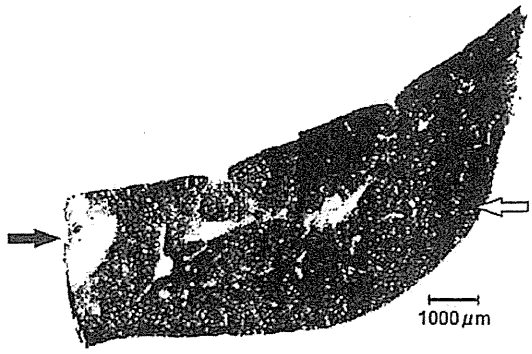

図4　抗ヒトサイトケラチン8/18抗体を用いた免疫組織化学染色

PXBマウスでは70％以上のマウス肝細胞がヒト肝細胞によって置換されている。
黒矢印：マウス肝細胞領域，白矢印：ヒト肝細胞領域

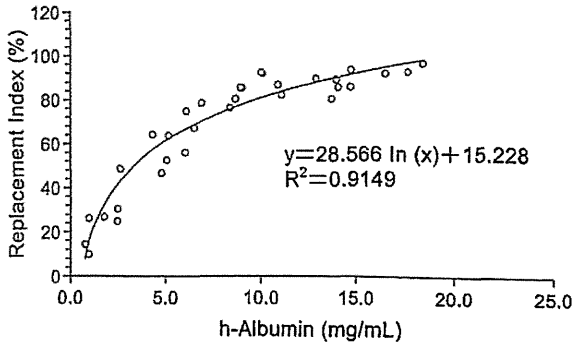

図5　PXBマウスの血中ヒトアルブミン濃度測定結果とヒト肝細胞置換率測定結果との相関曲線

血中ヒトアルブミン濃度とヒト肝細胞置換率が高い相関性を示す。

表1　ホストマウスおよびPXBマウスの飼育条件

仕　様	ISO14644-1 清浄度クラス6相当
温　度	20～26℃
湿　度	42～72％
室　圧	ホストマウスコロニーおよびPXBの生産飼育施設では陽圧制御，P2A実験施設では陰圧制御を実施
照明時間	12時間周期(8:00～20:00)
換　気	常時換気(HEPAフィルターろ過)
飼育ケージ	ポリカーボネート製マウス用ケージ
床　敷	実験動物用床敷ペパークリーン
飼　料	γ線照射滅菌した，げっ歯類用飼料チャールスリバーフォーミュラー(CRF-1)を自由摂取させている。PXBマウスには特別にビタミンCを添加したCRF-1を与えている。この他，SCIDの形質を持つキメラマウスおよびホストマウスへのカリニ肺炎病原体の感染を防御する目的で，スルフォメトキサゾールおよびトリメトプリム配合した特殊飼料を所定の期間中に給餌している。
飲　水	東広島市水道局より供給される水道水をオートクレーブ滅菌した後，次亜塩酸を添加したものをマウス用給水ボトルに容れ，自由摂取させている。

全ての凍結ヒト肝細胞ロットについて個別に実施されている。

3. PXBマウスの飼育

以上に紹介した方法で生産したPXBマウスの飼育は，ホストマウスと共に微生物学的清浄度が維持されたクリーンルームで行われている(表1)。これは，SCIDの形質を保有する動物を微生物感染から保護することを目的とした対応である。適切な飼育環境を与えることで，PXBマウスは10週間以上安定した体重推移(図6)と血中h-Alb濃度推移(図3)を維持することができる。ただし，死亡率は無処置の飼育期間中であっても1週間におよそ1.5％という数値を示している。

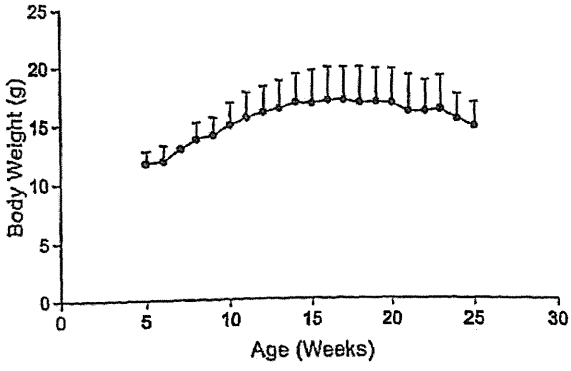

図6　PXBマウスの体重推移

10週齢を経過すると，15g以上の体重を維持する。

4. PXBマウスの実験への利用

PXBマウスには，野生型のマウスに準じて多様な実験処置を施すことが可能である。当所で実施している試験操作の中から，被験物質の投与と生体試料の採取方法について具体例を挙げる。被験物質の投与については，投与経路として経口，皮下，腹腔内，静脈内，筋肉内など一般的な経路の他に，麻酔下動物の静脈内への持続的な投与や肝臓への直接投与などの特殊な経路も選択できる。一般的な投与経路については，1日あたり複数回(3回を上限として設定)の投与や，反復投与(2週間の実施が最も多い)も実施可能である。採取可能な生体試料の項目は，経時／連続採取可能なものとして，血液，胆汁，尿および糞，解剖時には各種組織および器官の採取が可能である。このうち，血液は10μL/g BW/1 week，胆汁は5μL/h，尿は1.0 mL/24 h，糞は1.5 g/24 hが採取量の目安となっている。これらの操作は，一般状態観察および体重測定によって個体の健康状態を把握しながら実施されている。また，肝炎ウイルスなど感染性微生物を感染させたPXBマウスについては，作業者への感染リスクが大きくならないことを事前に判断した上で各種の処置や生体試料の採取を実施している。なお，当所では，これらPXBマウスの利用方法の詳細について，所内に設けた倫理委員会で審査・承認を行っている。

以降には，PXBマウスの実用例の一部として，特に医薬品開発に関連する薬物代謝，肝毒性および肝炎ウイルスについての研究成果を紹介する。

薬物代謝は，医薬品開発の過程においてヒトでの薬効と毒性を正しく理解するために重要な研究分野である。この分野においてPXBマウスに期待される点は，PXBマウス肝臓内のヒト肝細胞がヒト生体と同様の薬物代謝機能を有していることである。

現在までに当所において生産されたPXBマウスのうち，3ロットの異なるドナー肝細胞を用いて生産されたPXBマウスの肝臓について，DNAアレイ(Affymetrix GeneChip® Human Genome U133 plus 2.0 Array，38,500遺伝子を解析可能)を用いた遺伝子発現解析を実施したところ，PXBマウス肝臓内のヒト肝細胞では，当該アレイに搭載されたヒト遺伝子のうち，70％にあたる遺伝子の発現が確認され，このうち98％に相当する遺伝子については3ロット間の発現量に大きな差(2倍未満または1/2より大きい)は認められなかった。

ヒト肝細胞に発現している多数の分子のうち，薬物代謝酵素に注目すると，第I相反応を担うチトクローム P450(CYP)に含まれる主要な分子種であるCYP1A1，1A2，2A6，3A4，3A5，2C9，2C8，2C19，および2D6の発現が確認されており[3),11)-13)]また，アルデヒドオキシダーゼについても発現が確認されている[14)]。このうち，CYP2A6，3A4，2C8，2C9，2C19および2D6については，PXBマウスの置換率上昇に伴って酵素活性が上昇を示すことが報告されており[11)]，また，CYP2A6，2C19および2D6については，ドナーの遺伝子多型由来と考えられる酵素活性の差が確認されている[11),15)]。第II相反応を担う酵素については，グルクロン酸抱合酵素(UGT)，硫酸抱合酵素(SULT，CSTおよびTPST)，アセチル抱合酵素(NAT)，グルタチ

オン抱合酵素(GSTおよびMGST)，メチル抱合酵素(COMT，PNMT，TPMT，GAMT，PEMTおよびASMT)に含まれる主要な分子種の発現が確認されている[12),16)]。これらの酵素に含まれる分子種のうち，UGT2B7，SULT1A1，SULT1E1およびNAT2については，PXBマウスの置換率上昇に伴って酵素活性が高値を示すことも報告されており[16)]，NAT2の酵素活性についてはドナー肝細胞の遺伝子多型に由来すると考えられるドナー間差が確認されている[16)]。化学物質を肝細胞内外に輸送する機能を持つトランスポーターについても，ABCトランスポーター(ABCA)，Solute carrierファミリー22(SLC22)，有機アニオントランスポーター(OATP)についてmRNAの発現が確認されている[12)]。

このように，PXBマウス肝臓内のヒト肝細胞では，多数のヒト遺伝子およびタンパク質が発現して機能していることが確認されており，肝細胞を介する薬物代謝分野の研究において非常に有効なリソースであると考えられる。実際に，ヒトで利用されている医薬品に関連する研究として，Cefmetazole[17)]やWarfarin[18),19)]の体内動態(吸収，代謝，分布および排泄を含む)が，ヒト肝細胞の機能を反映していることが確認されている。また，ヒト臨床で薬物相互作用の1つとして問題となっている酵素誘導については，PXBマウスにRifampicinまたはRifabutinを反復投与した後にCYP3A4のmRNA発現量，タンパク質発現量およびタンパク質活性が増加することが確認されている[3),20)-22)]。これらの研究報告は，PXBマウスがヒト臨床での薬物相互作用の解析・研究において有用であることを示すデータと考えられる。

