表2　移植後再発症例

年齢	性別	Stage	ミラノ	個数	最大径(cm)	再発時期(月)	再発部位	治療	転帰(月)
46	男	Ⅲ	外	5	5	26	肺	切除	生存 (66)
31	男	Ⅲ	外	4	4	8	肺	放射線	死亡 (29)
59	男	Ⅲ	外	15	5.5	3	脳	切除・放射線	死亡 (17)
23	女	Ⅲ	外	23	22.5	8	横隔膜	化学療法	死亡 (28)
27	女	Ⅰ	内	4	2.1	44	肝	PEIT	生存 (48)
54	男	Ⅲ	内	3	4	7	副腎・骨	TAE・放射線	死亡 (17)
62	女	Ⅲ	外	7	3.5	3	肺	切除	死亡 (23)
59	男	Ⅲ	内	4	5.2	14	肝	RFA	死亡 (36)
56	男	Ⅱ	内	2	4.9	6	腹腔リンパ節		死亡 (7)
30	男	Ⅲ	外	9	9	22	肝	化学療法	死亡 (30)
54	男	Ⅱ	外	96	1.8	16	骨	放射線	生存 (20)
60	男	Ⅱ	内	15	3.0	18	骨	切除	生存 (20)
54	男	Ⅲ	外	380	4	6	骨	RFA	生存 (14)
51	女	Ⅲ	外	40	3	6	肺	化学療法	生存 (8)

Stageとミラノ基準は術前画像診断から判定，腫瘍の個数と最大径（cm）は摘出肝病理所見による．

例に生体肝移植を行った．患者背景（表1）では，男女比が71：32，年齢が22～69歳（中央値54歳）で，HCVまたはHBV関連肝硬変の合併が94例に見られた．移植前肝機能はChild-Pugh分類でAが16例，Bが38例，Cが49例で，MELD Scoreは10以下が21例，11～20が53例，21～30が24例，31以上が5例で中央値は14であった．原発性肝癌取扱い規約による術前の進行度分類ではStage Ⅰ，Ⅱ，Ⅲ，Ⅳ-Aがそれぞれ14，35，42，2例であった．脈管浸潤症例を除外しているため原則的にはStage Ⅳ-Aは適応外であるが，若年患者のために（22歳と30歳）例外的にVp3の腫瘍に対して2例の肝移植を行った．また，術前画像診断では腫瘍が指摘されなかった，または前治療によって腫瘍が完全にコントロールされていると診断されていた症例で，肝硬変を適応として移植を行ったところ摘出肝病理検査でviableな腫瘍が見つかった（incidental）症例が10例含まれている．このincidental 10例を除くと，ミラノ基準を満たす症例が50例，超える症例が43例であった．肝細胞癌が初発で肝移植が最初の治療であった症例は24例で，残り79例（77％）は移植前に他の治療を受けており，3回以上の治療歴のある症例は約半数（46例）を占めていた．再発予防対策として，Stage Ⅲ以上の症例には，ファルモルビシン10 mg/m²を術中から投与し，術後も体力の回復を待って可及的早期より同量を週に1度，10回まで投与することにしている．移植後観察期間は1～68カ月（中央値32カ月）である．

3．移植後生存率

全103例の4年累積生存率は65％であった（図1）．Incidental tumorを除く93例で術前診断においてミラノ基準を満たす症例（50例）と基準を超える症例（43例）を比較すると（図2），4年生存率はそれぞれ66％と61％であり，統計学的には有意差を認めなかった．

4．移植後再発

移植後の肝細胞癌再発はこれまで14例に見られ（表2），非癌死を打ちきりとした場合の全体の累積再発率は4年で25％であった．最初に再発が発見された臓器は，肺が4

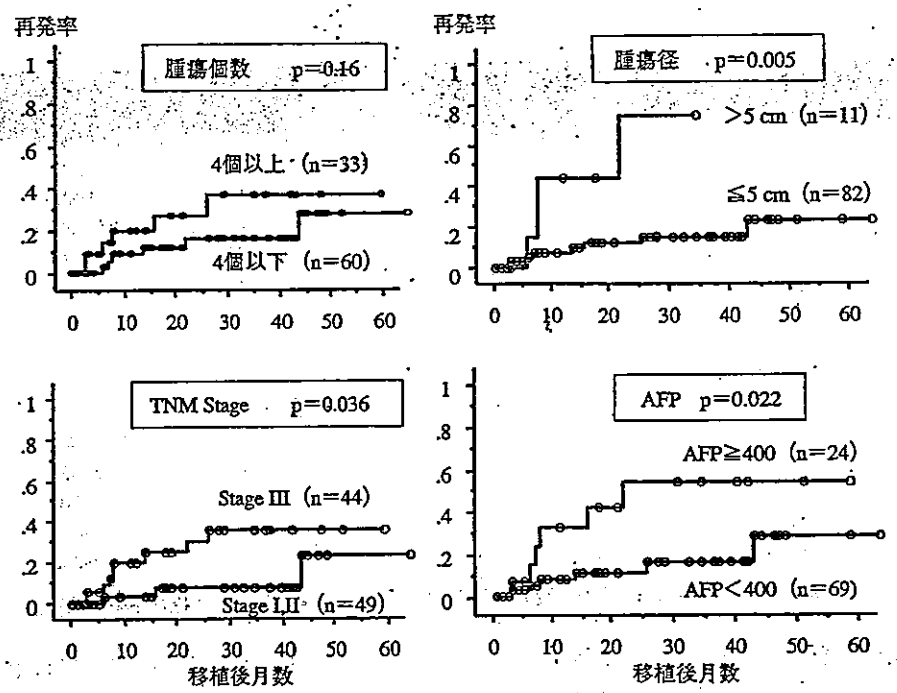

図3 術前腫瘍因子と移植後累積再発率
incidental 症例 (n = 10) を除くと，腫瘍最大径が 5 cm 超，進行度が stage Ⅲ，AFP 値が 400 ng/ml 以上の場合に有意に再発率が高値であった．

