipients	Median	(Range)
Gender (male/female)	19/28	
Age at LDLT (years)	19.6	(16.0–33.9)
Body weight at LDLT (kg)	52.0	(30.0–66.5)
Age at first portenterostomy (days)	65	(26–144)
Classification of biliary atresia		
I/II/III/unknown	8/1/33/5	
No. of previous operation (times)	2	(1–9)
MELD ² score	16.9	(8–37)
Follow-up period (months)	58.5	(4–136)
Donors		
Age (years)	47.0	(19–61)
Body weight (kg)	58.0	(40.8–90.0)
Relationship		
Father/mother/brother/sister	17/27/2/1	

LDLT = living donor liver transplantation; MELD = model for end stage liver disease.

Statistical analysis

Numerical values are presented as the median (range), unless stated otherwise. Univariate analyses were performed with the generalized Wilcoxon test. Actuarial 1- and 5-year graft survival curves were calculated with the nonparametric Kaplan-Meier method and comparison was made by the Wilcoxon test. Multivariate analysis used a logistic regression model. Numerical variables are shown as the mean (95% confidence interval, 95%CI) in Table 5. The SPSS software (SPSS 12.0 for Windows; SPSS, Chicago, IL) was used. A P value less than 0.05 was regarded as significant throughout the study. This study was approved by the institutional review board of Kyoto University Hospital, and informed consent was obtained from all patients.

Results

The primary indications for LDLT in the adult patients included refractory cholangitis (n = 44), jaundice (n = 42), gastrointestinal bleeding (n = 21), intrapulmonary shunting (n = 5), hepatic encephalopathy (n = 4), portopulmonary hypertension (n = 1) and hepatocellular carcinoma (n = 1) (Table 2). No other congenital anomalies were seen in any patient.

Table 3 shows surgical variables. Median duration of the adult recipient operation was 868.0 min (range, 555–1420 min) and median blood loss was 80.2 g/kg (range, 16.9–597.6 g/kg). Median cold and warm ischemic time was 133 min (range, 25–527 min) and 46 min (range, 23–76 min), respectively. Actual median GRWR of the adult patient was 1.05% (range, 0.48–1.85%). Significant differences between the pediatric and adult patients with BA

were seen in duration of operation, cold ischemic time and GRWR.

Blood type compatibility was a significant prognostic factor, with a 3-year survival of 74.3% in identical/compatible combinations (n = 43) but only 25.0% in incompatible combinations (n = 4) (p < 0.05). Five patients showed hypoxemia related to intrapulmonary shunting. Technetium-99 m macroaggregated albumin scintigraphy (19) evaluation of the intrapulmonary shunt ratio showed a significant difference in the survival of patients whose intrapulmonary shunt ratios were greater (n = 2) or less than 30% (n = 45), with 1-year survival rates of 0% vs. 75.5% (p < 0.05). Two of the five patients who had intrapulmonary shunting, with shunt ratios of 34.0% and 58.2%, died from sepsis. A shunt ratio of greater than 30.0% was associated with a significantly poorer prognosis (p < 0.05). One patient with portopulmonary hypertension died from progressive pulmonary hypertension on post-operative day 12, despite administration of NO and prostaglandin. Regarding MELD scores, significantly better patient survival was achieved with scores less than 20 points (n = 27) than with other scores (n = 20) (5- and 10-year survivals of patients with lower scores were 80.7% vs. a 5-year survival of 55.0% and a 10-year survival of 36.7% for patients with higher scores) (p < 0.05). No significant associations were seen between patient survival and the number of previous operations, previous laparotomy for portal hypertension, GRWR, mode of LT received or graft type.

Table 4 shows post-operative complications after LDLT. Intestinal perforation (31.9% in adults vs. 8.4% in children; p < 0.0001), intra-abdominal bleeding necessitating repeat laparotomy (27.7% in adults vs. 9.6% in children; p < 0.001) and biliary leakage (23.4% in adults vs. 7.9% in children; p < 0.001) were all significantly more common in adults. Repeat transplantation was indicated in two patients due to vascular complications 8 years after LDLT in one case and acute graft failure at 10 days in the other case. Fifteen of 47 adult patients (31.9%) experienced post-operative complications due to sepsis in 8 cases (secondary to either intestinal perforation or bleeding or both in 6 cases), vascular complications in 3, humoral rejection due to blood type

Table 2: Indications for living donor liver transplantation

Indication	n
Cholangitis	44
Jaundice	42
Gastrointestinal bleeding	21
Intrapulmonary shunting	5
Hepatic encephalopathy	4
Portopulmonary hypertension	1
Hepatocellular carcinoma	1