肝毒性研究分野では，医薬品や臨床試験段階にある化合物によって発生する肝障害が，ヒトの健康や製薬企業の医薬品開発に重大な影響を与えるため，PXBマウスを含む新しいリソースの登場によって，ヒトでの肝毒性発生を精度よく予測できることが期待されている。しかし，ヒト肝細胞キメラマウスには，肝毒性評価にあたって考慮すべき課題が3点挙げられる。1点目は，PXBマウスではuPA発現によってマウス肝細胞に障害が発生しているため，無処置でも血漿中ASTやALT測定値が高値を示し，またマウス肝細胞由来のASTとALTはヒト肝細胞に由来するこれら逸脱酵素と区別することが困難な点である(表2)。PXBマウスを利用してヒト肝細胞への毒性を検討する際には，これらマウス肝細胞由来の逸脱酵素の測定値のベースが高いことを踏まえ，被験物質の投与前後で同一個体での逸脱酵素の推移を確認することや組織観察によって肝障害の局在を確認することが必要と考えられる。2点目は，PXBマウスが免疫不全の形質を持つために，免疫系が関

表2　PXBマウスの血液生化学データ

項　目	単　位	平均 ± 標準偏差
GOT/AST	(U/l)	437.2 ± 504.8
GPT/ALT	(U/l)	280.3 ± 280.2
GGT	(U/l)	52.2 ± 24.0
CPK	(U/l)	275.4 ± 134.8
LDH	(U/l)	1165.9 ± 799.7
ALP	(U/l)	874.0 ± 421.1
LAP	(U/l)	86.6 ± 28.6
CHE	(U/l)	417.3 ± 82.4
AMYL	(U/l)	977.3 ± 363.3
BUN	(mg/dL)	35.6 ± 21.4
TCHO	(mg/dL)	135.3 ± 50.0
HDL-C	(mg/dL)	31.3 ± 15.2
TG	(mg/dL)	96.9 ± 37.3
TBIL	(mg/dL)	0.7 ± 0.4
DBIL	(mg/dL)	0.1 ± 0.0
GLU	(mg/dL)	118.3 ± 36.9
UA	(mg/dL)	3.5 ± 1.9
ALB	(g/dL)	2.7 ± 1.3
TP	(g/dL)	4.9 ± 0.4
CRE	(mg/dL)	255.6 ± 260.3
Ca	(mg/dL)	10.1 ± 1.2
IP	(mg/dL)	9.5 ± 1.6
Mg	(mg/dL)	2.6 ± 0.9

与する毒性の再現が困難な点，また3点目はPXBマウスの肝臓でヒト由来の成分は肝細胞のみであり，間質細胞はマウスに由来する点である。現在では，肝毒性発生の過程に免疫系や間質細胞が複雑に関与している可能性が示唆されているが，PXBマウスを用いてこのような肝毒性の全ての過程を再現することは困難である。現在，我々は業務提携先である積水メディカル㈱と共同して，PXBマウスを用いてヒト肝細胞を対象としたトキシコゲノミクスの構築を進めている。ヒト肝細胞での遺伝子発現の変化を基に，肝毒性発生を予想できるシステムとなることを期待したい。

肝炎ウイルス研究分野での利用は，PXBマウスの有効性が最も発揮されている例である。ヒト肝細胞キメラマウスが登場するまでは，チンパンジー以外に実用的なモデル動物が存在しなかった状況であったため，C型肝炎ウイルス(HCV)およびB型肝炎ウイルス(HBV)の2種類のウイルスに持続感染することが可能であり，またヒト臨床で使用されている代表的な治療薬の薬効が再現される点がPXBマウスの最大のアドバンテージとなっている。

HCVジェノタイプ1bを感染させて作製したHCV感染PXBマウスモデルを利用して，HCV治療薬であるPeg-IFNα-2aによる応答性を確認した試験の例を図7に示す。この試験では，HCV感染PXBマウスに，30μg/kgのPeg-IFNα-2aを1週間に2回の頻度で2週間反復皮下投与した後に2週間の休薬期間を設けた。その結果，Peg-IFNα-2aの投与によって血清中のHCV RNA濃度は速やかに減少し，投与終了後には血清中HCV RNA濃度の回復が確認された。HCV感染PXBマウスモデルを用いたHCV治療薬の評価に関しては，当社での検討の他にMyriocin(Serine palmitoyltransferase inhibitor)[23]，17-(dimethylaminoethylamino)-17-demethoxygeldanamycin(HSP90 inhibitor)[24]，DEBIO-025(Cyclophilin Inhibitor)[25]を検討した結果についても報告されている。

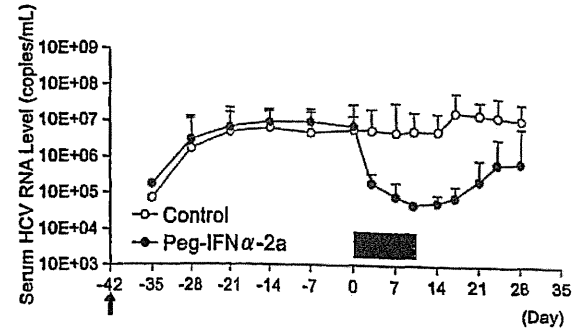

図7　HCV感染PXBマウスモデルでのPeg-IFNα-2a応答性

Peg-IFNα-2aにより血清中HCV RNA濃度が減少する。
矢印：HCV接種，灰色：Peg-IFNα-2a投与期間。

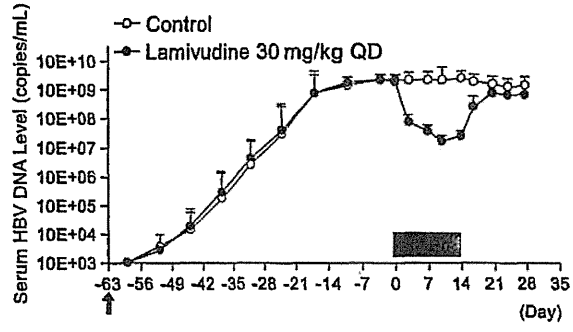

図8　HBV感染PXBマウスモデルでのLamivudine応答性

Lamivudineにより血清中HBV DNA濃度が減少する。
矢印：HBV接種，灰色：Lamivudine投与期間

HBVジェノタイプCを感染させて作製したHBV感染PXBマウスモデルを利用して，HBV治療薬であるLamivudineへの応答性を確認した試験の例を図8に示す。この試験では，Lamivudineの30 mg/kgを1日1回の頻度で2週間反復経口投与した後に2週間の休薬期間を設けた。その結果，Lamivudine投与によって血清中のHBV DNA濃度は緩やかに減少し，投与終了後は血清中HBV DNA濃度が速やかに回復した。HBV感染PXBマウスモデルに対するLamivudineの薬効に関しては，当社での検討の他に，Lamivudine耐性HBVを感染させたPXBマウスではLamivudineの抗HBV効果が減弱することが確認されている[26]。

5. おわりに

ヒト肝細胞研究のための新しいリソースとして，ヒト肝細胞キメラマウスは様々な研究分野で成果を上げている。今後，現状の uPA$^{+/+}$/SCID マウスが抱えるいくつかの問題点，PXB マウスの用途上の制約を克服すべく研究開発を続けていきたいと考えている。

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Ann Surg Oncol (2012) 19:2888–2896
DOI 10.1245/s10434-012-2338-y

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Prognostic Significance of Antithrombin III Levels for Outcomes in Patients with Hepatocellular Carcinoma After Curative Hepatectomy

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ABSTRACT

Background. Although several studies have shown that serum antithrombin III (ATIII) has anti-inflammatory effects, the prognostic value of ATIII in HCC is unknown. We investigated the influence of preoperative ATIII levels on the outcome of patients who underwent hepatectomy for hepatocellular carcinoma (HCC).

Methods. Data from 440 patients (314 patients with ATIII $\geq$70 % and 126 patients with ATIII <70 %) who underwent curative hepatectomy for HCC were retrospectively collected and analyzed. To overcome bias due to the different distribution of covariates for the 2 groups, propensity score matching was performed on the patients, and outcomes were compared.

Results. The propensity score analysis revealed that 65 patients with ATIII of $\geq$70 % (group 1) and 65 patients with ATIII of <70 % (group 2) had the same preoperative and operative characteristics (excluding the ATIII level). The overall survival rate and the disease-free survival rate was significantly higher in group 1 than in group 2 ($P = 0.005$ and 0.011, respectively). Multivariate analysis showed that ATIII was a significant favorable factor for overall survival and disease-free survival of patients with HCC after curative hepatectomy.