例，移植肝が3例，骨が3例で，その他脳，副腎，横隔膜局所，腹部リンパ節であった．再発病巣に対して切除や化学療法，放射線療法など積極的に治療しているが，再発後長期生存例は稀であり，特に移植後1年以内に再発した症例の予後は極めて悪い．

術前の腫瘍因子と再発率の関係を見ると(図3)，腫瘍最大径が5 cm超，進行度がstage Ⅲ，AFP値が400 ng/ml以上の場合に有意に再発率が高値であった．術前ミラノ基準を満たした群と超えた群で4年再発率を比較すると(図4)，19 % vs 35 %で有意差が見られた($p < 0.05$)．ただし術前画像診断と摘出肝の病理所見が異なる場合もあり，ミラノ基準内で再発した5例も摘出肝でみるといずれも基準を超えていた(表2)．

摘出肝での病理所見で再発の危険因子を検討したところ，腫瘍個数が4個以上，最大径が5 cm超，組織型が低分化，脈管浸潤陽性などが単変量解析で有意な危険因子であった．これらをCox比例ハザードモデルによる多変量解析にかけると，個数4個以上(risk ratio 12.048, $p = 0.004$)と組織型が低分化(risk ratio 4.385, $p = 0.011$)が有意の独立した再発危険因子と判断された．

4 考 察

生体肝移植後の肝細胞癌再発について最近いくつかの報告が見られるようになってきた[6~8]．The Mount Sinai Hospitalの36例の報告では19例(53 %)がミラノ基準(UNOS priority criteria)を超えており，平均観察期間450日でこれまで6例(うちミラノ基準外4例)に移植後再発を認めている[7]．われわれの初期の報告(2002年3月までの56例)ではミラノ基準外が25例(45 %)で，移植

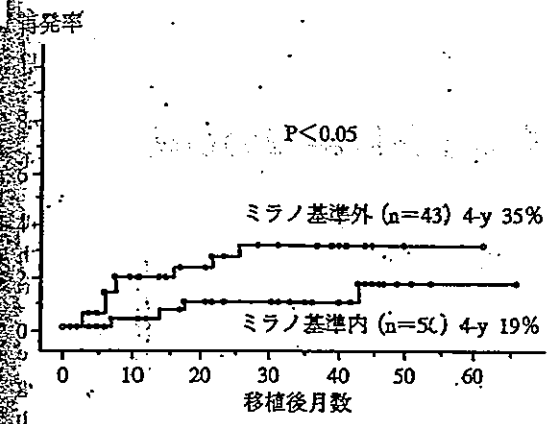

図4　ミラノ基準と移植後累積再発率
incidental 症例 (n = 10) を除くと，4 年再発率はミラノ基準内群 (n = 50)，基準外群 (n = 43) でそれぞれ 19 %，35 %であった (p = 0.048，log-rank test)．

後再発を 6 例に認めそのうちミラノ基準外症例が 5 例を占めていたが，統計的にはミラノ基準内外で再発率に有意差を認めていなかった．しかし，症例数が増加した今回の検討では，ミラノ基準を超えると再発の危険率が有意に高くなることが明らかとなり (35 % vs 19 %)，さらに術前所見で最大腫瘍径が 5cm を超える，進行度分類で Stage Ⅲ以上，AFP 値が 400 ng/ml 以上などが術後再発と関連した因子であることが示された．

一方，これら術前画像所見と実際の摘出肝の病理所見に相違が見られる場合もあり，特に最近の MD-CT 導入以前は小さな病変の描出感度が低く，腫瘍個数が過小評価されている症例が少なくなかった[6]．そのため摘出肝所見と術後再発の関係について単変量解析にて検討すると，腫瘍個数が 4 個以上という所見も含めて表 3 に示す腫瘍因子が有意な再発危険因子と判断され，これまでの脳死肝移植の解析[3,9-11]と同様の結果であった．肝移植後の肝細胞癌再発の機序を考察すると，全肝摘出以前に血行性またはリンパ行性に肝

表3　病理学的腫瘍因子と再発危険度
(Cox 比例ハザードモデルによる多変量解析)

因子	risk ratio	95% CI	P value
個数≧4個	12.048	2.155-66.666	0.004
最大径＞5 cm	1.626	0.434-6.060	0.471
組織型低分化	4.385	1.394-13.698	0.011
脈管浸潤陽性	1.148	0.322-4.081	0.831

外へ遊離または転移した癌細胞が移植後増大するものと考えられるが，組織学的脈管浸潤陽性はこのような進展様式を直接的に示唆する所見と考えられる．今回多変量解析の結果，組織学的分化度が低分化とならんで腫瘍個数が 4 個以上という所見が独立した有意の再発危険因子と判断されたが，同じ多発病変でも肝内転移と多中心性発癌では再発危険度は大きく異なるものと推測され，今後詳細な検討が必要と考えられる．