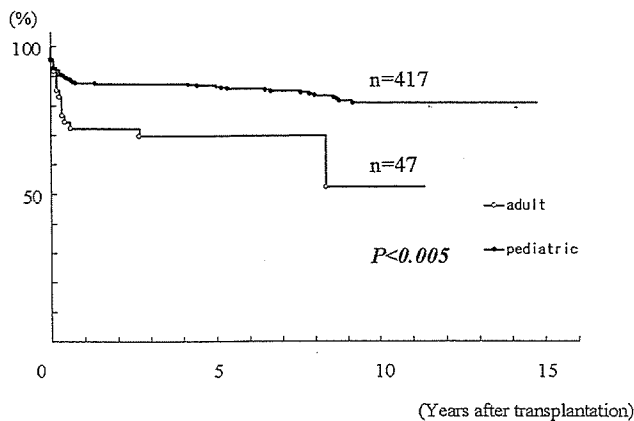
Table 3: Surgical variables

	Age ≥ 16 year (n = 47)		Age < 16 year (n = 417)		p-Value
	Median	(Range)	Median	(Range)	
Duration of operation (min)	868.0	(555–1420)	647.5	(324–1800)	<0.0001
Blood loss/body weight (g/kg)	80.2	(16.9–597.6)	106.1	(6.4–2007.0)	ns
Cold ischemic time (min)	133	(25–527)	104	(18–943)	<0.05
Warm ischemic time (min)	46	(23–76)	44	(20–145)	ns
GRWR (%)	1.05	(0.48–1.85)	2.65	(0.54–5.94)	<0.0001
Blood type combinations					
Identical: compatible: incompatible		38:5:4		284:74:59	
Graft type					
Right lobe: left lobe: left lateral: mono		31:16:0:0		6:63:332:16	

GRWR = graft-to-recipient weight ratio.

Table 4: Post-operative complications in patients with biliary atresia

Complications	Age \geq	Age $<$	p-Value
	16 year (n = 47) %	16 year (n = 417) %	
Intestinal perforation	31.9	8.4	<0.0001
Intraabdominal bleeding	27.7	9.6	<0.001
Hepatic artery thrombosis	2.1	4.8	ns
Portal vein thrombosis	4.3	3.8	ns
Biliary leakage	23.4	7.9	<0.001
Biliary stricture	2.1	5.5	ns
Need for retransplantation	4.3	6.5	ns

**Figure 1: Cumulative survival.**

incompatibility in 2, and portopulmonary hypertension and recurrence of hepatocellular carcinoma in 1 each. One surviving patient required several treatments per year due to autoimmune hepatitis.

The overall cumulative 1-, 5- and 10-year graft survival rates in adult patients with BA were 72.2%, 69.7 and 55.8%, respectively, while the corresponding patient survival rates were 72.2%, 69.7% and 52.3%. In contrast, the respective rates in pediatric patients with BA were 86.9%, 84.9% and 76.6% for grafts and 87.7%, 86.7% and 80.8% for patients (Figure 1). Survival after LDLT was thus significantly lower in adult patients with BA than in pediatric patients with BA ($p < 0.005$ for patient survival, $p < 0.01$ for graft survival).

We then investigated independent prognostic factors of survival for hospital death after LDLT using a multiple logistic regression model (Table 5). Results showed no significant relationships among age at LDLT (95% CI 0.926–1.276, OR 1.087), past laparotomy for portal hypertension (95% CI 0.104–4.590, OR 0.692), number of previous laparotomies (95% CI 0.243–1.133, OR 0.525), duration of operation (95% CI 0.998–1.006, OR 1.002), or GRWR (95% CI 0.110–4.497, OR 0.704). In contrast, posttransplant complications requiring repeat laparotomy (95% CI 1.072–131.386, OR 11.868) and MELD score (especially

Table 5: Results of logistic regression analysis

	p-Value	OR	95% CI
Age at LDLT	0.308	1.087	0.926–1.276
Pastlaparotomy for PH	0.703	0.692	0.104–4.590
No. of previous laparotomies	0.101	0.525	0.243–1.133
Duration of operation	0.427	1.002	0.998–1.006
GRWR	0.711	0.704	0.110–4.497
Relaparotomy post-LDLT	0.044	11.868	1.072–131.386
MELD score (over 20)	0.027	6.943	1.243–38.777

OR = odds ratio; CI = confidence interval; PH = portal hypertension.

over 20, 95% CI 1.243–38.777, OR 6.943) were shown to be independent prognostic factors.

Discussion

The Kasai operation for BA has been frequently used as the only radical treatment available, with jaundice disappearing in about 70% of treated patients (20). Long-term survival rates and favorable results have been reported (21). Early detection and treatment by the Kasai operation improve the prognosis for patients with BA (7) and it has been reported that if bilirubin levels normalize within 3 months of surgery, the patient is likely to survive for an extended period without developing portal hypertension or hypersplenism (22). In their series, Nio et al. (3) reported that 58 of 199 patients (29%) survived for more than 20 years after this operation, whereas Lykavieris et al. reported that only 63 of 271 patients (23%) survived this length (4). Long-term survival without LT would thus have been highly unlikely in the present patients.

About 50 years have passed since the Kasai operation was first attempted and a large body of data on long-term outcomes has now been accumulated. Several problems have been revealed, including two major complications, repeated cholangitis (23) and portal hypertension (esophageal varices, splenomegaly and hypersplenism). Other problems include the recurrence of jaundice, as well as hepatopulmonary syndrome and portopulmonary hypertension as a special type of portal hypertension. In our study, LDLT was necessary for almost all patients with repeated cholangitis in adulthood (44 of 47 patients, 93.6%) and for 44.7% (21 of 47) of patients in whom BA was complicated by portal hypertension (e.g. gastrointestinal bleeding).

We identified a number of differences in outcomes between adult and pediatric patients in our study. When classified by age, pediatric patients requiring LDLT showed a 10-year survival rate of 80.8%. Moreover, Fouquet et al. reported a rate of 82.0% in pediatric patients receiving deceased donor LT (10). Thus, a favorable postoperative course has been seen in pediatric patients after LT from both deceased and living donors. In contrast, our 10-year