Conclusions. The prognosis of patients with HCC was found to be associated with preoperative antithrombin III levels. ATIII may be useful for predicting outcomes of patients with HCC after curative hepatectomy.

Hepatic resection is a well-accepted therapy for hepatocellular carcinoma (HCC), but many patients develop cancer recurrence, with the cumulative 5-year HCC recurrence rate being over 60 %.[1,2] A high incidence of tumor recurrence after hepatic resection remains a major drawback. The risk factors for prognosis after resection of HCC have been extensively studied.

Antithrombin III (ATIII) is a heparin-binding protein and a major inhibitor of coagulation proteases, primarily thrombin and factor Xa.[3] ATIII has been reported to efficiently inhibit tumor angiogenesis in a mouse model.[4,5] In clinical settings, decreased plasma ATIII levels have been described in a variety of different cancers, including lung, colon, ovary, and prostate cancers.[6–8] However, few data are available on the impact of ATIII on outcomes of patients with HCC who underwent hepatectomy.

In this study, we aimed to investigate the effect of ATIII on survival and HCC recurrence in patients who underwent curative hepatic resection by both methods of one-to-one match study using propensity score and multivariate analysis.

METHODS

Between the years 2000 and 2008, a total of 440 patients with HCC underwent curative hepatectomy as an initial treatment at the Department of Gastroenterological Surgery, Hiroshima University Hospital, Hiroshima, Japan. The study was approved by the concerned institutional

Electronic supplementary material The online version of this article (doi:10.1245/s10434-012-2338-y) contains supplementary material, which is available to authorized users.

First Received: 19 November 2011;
Published Online: 31 March 2012

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review boards. Written informed consent was obtained from all patients. The patients were categorized into 2 groups on the basis of their preoperative ATIII level: ≥70 % (group 1, $n = 314$), and <70 % (group 2, $n = 126$).

The type of hepatectomy selected was based on liver function and tumor extent.[9,10] Liver function was assessed by the Child-Pugh classification and the indocyanine green retention rate at 15 minutes (ICG-R15).[11] If the liver function was sufficient, anatomic resection (segmentectomy, sectionectomy, or hemihepatectomy) was performed.[12,13] In patients with insufficient hepatic reserve, limited resection was performed. For example, right hemihepatectomy could be tolerated if the ICG-R15 was in the normal range. One-third of the liver parenchyma could be resected for patients with ICG-R15 of 10–19 %, segmentectomy was possible for patients with ICG-R15 of 20–29 %, and limited resection was possible for patients with ICG-R15 of ≥30 %.[10] Hepatectomy was performed using procedures described by Itamoto et al.[9] Postoperative follow-up included liver function tests, serum alfa-fetoprotein (AFP), hepatic ultrasonography on a 3-month basis, and computed tomographic scans every 6 months. Follow-ups were performed in outpatient clinics or by the patients' general practitioner. Patients with intrahepatic recurrence were managed with ablative therapies such as radiofrequency ablation and percutaneous ethanol injection therapy, transarterial chemoembolization (TACE), or surgery, including living donor liver transplantation. Data were updated until June 2011 and survival was computed from the date of the initial surgery.

Definitions

Normal ATIII is defined as a level of ≥70 % in this study, because it has been shown that anti-thrombin activity of heparin was significantly decreased in the serum ATIII level-dependent manner when plasma ATIII levels were <70 %.[14,15] Major hepatectomy was defined as the resection of 3 or more Couinaud segments. Curative hepatectomy was defined as the removal of all recognizable tumors. All postoperative complications were reviewed for at least 30 days after surgery. The complications were graded according to the method described by Clavien et al.[16] Complications were considered morbid if they were of grade IIIA or greater. Postoperative mortality was defined as any death that occurred within 30 days of surgery.

Receiver-Operating Characteristic (ROC) Analysis

ROC curve analysis was performed to determine the optimal cutoff values for subsequent analyses. Each cutoff value was determined by seeking the most optimal combination of high sensitivity and specificity values, while maintaining the lowest likelihood ratio of a negative test and the highest likelihood ratio of a positive test.

Statistical Analysis

For continuous variables, parametric analyses were performed using Student's t test, and Mann–Whitney U test was used for non-parametric analyses. Categorical variables and postoperative courses were compared using χ^2 tests with Yates correction. The Kaplan–Meier method was used for analyses of overall survival and disease-free survival, whereas comparisons between groups were performed using the log-rank test. For factors determined to be significant for overall and disease-free survival using univariate analysis, we performed multivariate analyses using the Cox proportional hazards model. An initial Cox proportional hazards model was applied to the entire study population to identify poor prognostic predictors. To overcome bias due to the different distribution of covariates among patients from the 2 groups, a one-to-one match was created using propensity score analysis.[17,18] The propensity score represents the probability of each individual patient being assigned to a particular condition in a study given a set of known covariates. Propensity scores are used to reduce selection bias by equating groups on the basis of these covariates and are used to adjust for selection bias in observational studies through matching. Variables entered in the propensity model were age, sex, anti-hepatitis C virus (HCV) antibody, and liver function test including total bilirubin, prothrombin time, ICG-R15 %, albumin, and Child–Pugh classification. Tumor size, number of tumor, vascular invasion, and AFP were used as tumor factors. Operative bleeding, operative time, transfusion, and type of hepatectomy were used as operative factors. The model was then used to obtain a one-to-one match by using the nearest-neighbor matching method.[19,20] Once the matched groups were obtained, overall and disease-free survival analyses were performed within each matched subgroup to assess the influence of preoperative ATIII level on prognosis after adjusting the confounding factors. A difference was considered significant if the P value was <0.05. Statistical analyses were performed using the SPSS statistical software version 16 (Chicago, Illinois, USA).

RESULTS

ROC Curve Analysis for Cutoff Value of ATIII

The optimal cutoff values of ATIII for survival and recurrence were determined by ROC curve analysis, respectively. A cutoff value of survival was 72 % of ATIII

with a sensitivity of 47 % and specificity of 72 %. A cutoff value of recurrence was 69 % of ATIII with a sensitivity of 36 % and specificity of 81 %. ATIII value of 70 % has been chosen as a cutoff level in this study, since normal ATIII is defined as a level of ≥70 % (Supplementary Figs. 1 and 2).

Clinicopathological Characteristics and Postoperative Course of the Entire Study Group

Differences between the characteristics of patients in the 2 groups are shown in Table 1. Specifically, patients in group 1 had higher prothrombin time (PT) activity, lower serum bilirubin, lower ICG-R15, lower proportion of patients with Child–Pugh class B, greater maximum tumor diameter, and higher frequency of microvascular invasion. The level of preoperative ATIII in group 1 was significantly higher than that in group 2 (88.4 vs. 59.6 %; $P < 0.001$).

In the entire study population, the overall survival rate of patients in group 1 was significantly higher than that of patients in group 2 ($P < 0.001$): in group 1, the 3- and 5-year overall survival rates were 85.0 and 75.8 %, respectively, whereas in group 2, they were 77.1 and 53.1 %, respectively (Fig. 1a). Furthermore, the disease-free survival rate of patients in group 1 was significantly higher than that of patients in group 2 ($P < 0.001$): the 1-, 2-, and 3-year disease-free survival rates were 75.3, 60.1, and 48.1 %, respectively, in group 1 and 63.5, 43.6, and 27.4 %, respectively, in group 2 (Fig. 1b). Postoperative complications did not differ between the 2 groups (Table 2). Table 2 shows the patterns of cancer recurrence and the treatment details of the recurrences in both groups. The overall recurrence rate was also significantly lower in group 1 than in group 2 ($P < 0.001$): 53.5 versus 70.6 %. Regarding treatment for HCC recurrence, the proportion of patients in whom repeat hepatectomy was selected for treatment in group 1 tended to be higher than that in group 2 ($P = 0.07$). Furthermore, the proportion of patients in whom living donor liver transplantation was selected for treatment in group 1 was significantly lower than that in group 2 ($P = 0.038$).