今回の検討では，ミラノ基準を超えると確かに再発率は有意に上昇するが，生存率には有意差を認めなかった．これは手術関連の合併症による移植後早期死亡が少なくないこととも関連しているが，今後追跡期間の延長と症例数の増加に伴い生存率にも有意差が見られるようになると予想される．事実，日本全国の移植施設へのアンケート調査の結果では[8]，2003 年 12 月までに 29 施設で 316 例の肝細胞癌に対する生体肝移植が実施されたが，移植後 3 年生存率はミラノ基準内症例で 79.4 %，基準外症例で 60.0 % (P = 0.0222) と有意差が認められた．しかし一方で，今回の検討ではミラノ基準外症例 43 例のうち実際に 9 名が 3 年以上無再発で生存している (無再発生存率 56 %) という結果も見過ごせない．今後はこのような知見を基に肝細胞癌に対する生体肝移植の適応を考えていかなければならないが，できるだけ多くの

患者の救命を図るという立場に立つならばミラノ基準を超えて適応基準を拡大することは可能であると思われる．生体ドナーのリスク，負担という問題に対して倫理的，医学的に慎重に対処したうえで，ミラノ基準を超える進行肝細胞癌患者にとっても根治性が望める治療法として生体肝移植の有効性を今後も検討していく必要がある．

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特集

肝細胞癌
(第104回日本外科学会総会より)

当科における肝癌に対する生体肝移植の成績

上田幹子*1　高田泰次*1　田中紘一*1

Living Donor Liver Transplantation for Hepatocellular Carcinoma at Kyoto University Hospital: Ueda M, Takada Y and Tanaka K (Dept of Transplantation and Immunology, Faculty of Medicine, Kyoto Univ)

From February 1999 to December 2003, 82 HCC patients (male; 60, female; 22, age; 21～69y. o.) received living donor liver transplantation (LDLT). The indication of LDLT for HCC was unresectable tumor with no extrahepatic lesions nor major hepatic involvement. Half of the patients had advanced carcinoma, which exceeded the so-called Milan criteria. Patient survival at 3-year after LDLT was 66%. Ten patients had recurrence after LDLT. Recurrence rate at 3-year was 18%, with 0% for patients within pathological Milan criteria and with 35% for patients out of pathological Milan criteria. LDLT for unresectable HCC seems to be acceptable even for advanced HCCs.

Key words: Hepatocellular carcinoma, Living donor liver transplantation, Pilot study, Survival, Advanced HCC

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はじめに

肝細胞癌は，多くが慢性肝炎や肝硬変などの背景疾患を持ち，癌の治療を行っても再発を繰り返すのみならず，いつか癌が制御不能な段階に至る．癌を背景疾患とともに治癒させる可能性のある治療として，諸外国では脳死肝移植が行われてきた．しかし，進行肝癌の成績は不良であり[1,2]，現在はミラノ基準（5 cm 以下1個，または3 cm 以下3個以内）[3]をゴールドスタンダードとして早期肝癌に適応が限定され，せいぜいそれをやや拡大した基準（UCSF 基準など）[4]までが用いられている．ドナー不足を背景とする脳死肝移植では，公平にかつ有効に臓器を配分する原則に則る必要がある[5]．しかし，生体肝移植では，公平な臓器配分は不要であり，適する生体ドナーのいる肝癌患者では，有効な移植をめざすことになる．当科では，1999年2月から，肝癌患者に対する生体肝移植を開始した．肝癌に関する生体肝移植の役割や適応は不明であったため，肝内に腫瘍が限局して大血管浸潤がなければ，早期肝癌に限らず移植を行う方針でパイロットスタディーを行った．この考えの背景は，生体ドナーを持つ患者は，進行癌であっても，生体肝移植により生存や治癒の最大限のチャンスを持っており，それを制限しない，ということであった．そうした中で行ってきた当科での肝癌に対する生体肝移植の成績を以下に紹介する．

1. 患　者

1999年2月から2003年12月末までに82人の肝癌患者に生体肝移植を行った．移植直前の画像では腫瘍が認められなかったが，摘出肝に肝癌のみつかった患者9人を含んでいる．男性60人，女性22人で，年齢は21～69歳（中央値；54歳）であった．背景疾患はC型肝炎が最も多かった（表1）．手術の適応を決める最終評価は，術前数週間以内に撮影した頭部・胸部・腹部CTと骨シンチに基づき，肝内腫瘍の画像診断と，遠

*1 京都大学大学院医学研究科移植免疫医学講座

表1　1999年2月～2003年12月末までに生体肝移植を受けた肝癌患者（82人）

男性；60人，女性；22人		
年齢；21～69歳（中央値；54歳）		
背景疾患；C型肝炎		44人
	B型肝炎	27人
	B+C型肝炎	2人
	アルコール性	3人
	その他	6人

Child A；13人，Child B；31人，Child C；38人

隔病巣がないことを確認した．画像診断によるミラノ分類，UICC分類，日本肝癌研究会による肝癌ステージ分類（第4版）をそれぞれ図1に示す．ミラノ基準を超えた肝癌が半数を占める．UICC分類ではStage ⅣAが半数を占め，肝癌研究会分類ではStage Ⅲが半数を占めた．肝癌に対する過去の治療歴を持つ症例は76％で（図2），癌の初診から移植までの期間は1カ月～91カ月まで（平均26.4カ月）あった．術前の画像診断で腫瘍が認められず摘出肝において腫瘍がみられた9例中，5例は過去に肝癌の治療歴を持ち，いわゆる肝移植後に初めて肝癌が診断されたincidentalなものは4例であった．海外の脳死移植における手術適応を超える進行症例が半数含まれていた．AFP値は，0.9 ng/ml～20,362 ng/ml（中央値；28 ng/ml）であった．なお，肝予備能としてChild分類では，Child A 13人，Child B 31人，Child C 38人であった．

2.　治療成績

1）生存率

移植後累積生存率を図3に示す．移植後1, 2, 3年生存率はそれぞれ76, 73, 66％であった．同時期に行った非腫瘍症例における生存率は，いずれも75％で，両群間に有意差はみられなかった．24人が死亡したが，癌の再発によるものが6人（％）で，その他の合併症による死亡が18人（12％）であった．腫瘍症例において術後1年以降に生存率が減るのはすべて再発死によるものであった．

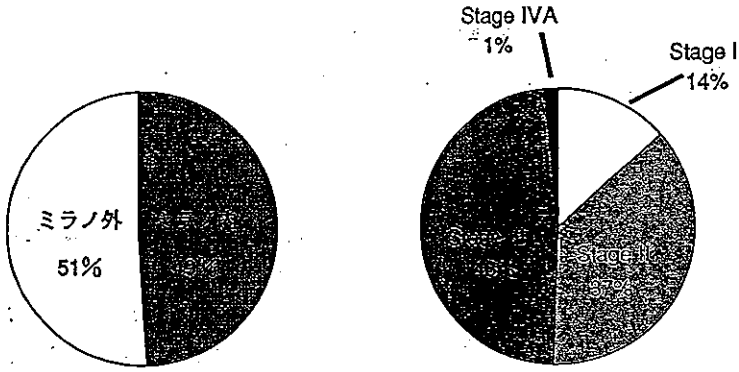
術前に腫瘍が診断されていた73例で，肝癌病期による生存率，およびミラノ基準による生存率を同じく図4に示す．肝癌病期の4群とミラノ基準内と基準外の2群のいずれも，生存率に有意差はみられなかった．

2）再発率

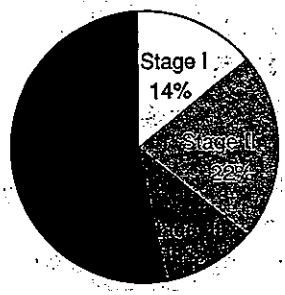
全82症例中10例が再発した．3年再発率は18％であった（図5）．移植前に腫瘍の認められた73例についてみると，3年再発率は20％であった．ミラノ基準内症例と基準外症例の3年累積再発率はそれぞれ7.6％と33.3％で，両群間に有意差がみられた．これを，摘出肝の病理所見でミラノ基準を満たすものと，基準外のものに分けると，基準内の再発率は0％，基準外の再発率が35％であった（図6）．病理所見から再発の危険因子をみたところ，腫瘍の個数が4個以上，最大径5 cm以上，低分化型，脈管浸潤あり，の4項目が危険因子であった（図7）．これに有意な危険因子である移植前AFP値400 ng/mlを加えた5項目で多変量解析を行ったところ，低分化型および個数4個以上が危険因子となっていた（表2）．

3）他治療法との成績の比較

最近，わが国で，背景疾患を持つ肝癌に対して，腫瘍の進行度と背景肝の病気の進行度をそれぞれ点数化して，両者を合計するJISスコアが提唱されている（表3）．近畿大学の工藤先生らは，肝癌が始めて診断された日からの生存曲線をJISスコア別に表した．この中に含まれる患者は，できる限り，局所療法や肝動脈塞栓療法，肝切除などを受けている．つまり，移植以外の治療を積極的に受けた患者の生存曲線である．本シリーズにおける移植患者でJIS曲線を求めてみた（図8）．ただし，腫瘍が初めて診断された日からの生存曲線ではなく，移植日からの曲線で表されている．移植成績はJISスコアとの相関はなく，最も成績が悪かったJIS 3点の3年生存率62％と，工藤らの移植外治療による3年生存率を比較すると，JIS 3点におけるそれは40％であり，JIS 4点，JIS 5点を含めて移植後の生存率が上回っている．一方，JIS 0～2点では，他治療による成績が上回っている．