Results after Propensity Score Match

The characteristics of propensity score-matched patients are shown in Table 1. Sixty-five of the 314 patients with preoperative ATIII levels ≥70 % were matched with 65 of the 126 patients with preoperative ATIII levels <70 % after covariate adjustment. Therefore, 249 patients in group 1 and 61 patients in group 2 were excluded because their propensity scores could not be matched. The study group of 130

TABLE 1 Baseline characteristics and operative data on patients who underwent hepatectomy

Characteristic	Whole study series			Propensity matched series		
	ATIII ≥ 70 % ($n = 314$)	ATIII < 70 % ($n = 126$)	P	ATIII ≥ 70 % ($n = 65$)	ATIII < 70 % ($n = 65$)	P
ATIII (U/ml)	88.4 ± 13.2	59.6 ± 8.5		82.5 ± 11.2	62.3 ± 6.0	
Age (years)	65.2 ± 10.3	64.6 ± 9.4	0.578	65.2 ± 8.2	64.5 ± 10.1	0.685
Sex (M/F)	238/76	85/41	0.074	44/21	42/23	0.711
Anti-HCV antibody positive	185 (59.2 %)	93 (70.8 %)	0.004	47 (72.3 %)	45 (69.2 %)	0.699
Prothrombin time (%)	90.7 ± 16.1	79.1 ± 15.1	<0.001	85.6 ± 14.1	83.8 ± 11.6	0.424
T-Bil (mg/dl)	0.78 ± 0.31	0.91 ± 0.34	<0.001	0.82 ± 0.29	0.83 ± 0.29	0.831
Albumin (g/dl)	4.00 ± 0.42	3.50 ± 0.45	<0.001	3.72 ± 0.36	3.72 ± 0.33	0.939
ICG-R15 (%)	14.8 ± 8.5	23.6 ± 9.7	<0.001	18.4 ± 10.6	18.5 ± 6.8	0.958
Child–Pugh grade, A/B	303/11	96/30	<0.001	60/5	62/3	0.465
Extent of hepatic resection, major/minor	54/260	13/113	0.069	9/56	8/57	0.794
Operation time (min)	292.8 ± 101.8	283.5 ± 105.1	0.394	277.0 ± 84.3	273.9 ± 76.5	0.585
Blood loss (ml)	380.8 ± 478.4	466.8 ± 633.9	0.123	357.5 ± 415.1	357.8 ± 257.8	0.498
Transfusion	18 (5.7 %)	13 (10.3 %)	0.089	2 (3 %)	2 (3 %)	1
AFP (ng/ml)	4568.3 ± 34234	856.3 ± 3546	0.225	884.7 ± 4657	410.8 ± 994.4	0.788
No. of tumors	1.75 ± 1.88	1.62 ± 0.92	0.44	1.65 ± 1.46	1.63 ± 0.91	0.529
Maximum tumor diameter (mm)	39.7 ± 31.4	32.4 ± 27.7	0.024	33.1 ± 23.3	34.0 ± 24.4	0.407
Vascular invasion	94 (29.9 %)	27 (21.4 %)	0.071	16 (24.6 %)	17 (26.1 %)	0.84

Data are reported for whole study and for the matched study population after propensity score analysis. Continuous variables are expressed as mean ± standard deviation

ATIII anti-thrombin III, *HCV* hepatitis C virus, *T-Bil* total bilirubin, *ICG-R15* indocyanine green retention rate at 15 min, *AFP* alfa-fetoprotein

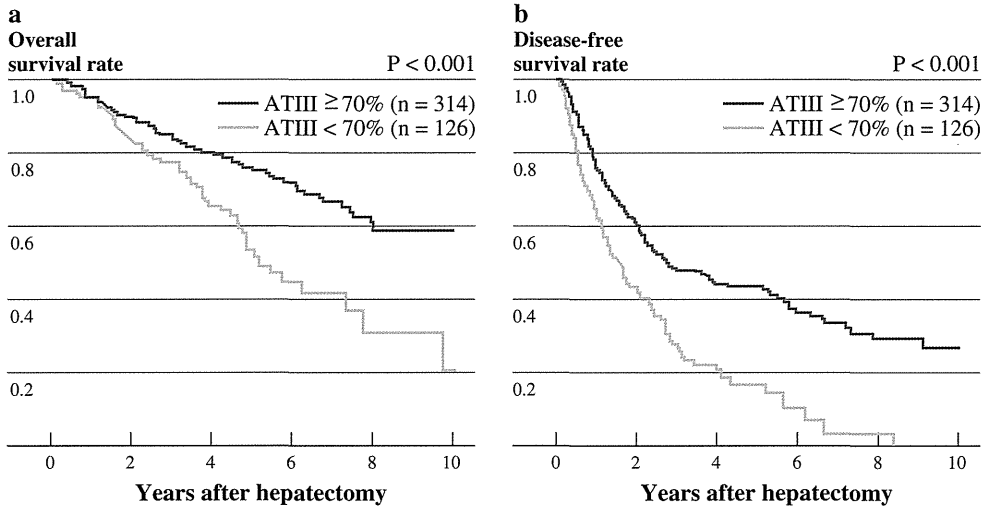

FIG. 1 Outcomes of the entire study population of 440 patients who underwent liver resection for HCC by stratified with the level of ATIII. **a** Kaplan–Meier curves for the overall survival rate after hepatectomy. Overall survival rates of HCC patients with serum ATIII level of more than 70 IU/ml (group 1, $n = 314$) at 3 and 5 years (85.0 and 75.8 %, respectively) were significantly lower than those of the serum ATIII level of <70 IU/ml (group 2, $n = 126$) at 3 and 5 years (77.1 and 53.1 %, respectively) ($P < 0.001$). **b** Kaplan–Meier curves for the disease-free survival rate after hepatectomy. Disease-free survival rates of group 1 at 1, 2, and 3 years (75.3, 60.1, and 48.1 %) were significantly lower than those of the group 2 at 1, 2, and 3 years (63.5, 43.6, and 27.4 %) ($P < 0.001$)

patients was well matched. In particular, all covariates that significantly affected overall survival in the entire study group were equally distributed over the 2 matched groups. Matched patients in groups 1 and 2 had similar anti-HCV antibody positivity (72.3 vs. 69.2 %; $P = 0.699$), PT activity (85.6 vs. 83.8 %; $P = 0.424$), ICG-R15 (18.4 vs. 18.5 %; $P = 0.958$), serum AFP levels (884.7 vs. 410.8 ng/ml; $P = 0.778$), maximum tumor diameter (33.1 vs. 34.0 mm; $P = 0.407$), number of tumors (1.65 vs. 1.63; $P = 0.529$), and microvascular invasion (24.6 vs. 26.1 %; $P = 0.840$). Other clinical variables and tumor characteristics were also similar in both groups. The preoperative ATIII level of patients in group 1 was significantly higher than that of patients in group 2 (82.5 vs. 62.3 %; $P < 0.001$). The postoperative course of the matched study groups is shown in Table 2. Postoperative complications did not differ between the 2 groups. The mean follow-up period ± standard deviation of groups 1 and 2 was 37.8 ± 36.2 and 34.3 ± 31.0 months, respectively. The overall survival rate of patients in group 1 was significantly higher than that of patients in group 2 ($P = 0.005$): in group 1, the 3-, and 5-year overall survival rates were 92.5 and 83.4 % respectively, whereas in group 2, they were 75.8 and 57.1 %, respectively (Fig. 2a). Furthermore, the disease-free survival rate of patients in group 1 was significantly higher than that of patients in group 2 ($P = 0.012$): the 1-, 2-, and 3-year disease-free survival rates were 74.3, 52.6, and 37.0 %, respectively, in group 1 and 55.6, 43.0, and 29.1 %, respectively, in group 2 (Fig. 2b).

Table 2 shows the patterns of cancer recurrence and the treatment details of the recurrences in both groups. The overall recurrence rate in group 1 tended to be lower than that of group 2 (58.5 vs. 73.8 %; $P = 0.064$). Regarding treatment for HCC recurrence, the proportion of patients in whom repeat hepatectomy was selected for treatment in group 1 tended to be higher than that in group 2 ($P = 0.093$). Furthermore, the proportion of patients in whom TACE was selected for treatment in group 1 was significantly lower than that in group 2 ($P = 0.041$).

Table 3 shows the results from the univariate and multivariate analyses of prognostic factors for overall survival in the whole study. Factors found to be significant in the univariate analysis were PT activity, serum ATIII level, serum albumin level, Child–Pugh grade, extension of hepatectomy, operation time, transfusion, serum AFP level, multiple tumors, tumor size, and microscopic vascular invasion. Multivariate analysis revealed that PT activity, serum ATIII level, serum AFP level, multiple tumors, and microscopic vascular invasion were the independent prognostic factors of overall survival. Table 4 shows the results from the univariate and multivariate analyses of prognostic factors for disease-free survival in the whole study. Factors found to be significant in the univariate analysis include HCV antibody, PT activity, serum ATIII level, serum total bilirubin level, serum albumin level, ICG-R15, Child–Pugh grade, operation time, serum AFP level, multiple tumors, and microscopic vascular invasion. Multivariate analysis revealed that PT activity, serum

TABLE 2 Follow-up data including postoperative complications after curative hepatectomy

Characteristic	Whole study series			Propensity matched series		
	ATIII (≥70 U/ml) ($n = 314$)	ATIII (<70 U/ml) ($n = 126$)	P	ATIII (≥70 U/ml) ($n = 65$)	ATIII (<70 U/ml) ($n = 65$)	P
Mean follow-up duration (years)	4.09 ± 2.87	3.55 ± 2.30		4.3 ± 2.56	3.46 ± 2.21	
Operative complications						
Clavien–Dindo grade[a]			0.3			1
IIIa	12 (3.8 %)	6 (4.8 %)		2	2	
IIIb	4 (1.3 %)	2 (1.6 %)		0	0	
IVa	0	2 (1.6 %)		0	0	
IVb	2 (0.6 %)	0		0	0	
V	1 (0.3 %)	1 (0.8 %)		0	0	
90-day mortality	10 (3.2 %)	5 (4.0 %)	0.68	2 (3.1 %)	4 (6.2 %)	0.4
Overall recurrence	168 (53.5 %)	89 (70.6 %)	<0.001	38 (58.5 %)	48 (73.8 %)	0.064
First recurrence time (years)	1.83 ± 1.76	1.59 ± 1.57	0.28	1.63 ± 1.40	1.43 ± 1.46	0.52
Recurrence pattern[a]						
Intrahepatic	130 (41.4 %)	81 (64.3 %)	<0.001	34 (52.3 %)	44 (67.7 %)	0.073
Single	58 (18.5 %)	40 (31.7 %)	0.002	14 (21.5 %)	23 (35.4 %)	0.08
Multiple	72 (22.9 %)	41 (32.5 %)	0.037	20 (30.8 %)	21 (32.3 %)	0.85
Extrahepatic	38 (12.1 %)	8 (6.3 %)	0.075	4 (6.2 %)	4 (6.2 %)	1
Main treatment for first recurrence[b]						
Hepatectomy	43 (25.6 %)	14 (15.7 %)	0.07	16 (42.1 %)	12 (25.0 %)	0.093
RFA	28 (16.7 %)	20 (22.5 %)	0.256	7(18.4 %)	10 (20.8 %)	0.78
PEI	7 (4.2 %)	3 (3.4 %)	0.754	4 (10.5 %)	3 (6.3 %)	0.471
TACE	55 (32.7 %)	36 (40.4 %)	0.546	6 (15.8 %)	17 (35.4 %)	0.041
LDLT	2 (1.2 %)	5 (5.6 %)	0.038	1 (2.6 %)	1 (2.1 %)	0.867
Other	22 (13.1 %)	5 (5.6 %)	0.063	1 (2.6 %)	3 (6.3 %)	0.429
No treatment	11 (6.5 %)	6 (6.7 %)	0.953	0	2 (4.2 %)	0.203

ATIII serum antithrombin III, *RFA* radiofrequency ablation, *PEI* percutaneous ethanol injection, *TACE* transcatheter arterial chemoembolization, *LDLT* living donor liver transplantation

[a] Data are expressed as the number of patients (percentage of total patients)

[b] Data are expressed as the number of patients (percentage of patients with recurrence)

ATIII level, serum AFP level, multiple tumors, and microscopic vascular invasion were the independent prognostic factors of overall survival.

DISCUSSION

To our knowledge, this is the first study that investigates the influence of ATIII on HCC patients using propensity score analysis. The present study demonstrated that when other prognostic variables were appropriately adjusted for, overall and disease-free survival after hepatectomy was significantly prolonged in HCC patients with high preoperative levels of ATIII. Therefore, a low preoperative level of ATIII may be considered a risk factor for tumor recurrence and prognosis. The results of this study are in agreement with certain studies, which showed that a decrease in plasma ATIII levels was a risk factor for tumor recurrence and prognosis in patients with several cancers including lung, colon, ovary, and prostate cancers.[6–8]

The serpin ATIII controls a number of important coagulation enzymes, including factor Xa and thrombin, with the aid of its cofactor, heparin. Heparin activates antithrombin by inducing conformational changes in the protein that specifically enhances binding. While the classical function of ATIII is of an anticoagulant regulator of blood clotting proteinases such as thrombin, recent studies demonstrate its ability to attenuate inflammatory responses by inhibiting cytokines and other inflammatory mediators found within serum and tissue.[3] ATIII has also been reported to suppress the invasion and metastasis of several cancers. Recent studies by Kurata et al.[21] indicate that ATIII prevented hepatic ischemia/reperfusion-induced metastasis of colon cancer cells in a rat model by blocking tumor necrosis factor alpha production. Macrophage inhibitory factor (MIF) has been known to be associated

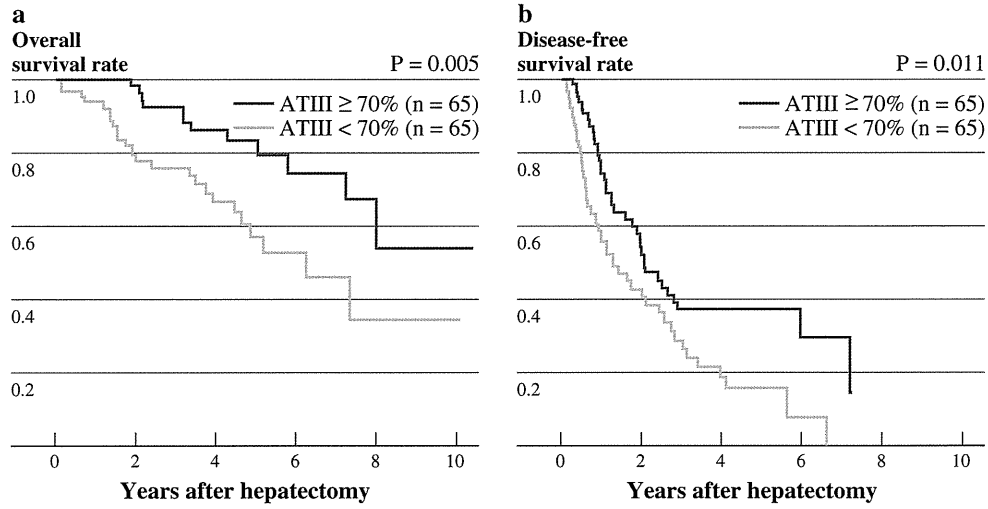

FIG. 2 Outcomes of the matched study population of 130 patients who received liver resection for HCC by stratified with the level of ATIII. **a** Kaplan–Meier curves for the overall survival rate after hepatectomy. Overall survival rates of HCC patients with serum ATIII level of >70 IU/ml (group 1, $n = 65$) at 3 and 5 years (92.5 and 83.4 %, respectively) were significantly lower than those of the serum ATIII level of <70 IU/ml (group 2, $n = 65$) at 3 and 5 years (75.8 and 57.1 %, respectively) ($P = 0.005$). **b** Kaplan–Meier curves for the disease-free survival rate after hepatectomy. Disease-free survival rates of group 1 at 1, 2, and 3 years (74.3, 52.6, and 37.0 %) were significantly lower than those of the group 2 at 1, 2, and 3 years (55.6, 43.0, and 29.1 %) ($P = 0.012$)

with some cancer cell proliferation and invasion. ATIII has been identified as an endogenous MIF-binding protein by forming ATIII–MIF complexes, which reduces MIF biological activity.[22] Recent evidence has shown that thrombin contributes to a more malignant phenotype in vivo by activating tumor-platelet adhesion, tumor adhesion to the subendothelial matrix, tumor implantation, tumor growth, and tumor-associated angiogenesis.[23–25] Kaufmann et al.[26] have shown that some HCC cell lines express a thrombin receptor, proteinase-activated receptor (PAR), and a thrombin-induced increase in HCC cell migration by mediating PAR. Rullier et al.[27] have shown that PAR-1 positive tumor cells are found in HCC. These results suggest that ATIII can suppress proliferation and migration of HCC cells by inhibiting thrombin-induced tumor growth and angiogenesis. It has been also shown that the expression of osteopontin is increased significantly in HCC, and is closely associated with poor prognosis, early recurrence, and metastasis.[28,29] Thrombin cleaves osteopontin into 2 fragments of approximately equivalent size. Osteopontin fragments generated by thrombin cleavage enhance proliferation and adhesion of HCC cells through the activation of integrin β-focal adhesion kinase signaling.[30] Thrombin has been shown to contribute to tumor progression in both a coagulation-dependent and coagulation-independent manner.[31] Further basic and clinical studies are needed to elucidate the antitumor mechanisms of ATIII.

In this study, repeat hepatectomy rather than TACE was selected as a recurrence treatment in more patients with normal level of ATIII, while more patients with decreased level of ATIII underwent TACE rather than hepatectomy for recurrence. This result was thought to be due to high occurrence of early recurrence within 1 year of surgery in patients with decreased level of ATIII. Many cases of recurrence within 1 year after primary hepatectomy are thought to be intrahepatic metastasis from the primary HCC, and survival rate in patients with early recurrence showed worse outcome.[32,33] In our series, most patients who had early recurrence within 1 year of primary hepatectomy were unlikely to receive repeat hepatectomy. Portolani et al.[2] have reported that curative treatment including surgery, percutaneous ethanol injection, and radiofrequency ablation, was feasible in 29.3 % in the early recurrence, while it was 67.6 % in the late recurrence: the proportion of patients who underwent curative treatment for HCC recurrence was significantly higher in the late recurrence than in the early recurrence ($P < 0.05$).

In this study, we have chosen the cutoff for ATIII based on the lower limit of normal level. The optimal cutoff values of ATIII for survival and recurrence determined by ROC curve analysis were 69 and 72 %, respectively. These results indicate that the cutoff value of ATIII with 70 % is valid in this study, and these results are consistent with that normal ATIII is defined as a level of $\geq$70 %.