肝癌取り扱い規約4版による



UICC分類

図1　腫瘍病期（n＝73）

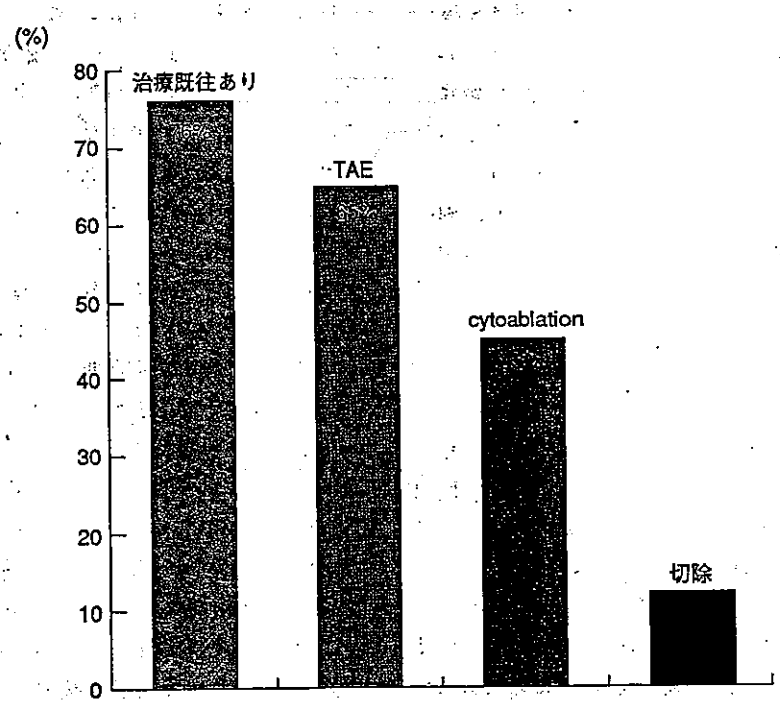


図2　過去の治療歴

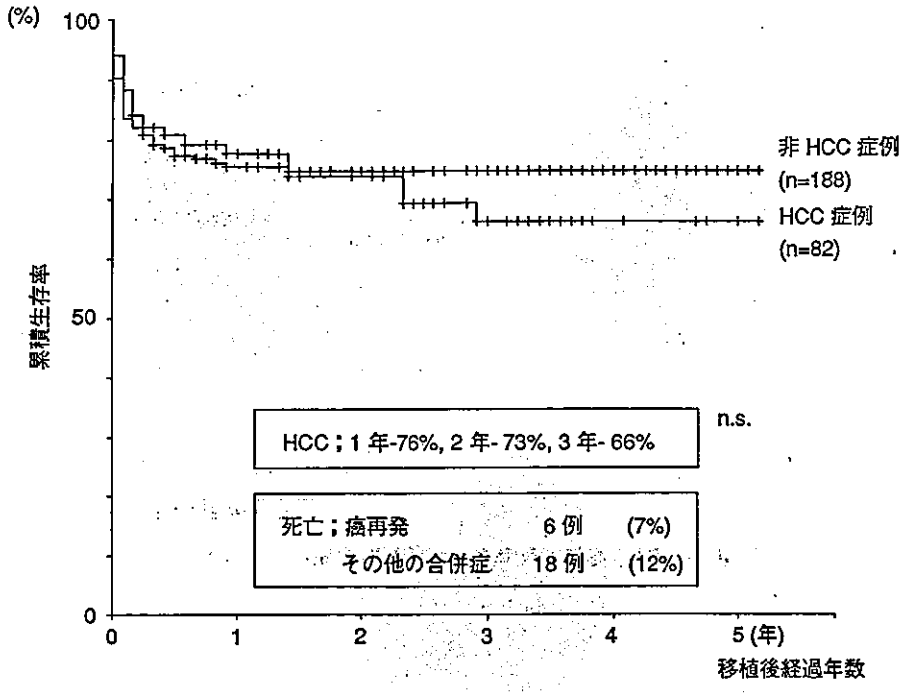


図3　患者生存率

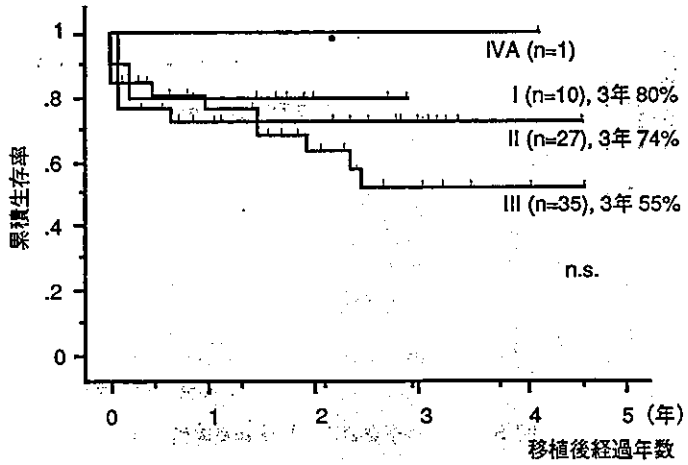


図4a　癌病期と移植後累積生存率（日本肝癌研究会規約による）

3. 考　察

肝に限局する肝細胞癌に対する生体肝移植のパイロットスタディーを開始してから5年になった．海外での肝移植が早期肝癌へより絞られる傾向を持つようになってきているのに対して，当科では半数が進行肝癌であった．ドナー臓器の公平配分を考慮する必要がないことが生体肝移植の特徴であり，進行肝癌に対する治療のチャンスを奪わないという考えにたっているため，進行肝癌の比率が高い．肝移植では，他の消化器手術や肝臓外科手術に比べて術後早期の死亡率は高く10～30%みられる．術前の全身状態が不良であることを背景とし，外科的合併症や免疫抑制療法下の治療抵抗性感染症によるもの，場合によっては拒絶反応などがその原因となっている．この時期を

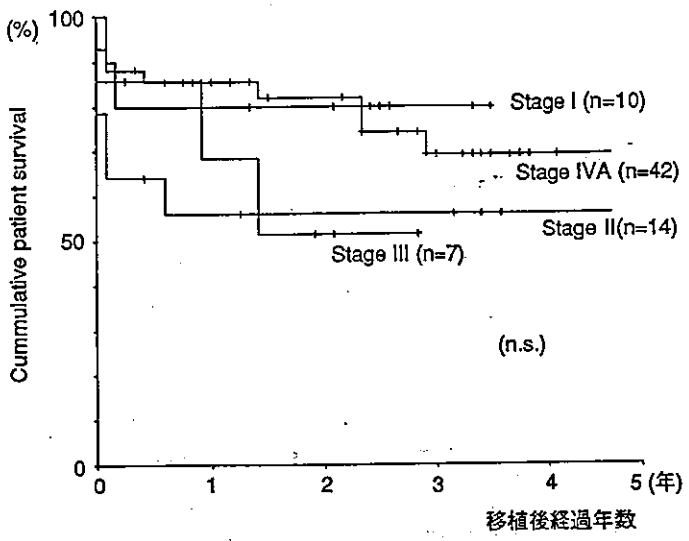


図4b　癌病期と移植後累積生存率（UICC分類による）（n＝73）

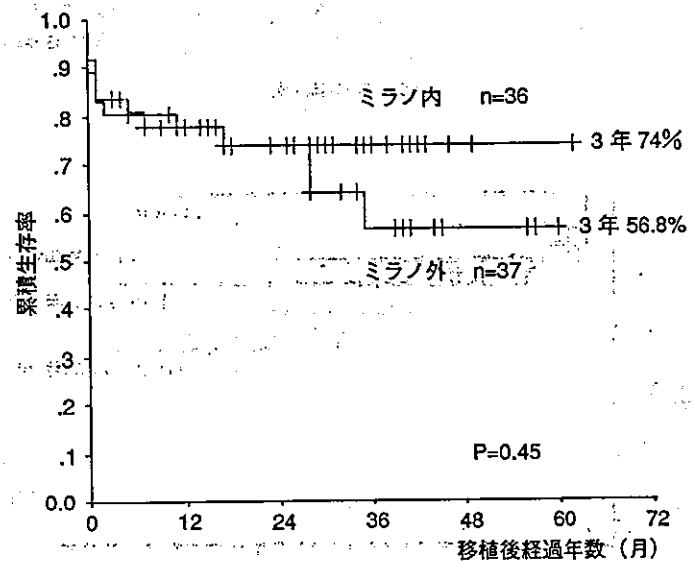


図4c　ミラノ基準による移植後累積生存

乗り切った患者でのその後は，全体で約2割の再発率，進行癌だけをとれば35%の再発率となっている．全く治療をしなければ，肝硬変か癌のために死亡していたと考えられる患者さんの3分の2が，生体肝移植により無再発で数年生存している現状は，それなりに有効な治療であると思われる．低分化，血管浸潤，腫瘍の最大径，腫瘍の個数，腫瘍マーカー値などがそれぞれに再発の危険因子となっていたが，その中でも低分化型肝癌が最も強い危険因子となっていた．これらは，以前から報告されている海外のものと同じである．現状で術前に知りうるものは腫瘍の最大径，腫瘍の個数，腫瘍マーカーの3つで，低分化型，血管浸潤は知りえない．特に低分化型における再発が高率であることから，イタリアのパドア大学では，移植前に全例腫瘍の肝生検を行って，低分化型肝癌を除き，その他の肝癌は個数や最大径にかかわらず移植する方針としている[6]．