Before matching by using the propensity score, the clinical characteristics of the entire study population that can strongly influence outcomes differed significantly between the 2 groups. The proportion of patients with better liver function was higher in group 1 than in group 2, and the proportion of patients with advanced HCC also

TABLE 3 Univariate and multivariate analysis of predictive variables of overall survival in the whole study

Variable	Univariate analysis		Multivariate analysis		
	5-year survival rate (%)	*P*	Hazard ratio	95 % CI	*P*
Age					
≥70 ($n = 167$); <70 ($n = 273$)	68.2; 69.7	0.959			
Gender					
Male ($n = 323$); female ($n = 117$)	69.9; 68.9	0.646			
Anti-HCV antibody					
Positive ($n = 279$); negative ($n = 161$)	68.5; 70.1	0.357			
Prothrombin time (%)					
≥80 ($n = 307$); <80 ($n = 133$)	77.0; 56.7	<0.001	1.62	1.069–2.457	0.023
ATIII (%)					
≥70 ($n = 314$); <70 ($n = 126$)	75.8; 53.1	<0.001	1.596	1.012–2.516	0.044
T-Bil (mg/dl)					
≤1.0 ($n = 307$); >1.0 ($n = 133$)	72.1; 63.0	0.196			
Albumin (g/dl)					
≥4.0 ($n = 332$); <4.0 ($n = 108$)	71.7; 61.7	0.048	1.034	0.621–1.719	0.898
ICG-R15 (%)					
>15 ($n = 213$); ≥15 ($n = 227$)	73.2; 65.6	0.093	1.088	0.729–1.623	0.681
Child–Pugh grade					
A ($n = 399$); B ($n = 41$)	71.9/50.8	0.001	0.792	0.418–1.501	0.475
Extend of hepatic resection					
Major ($n = 373$); minor ($n = 67$)	70.5; 61.2	0.023	0.751	0.449–1.283	0.237
Operation time (h)					
≥6 ($n = 280$); >6 ($n = 180$)	73.2; 62.5	0.008	0.844	0.553–1.289	0.433
Blood loss (ml)					
<1,000 ($n = 34$); ≥1,000 ($n = 406$)	69.9; 60.2	0.065	1.3	0.607–3.402	0.414
Transfusion					
No ($n = 409$); yes ($n = 31$)	71.2; 40.0	<0.001	0.641	0.293–1.296	0.122
AFP (ng/ml)					
≤100 ($n = 313$); >100 ($n = 127$)	76.2; 53.2	<0.001	0.571	0.417–0.924	0.004
No. of tumor					
Single ($n = 286$); multiple ($n = 154$)	77.0; 54.7	<0.001	0.532	0.386–0.824	0.001
Tumor size (5 cm)					
≥5 ($n = 365$); <5 ($n = 75$)	70.8; 62.3	0.033	0.917	0.505–1.664	0.776
Vascular invasion					
No ($n = 319$); yes ($n = 121$)	76.1; 51.8	<0.001	2.05	1.303–2.901	0.0001

HCV hepatitis C virus, *ATIII* anti–thrombin III, *T-Bil* total bilirubin, *ICG-R15* indocyanine green retention rate at 15 min, *AFP* alfa-fetoprotein

tended to be higher in the group 1 than in the group 2. To overcome bias due to the different distribution of the severity of liver function impairment between the 2 groups, a one-to-one match was created using propensity score analysis. After matching by propensity score, prognostic variables were appropriately handled, and there was no significant difference in prognostic factors excluding ATIII between the 2 matched groups. This study had a limitation related to the small sample size after propensity score matching. Two hundred forty-nine patients in group 1 and 61 patients in group 2 were excluded by propensity score matching, because their propensity scores could not be matched. Thus, further examination with a larger number of patients may be necessary.

Multivariate analysis agreed with that in previous publications, showing that vascular invasion, multiple tumors, and tumor marker such as AFP were independent prognostic factors associated with overall and disease-free survival rates. These results were compatible with previous reports.[34,35] Regarding with liver function, PT activity and

TABLE 4 Univariate and multivariate analysis of predictive variables of disease-free survival in the whole study

Variable	Univariate analysis		Multivariate analysis		
	5-year survival rate (%)	*P*	Hazard ratio	95 % CI	*P*
Age					
≥70 (n = 167); <70 (n = 273)	36.4; 39.7	0.377			
Gender					
Male (n = 323); female (n = 117)	36.6; 39.2	0.357			
Anti-HCV antibody					
Positive (n = 279); negative (n = 161)	34.4; 45.9	0.0164	0.728	0.482–1.101	0.133
Prothrombin time (%)					
≥80 (n = 307); <80 (n = 133)	47.5; 20.5	<0.001	1.621	1.074–2.447	0.021
ATIII (%)					
≥70 (n = 314); <70 (n = 126)	45.7; 20.0	<0.001	1.596	1.012–2.516	0.044
T-Bil (mg/dl)					
≤1.0 (n = 307); >1.0 (n = 133)	43.1; 29.1	0.023	1.009	0.677–1.505	0.965
Albumin (g/dl)					
≥4.0 (n = 332); <4.0 (n = 108)	42.3; 27.4	<0.001	1.043	0.630–1.727	0.871
ICG-R15 (%)					
>15 (n = 213); ≥15 (n = 227)	46.9; 30.3	<0.001	1.088	0.729–1.623	0.681
Child–Pugh grade					
A (n = 399); B (n = 41)	40.6; 21.3	0.034	0.518	0.438–1.517	0.518
Extent of hepatic resection					
Major (n = 373); minor (n = 67)	37.5; 43.6	0.349			
Operation time (h)					
≥6 (n = 280); >6 (n = 180)	40.1; 35.8	0.018	0.84	0.559–1.261	0.4
Blood loss (ml)					
<1000 (n = 34); ≥1000 (n = 406)	40.6; 38.4	0.276			
Transfusion					
No (n = 409); yes (n = 31)	41.0; 38.4	0.262			
AFP (ng/ml)					
≤100 (n = 313); >100 (n = 127)	39.4; 27.0	0.004	0.568	0.386–0.836	0.004
No. of tumors					
Single (n = 286); multiple (n = 154)	43.9; 29.1	<0.001	0.555	0.379–0.811	0.002
Tumor size (5 cm)					
≥5 (n = 365); <5 (n = 75)	37.7; 48.0	0.372			
Vascular invasion					
No (n = 319); yes (n = 121)	76.1; 39.2; 28.751.8	0.004	2.031	1.368–3.015	0.0001

HCV hepatitis C virus, *ATIII* anti–thrombin III, *T-Bil* total bilirubin, *ICG-R15* indocyanine green retention rate at 15 minutes, *AFP* alfa-fetoprotein

ATIII level were significant factors in multivariate analysis. Operative variables such as extension of hepatectomy, blood loss, and transfusion, were associated with poor outcomes in univariate analyses, but these factors were not significant factors in the multivariate analysis.

In conclusion, one-to-one matching study using propensity scores and multivariate analysis showed that the ATIII level was associated with favorable outcomes in HCC patients after curative hepatectomy. ATIII may be useful for predicting outcomes of patients with HCC after curative hepatectomy.

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Safety and Feasibility of Diet-Treated Donors With Steatotic Livers at the Initial Consultation for Living-Donor Liver Transplantation

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Background. The purpose of this study was to evaluate both safety of diet-treated donors and the feasibility of their use for living-donor liver transplantation (LDLT).

Methods. A total of 128 living donors were enrolled in this study between April 2003 and March 2010. Of them, 41 were diagnosed with hepatic steatosis at the initial consultation. Donor selection was based on the findings of liver biopsy accompanied with normalization of liver function tests after diet treatment consisting of an 800 to 1400 kcal/day diet and a 100 to 400 kcal/day exercise without drug treatment, targeting body mass index of 22 kg/m^2.

Results. Body mass index of diet-treated donors was significantly reduced with diet from 23.3±0.6 to 21.9±0.4 kg/m^2 (P<0.0001). Liver function tests associated with fatty liver, including alanine aminotransferase, gamma-glutamyl transpeptidase, and total cholesterol levels, also improved with diet (P=0.0128, 0.0016, and 0.0004, respectively). The liver biopsy results of most of these donors showed stage 0/1 fibrosis and minimal/mild steatosis after the diet therapy. Surgical outcomes, including postoperative liver function tests, perioperative complications, and liver regeneration rates, did not significantly differ between nondiet-treated and diet-treated donors. Surgical outcomes and the overall survival did not significantly differ between recipients of grafts from nondiet-treated and diet-treated donors.

Conclusion. The use of diet-treated donors for living-donor liver transplantation is feasible with respect to donor safety and the outcome of the recipient when strict selection criteria are used.

Keywords: Diet, Steatosis, Living donor liver transplantation, Biopsy, Fatty liver.