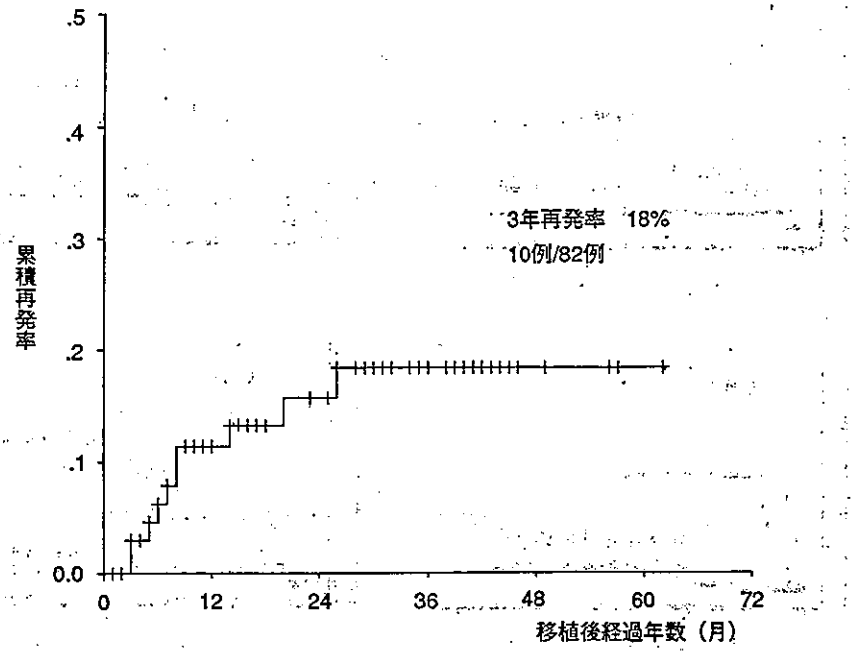


図5　移植後累積再発率

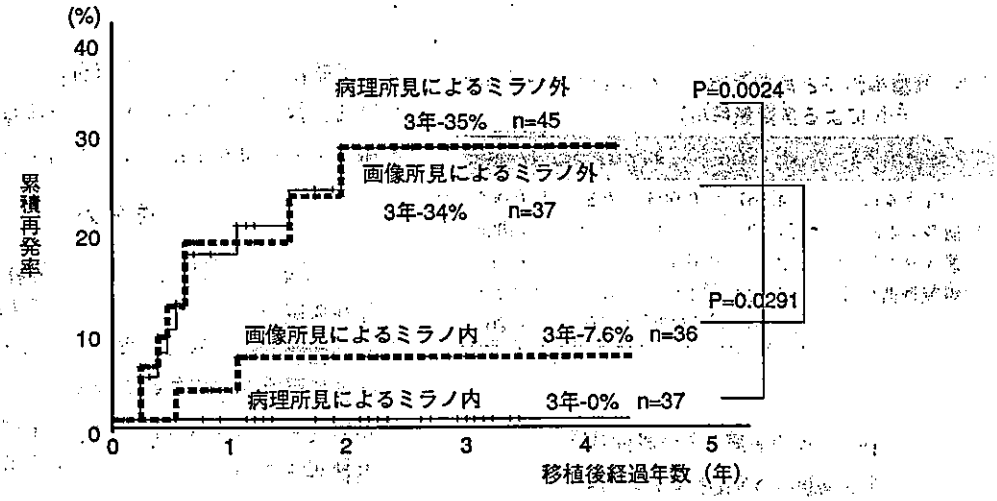


図6　移植後累積再発率

48人の患者を対象として，42%が術前にUICC分類のStage ⅢまたはⅣAに分類され，38%がミラノ基準外であったが，3人が術後1年以内に再発し，そのうちの1人は再発巣にPEITを繰り返して88カ月間腫瘍をコントロールできている．1, 2, 3年無再発生存率はいずれも92%と高率であった．肝右葉を用いる成人間生体肝移植において，ドナーの合併症は約19%にのぼり[7]，特に胆道合併症などの治療は，ドナーに負担をかける．健常人にこういった負担をかける可能性があり，腫瘍が肝内にとどまっていても，あまりにも術後の再発の危険の高い症例は，今後除外するべきと考えられ，今後の検討課題である．また，術前の腫瘍診断は，画像においてなされるのがほとんどであり，近年，機器の進歩によりCT画像などはかなり精度がよくなっている．できるだけ術

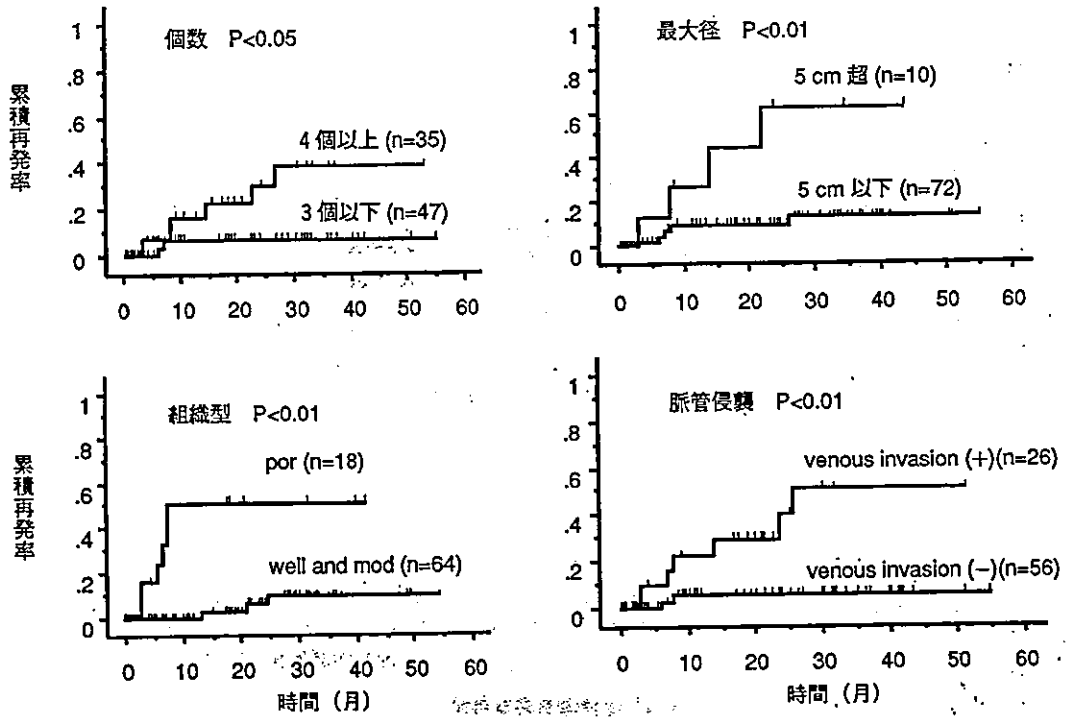


図7　病理所見からみた危険因子

表2　腫瘍因子と再発危険度（Cox比例ハザードモデルによる多変量解析）

腫瘍因子	risk ratio	95%CI	P value
AFP>400	4.367	0.905～20.833	0.067
個数≧4個	11.494	1.337～100.00	0.026*
最大径>5 cm	2.242	0.494～10.204	0.295
組織型低分化	9.528	1.821～50.000	0.008*
vp(+)	2.660	0.476～14.925	0.265

表3　他の治療法との成績の比較

肝細胞癌の予後を規定する因子
- 腫瘍の進行度
- 背景肝疾患の進行度

↓（患者の肝予備能）

両者を組み合わせたScoring System

JIS (Japan Integrated Staging) Score

予後	0	1	2	3
TNM分類	I	II	III	IV
Child-Pugh分類	A	B	C	

前に正確に診断することが望ましく，これまでに当科で施行した症例の摘出肝の所見と術前の画像所見との検討を現在行っているところである．

まとめ

1999年より当科で行ってきた肝癌に対する生体肝移植のパイロットスタディーの結果をまとめた．腫瘍が肝にのみとどまっている段階で，数や大きさに限定せずに移植を施行してきたが，いわゆるミラノ基準を超える進行癌症例の術後3年累積再発率は35%であった．進行癌の3分の2は，無再発で3年生存していることになる．ある程度，進行癌に対しても生体肝移植が有効であると考えられるが，今後，さらに再発の危険の高い症例をどのようにして除外していくか，などが検討課題である．