(*Transplantation* 2012;93: 1024–1030)

Liver transplantation is the only treatment option for patients with end-stage liver disease. However, the shortage of organs remains a serious problem, and annual death rates per 1000 patient-years at risk is 113.6 while on the waiting list (United Network for Organ Sharing at www.unos.org. accessed in May 2009). Many liver transplantation centers have been forced to modify their criteria for acceptable donors to increase the donor pool. A modified extended criteria donor has been applied to deceased-donor liver transplantation (DDLT), including older donors, donors with prolonged ischemia, donation after cardiac death, those with liver infected with certain viruses, obese donors, and those with steatotic (fatty) livers (*1*).

Implantation of donor livers with severe fatty infiltration is frequently associated with a high incidence of severe ischemic damage, resulting in primary dysfunction and/or primary nonfunction after DDLT (*2–6*).

Meanwhile, living-donor liver transplantation (LDLT) has been accepted and established as an alternative to DDLT (*7*) since it was first successfully performed in 1989 (*8*). Soejima et al. (*9*) described the feasibility of using a steatotic graft even in LDLT with respect to primary nonfunction and reported the effectiveness of short-term treatments consisting of a protein-rich diet, exercise, and bezafibrate for 2 to 8 weeks for donors with a fatty liver (*10*). The obvious advantages of LDLT are the reduction in the mortality of patients on the transplant waiting list and the provision of sufficient preparation time, which is a great merit in scheduling the transplantation (*11*, *12*). In our institute, a candidate of living donors with a fatty liver at the initial

This work was supported by Grants-in-Aid for Scientific Research No. 21791289 from the Japan Society for the Promotion of Science.

The authors declare no conflicts of interest.

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Received 19 April 2011. Revision requested 10 May 2011.

Accepted 23 January 2012.

ISSN: 0041-1337/12/9310-1024

DOI: 10.1097/TP.0b013e31824c9e25

TABLE 1. Effects of diet on donors

	Nondiet treated (N=87)		Diet treated (N=41)		
		P	Initial consultation	*P*	Postdiet
BMI (kg/m^2)	21.8±0.3	0.0163	23.3±0.6	< 0.0001	21.9±0.4
T. Bil (mg/dL)	0.9±0.0	0.2870	0.8±0.1	0.2556	0.8±0.1
D. Bil (mg/dL)	0.1±0.0	0.3256	0.1±0.1	0.2323	0.1±0.1
AST (IU/L)	18±1	0.0016	22±1	0.1042	20±1
ALT (IU/L)	18±1	0.0007	28±3	0.0128	21±1
γ-GTP (IU/L)	24±2	0.0003	41±6	0.0016	28±4
PT-INR	0.98±0.01	0.1006	0.96±0.01	0.0435	0.98±0.01
Alb (g/dL)	4.8±0.0	0.9389	4.8±0.1	0.0074	4.7±0.1
T-cho (mg/dL)	186±4	0.0002	213±6	0.0004	173±9
TG (mg/dL)	80±5	0.0021	110±9	0.6506	108±13

Continuous variables are expressed as means±standard error.

BMI, body mass index; T. Bil, total bilirubin; D. Bil, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; PT-INR, prothrombin time-international normalized ratio; Alb, albumin; T-cho, total cholesterol; TG, triglyceride.

consultation in the outpatient clinic is examined for his or her potential as a donor after administering a diet treatment. Herein, we refer to these donors as "diet-treated donors." Few studies have analyzed the outcomes of LDLT using diet-treated donors with steatotic livers (*13*).

The aim of this study was to evaluate both safety of the donors and the outcomes of the recipients undergoing LDLT from diet-treated donors.

RESULTS

Effects of Diet on Donors

A total of 87 donors did not receive diet treatment (nondiet-treated donors), and 41 donors were treated with a diet (diet-treated donors). The mean body mass index (BMI) and the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GTP), total cholesterol (T-cho), and triglyceride (TG) were significantly higher in diet-treated donors at the initial consultation than in nondiet-treated donors. After the diet, BMI was significantly reduced from 23.3±0.6 to 21.9±0.4 kg/m^2 (P<0.0001) for a median period of 2.9 (range, 0.2–13.6) months, which was limited by the critical status of the recipients. Factors associated with hepatic steatosis, including ALT, γ-GTP, and T-cho levels, also improved with the diet treatment (P=0.0128, 0.0016, and 0.0004, respectively), whereas the level of albumin decreased significantly (P=0.0074) (Table 1).

The results of the preoperative liver biopsy are presented in Table 2. In most of the diet-treated donors, a liver biopsy performed after the diet showed stage 0/1 fibrosis and minimal or mild steatosis. One diet-treated donor had stage 2 perisinusoidal/pericellular fibrosis and a minimal grade of macrovesicular steatosis. No complications associated with liver biopsy were reported.

Preoperative Characteristics of Donors and Recipients

The diet-treated donors were significantly older than the nondiet-treated donors (40.2±1.6 years vs. 35.5±1.4 years, P=0.0484). The mean values of BMI, total bilirubin (T. Bil), AST, ALT, and prothrombin time-international normalized ratio (PT-INR) of the donors measured just before the operation were comparable between the two groups. Although the model for end-stage liver disease (MELD) score was not significantly different between the two groups, it was likely to be higher in the recipients of grafts from nondiet-treated donors than in those of grafts from diet-treated donors (18.1±0.9 vs. 15.2±1.1, P=0.0552) (Table 3). In those of grafts from diet-treated donors, mean MELD score was increased from 13.3 to 15.2 during the diet period.

Surgical Demographics of Donors and Recipients

There were no significant differences between the two groups with respect to graft type and surgical data of donors and recipients, including operative time, blood loss, blood transfusions, graft-to-recipient weight ratio, and cold ischemic time (Table 4).

Donor Postoperative Data

There were no significant differences in perioperative laboratory data on T. Bil, AST, and ALT. Just PT-INRs on postoperative days 1, 2, and 3 were significantly higher in nondiet-treated donors than in diet-treated donors. However, there were no significant differences after postoperative day 5 (Fig. 1). Perioperative complications categorized according to the Clavien's grading system (*14*) showed no

TABLE 2. Results of the liver biopsy

	Stage				
Grade	0	1	2	3	4
Minimal	9	29	1	0	0
Mild	0	2	0	0	0
Moderate	0	0	0	0	0
Severe	0	0	0	0	0

Minimal, ≤10%; mild, 11%–20%; moderate, 21%–30%; severe, >30%.

TABLE 3. Preoperative demographics of donors and recipients

	Nondiet treated (N=87)	Diet treated (N=41)	*p*
Donor			
Age	35.5±1.4	40.2±1.6	0.0484
Gender			
Male	52	27	0.5088
Female	35	14	
Body weight (kg)	59.5±1.1	60.1±1.4	0.7191
BMI (kg/m^2)	21.8±0.3	21.9±0.4	0.7657
Liver function test			
T. Bil (mg/dL)	0.9±0.0	0.8±0.1	0.6782
AST (IU/L)	18±1	20±1	0.1212
ALT (IU/L)	18±1	21±1	0.1088
PT-INR	0.98±0.01	0.98±0.01	0.6924
Relation to the recipient			
Child	50	22	0.2146
Spouse	14	11	
Sibling	11	7	
Parent	8	1	
Others (son in law, niece, and nephew)	4	0	
Recipient			
Age	52.5±1.1	52.8±1.5	0.8715
Gender			
Male	54	26	0.8833
Female	33	15	
Body weight (kg)	63.5±1.3	64.3±2.0	0.7253
Indications			
HCC	37	23	
LC due to HCV	17	5	
FHF	7	1	
LC due to alcohol abuse	4	3	
LC due to HBV	4	2	
Secondary biliary cirrhosis	4	0	
PBC	3	3	
PSC	3	0	
AIH	3	1	
Wilson disease	1	1	
Liver failure posthepatectomy	1	1	
NASH	1	0	
Metastatic liver tumor (insulinoma)	1	0	
Retransplantation	1	0	
Budd-chiari syndrome	0	1	
MELD score	18.1±0.9	15.2±1.1	0.0552
ABO incompatibility			
Identical/compatible	80	35	0.2496
Incompatible	7	6	

Continuous variables are expressed as means±standard error.

BMI, body mass index; T. Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT-INR, prothrombin time-international normalized ratio; HCC, hepatocellular carcinoma; LC, liver cirrhosis; HCV, hepatitis C virus; FHF, fulminant hepatic failure; HBV, hepatitis B virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AIH, autoimmune hepatitis; NASH, nonalcoholic steatohepatitis; MELD, Model for End-Stage Liver Disease.

significant differences between the two groups. Perioperative complications in patients with a Clavien grade III or higher included an intraabdominal hematoma in one nondiet-treated donor, biliary leakages in two nondiet-treated donors, and a biliary stenosis in one diet-treated donor. For the right lobe graft, liver regeneration rates on postoperative day 7 were 1.41±0.03 in nondiet-treated donors and 1.44±0.04 in diet-treated donors (P=0.574). For the remaining grafts,

liver regeneration rates were also comparable between the two groups.

Overall Survival in Recipients

There were no significant differences in overall survival between recipients of grafts from nondiet-treated and diet-treated donors. The 1-, 3-, and 5-year survival rates were 79%, 74%, and 70% for recipients of grafts from nondiet-treated donors, whereas the corresponding values were 68%, 68%, and 68% for recipients of grafts from diet-treated donors, respectively (P=0.455).

Biliary Complications in Recipients

Biliary complications in the recipients, including stricture, leakage, and stricture after leakage, showed no statistically significant differences between nondiet-treated and diet-treated donors. The number of patients with biliary diversion was also comparable (data not shown).

DISCUSSION

The condition of both donors and recipients is a critical issue in LDLT. Although safety of donors should be of the highest priority (*15*, *16*), there is considerable controversy with respect to that of extended criteria donors. In particular, it has not been well elucidated if fatty liver affects the donor safety, whereas steatotic liver grafts have been well analyzed and there is still controversy regarding the outcome of recipients (*4–6*).

The incidence of obesity has increased dramatically in developed countries in the last few decades. There has also been a simultaneous rise in the frequency of metabolic syndrome. Nonalcoholic fatty liver disease is characterized by an elevated intrahepatic TG content, with varying degrees of inflammation and fibrosis. A clear differentiation between a simple fatty liver and nonalcoholic fatty liver disease is difficult in the absence of liver biopsy results. Macrovesicular steatosis can lead to inflammation and fibrosis, and the likelihood of graft damage in recipients of a liver graft from a donor with macrovesicular steatosis is high (*17*, *18*). Therefore, the criteria of fatty liver were widened in this study. They were based only on the imaging studies including computed tomography (CT) and/or ultrasound. Oliva et al. (*19*) reported that liver-spleen ratio of less than 1.2 covered all the cases with fatty liver, whereas some authors underlined 1.0 or 1.1 as the cutoff line for fatty liver (*20*, *21*). The authors followed the criteria of Oliva et al. Ruhl and Everhart (*22*) reported that the proportion of elevated ALT activity due to excess weight and obesity (BMI>25 kg/m^2) was 65%. Rinella et al. (*23*) reported a significant correlation between BMI and the degree of macrovesicular steatosis and found that patients with a BMI of less than 25 kg/m^2 did not show macrovesicular steatosis. Moreover, Peng et al. (*24*) reported that patients with a BMI of less than 23 kg/m^2 were likely to display no or mild steatosis. Consequently, the target BMI value in this study was set to 22 kg/m^2. In this study, the results of liver function tests related to hepatic steatosis were significantly improved after the diet treatment. In addition, the histopathological results of the liver biopsies performed after the diet treatment showed less than 20% of macrovesicular steatosis. The main objective of the liver biopsy is to ensure donor safety, which is considered more important than the preservation of graft function (*25*).

TABLE 4. Surgical demographics of donors and recipients

	Nondiet treated (N=87)	Diet treated (N=41)	*p*
Donor			
Graft type			
Right lobe without MHV	68	29	0.4509
Left lobe with MHV	17	10	
Left lobe without MHV	1	0	
Posterior section	1	2	
Operative time (min)	408±7	409±10	0.9253
Blood loss (mL)	227±15	241±35	0.6772
Allogenic blood transfusion	0	0	
Autologous blood transfusion			
Yes	6	5	0.3183
No	81	36	
Recipient			
Operative time (min)	725±14	752±17	0.2417
Blood loss (mL)	4153±272	4566±612	0.4755
PRBC (U)	7.6±0.8	8.4±1.6	0.6323
FFP (U)	6.6±0.9	5.7±1.2	0.5765
GW (g)	581±14	609±26	0.3109
GRWR (%)	0.94±0.02	0.96±0.05	0.6632
CIT (min)	98±4	100±5	0.7346

Continuous variables are expressed as means±standard error.

MHV, middle hepatic vein; PRBC, packed red blood cell; FFP, fresh-frozen plasma; GW, graft weight; GRWR, graft-to-recipient weight ratio; CIT, cold ischemic time.

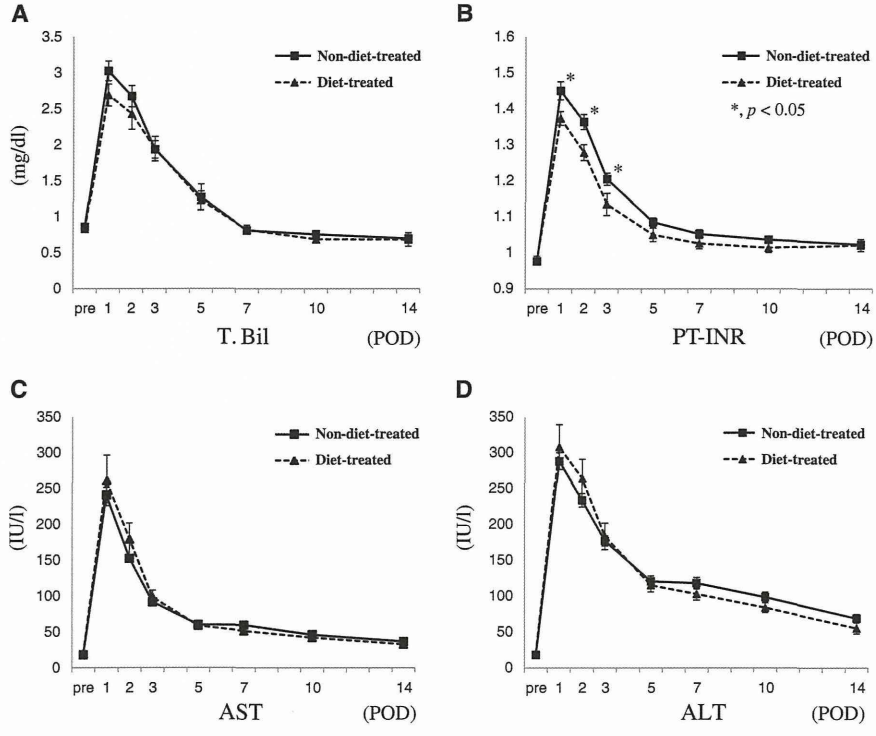

FIGURE 1. Perioperative data on donors. (A) T. Bil. (B) PT-INR. (C) AST. (D) ALT. T. Bil, total bilirubin; PT-INR, prothrombin time-international normalized ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Nakamuta et al. (*10*) reported the effectiveness of a short-term intensive treatment protocol for donors with steatosis. However, the donors in that study were subjected to two invasive liver biopsies. Although a liver biopsy performed before the start of a treatment can be useful to assess the effects of the treatment, it is not necessary for the final decision of inclusion of the donor. In this study, donors were treated with a diet with the target of achieving a BMI of 22 kg/m^2. They were subjected to only one biopsy with the exception of one donor who did not meet the diet goal. In addition, only one candidate was excluded for the safety because repeated liver biopsy revealed the findings of inflammation. The numbers of diet-treated donors in this study are much larger than those reported by Nakamuta et al. The most appropriate method and diet period to ensure successful LDLT are yet to be determined.

With respect to safety of donors, moderate or severe macrovesicular steatosis is generally considered among the exclusion criteria to prevent complications (*25–27*). Consistent with this strategy, postoperative laboratory data, including T. Bil, AST, and ALT levels, and perioperative complications graded according to Clavien's scale were comparable between the nondiet-treated and diet-treated groups. Only PT-INRs on postoperative days 1, 2, and 3 were significantly higher in the nondiet-treated donors than in the diet-treated donors. Although we cannot provide a clear explanation for this difference, an association between the condition of the liver after diet and coagulation disorders is suspected and should be investigated.

The relationship between macrovesicular steatosis and remnant liver regeneration after hepatectomy remains unclear (*27–30*). The present data indicate that steatosis up to mild macrovesicular infiltration does not impair liver regeneration after hepatectomy.

To summarize the results of donors, diet-treated donors are going well, compared with nondiet-treated donor. However, attention should be paid continuously that donor mortality can occur in the high-risk donor candidate.

Although Hayashi et al. (*13*) reported successful results in recipients of grafts from five diet-treated donors, they did not compare the outcome of recipients of grafts from diet-treated donors with that of recipients of grafts from nondiet-treated donors. In the present series, there were no significant differences in overall survival between the two groups, although survival in the nondiet-treated group was slightly better than that in the diet-treated group. Factors including donor age, preoperative MELD score in the recipients, ABO incompatibility, and other factors might affect the overall survival of the recipients. The limited size of the group included in this study makes it difficult to draw firm conclusions with respect to the impact of the use of diet-treated donors on overall survival.

Biliary complications are still considered the Achilles' heel of liver transplantation. Baccarani et al. (*31*) reported that a steatotic graft with more than 25% of macrovesicular infiltration is a risk factor for the development of biliary complications. In our series, there were no significant differences in biliary complications between the two groups, which could be attributed to the strict selection criteria, thus emphasizing that liver biopsy results after the diet treatment should show less than 20% of macrovesicular steatosis with minimal perisinusoidal fibrosis.