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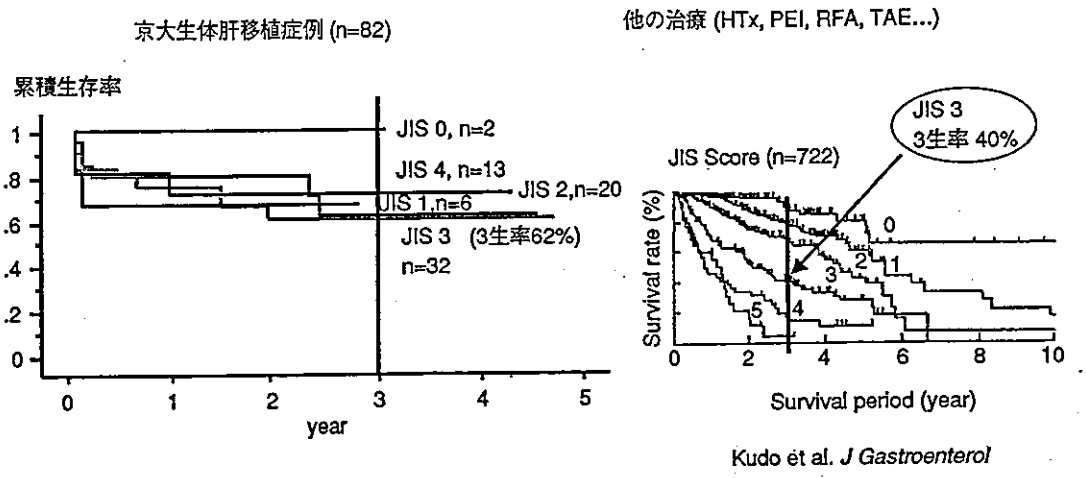

図8　JIS scoreによる生存率の比較

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TRANSPLANTATION

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A DIFFERENT AMYLOID FORMATION MECHANISM: DE NOVO OCULOLEPTOMENINGEAL AMYLOID DEPOSITS AFTER LIVER TRANSPLANTATION

YUKIO ANDO,[1,6] HISAYASU TERAZAKI,[2] MASAAKI NAKAMURA,[1] EIKO ANDO,[3] KATSUKI HARAOKA,[2] TARO YAMASHITA,[4] MITSUHARU UEDA,[4] HIROAKI OKABE,[1] YUTAKA SASAKI,[2] HIDENOBU TANIHARA,[3] MAKOTO UCHINO,[4] AND YUKIHIRO INOMATA[5]

Background. **Liver transplantation has served as a treatment for patients with familial amyloidotic polyneuropathy (FAP) because variant transthyretin (TTR), the pathogenic protein of FAP, is predominantly produced by the liver. However, the effect on amyloid formation of TTR that is synthesised by the retina and the choroid plexus remains to be elucidated in FAP patients with liver transplants.**

Objective. **To investigate changes in ocular tissues and the central nervous system (CNS) of FAP patients after liver transplantation.**

Design. **Clinical study.**

Setting. **Graduate School of Medical Sciences, Kumamoto University, Japan.**

Intervention. **Transplantation of livers from cadaveric or living donors.**

Measurements. **Preoperative measures and postoperative (16–108 months) follow-up of clinical data, including routine ophthalmologic, neurologic, and laboratory evaluations.**

Results. **In 22 patients with FAP related to the amyloidogenic TTR (ATTR) Val30Met and 3 patients with FAP ATTR Tyr114Cys, after liver transplantation, 3 patients began to show evidence of de novo glaucoma, and 1 had vitreous opacity that was caused by the variant TTR. Another three patients showed new amyloid deposits in the pupillary margin, which could lead to glaucoma and vitreous opacity. As for changes in the CNS and levels of total protein and TTR in cerebrospinal fluid (CSF), after liver transplantation, two FAP ATTR Tyr114Cys patients exhibited de novo amyloid deposition in the leptomeninges, and total protein and TTR levels in CSF were significantly increased.**

Conclusions. **Oculoleptomeningeal involvement in FAP was not prevented by liver transplantation because variant TTR produced by the retina and the choroid plexus forms amyloid fibrils in situ.**

Since 1990, liver transplantation has been used to treat familial amyloidotic polyneuropathy (FAP) (*1*) because amyloidogenic transthyretin (ATTR), the pathogenic protein of FAP, is predominantly synthesised by the liver. By the end of 2000, more than 500 FAP patients had undergone the surgery, with approximately 80% of the patients surviving (*2*). Liver transplantation is now considered a promising therapy for prevention of deterioration of neurologic complications of FAP patients (*1, 3–7*). After transplantation, variant TTR levels in serum have decreased to below 1% of those before transplantation, and clinical findings have improved, or at least the patients' conditions did not deteriorate (*8*). However, TTR is also synthesised by the choroid plexus and the retina (*9, 10*), and the role of TTR synthesised by these tissues in FAP patients remains to be elucidated. Moreover, ocular manifestations are common in patients with FAP ATTR Val30Met (*11, 12*), and these symptoms may become serious problems after liver transplantation.

Advances in molecular genetics and protein chemistry techniques have resulted in more than 80 different points of mutation in TTR being reported (*13*). Among the hereditary systemic amyloidoses, the type FAP ATTR Val30Met is the most common (*14–16*). FAP ATTR Val30Met is characterized by progressive sensorimotor peripheral neuropathy in addition to symptoms in the gastrointestinal tract, heart, kidney, and autonomic nervous system (*14, 15*). Liver transplantation has been reported to halt the progression of these clinical manifestations (*1, 3–7*). However, changes in manifestations in the eye and central nervous system (CNS) have not been carefully evaluated, and no precise or long-term follow-up documentation is available.

In the Kumamoto district, from 1994 to the end of 2002, 25 FAP patients underwent liver transplantation. All patients are still alive, and 84% have resumed their normal daily activities. Neurologic and laboratory examinations revealed that therapy has thus far prevented progression of systemic symptoms of FAP, and autonomic dysfunction partially improved after surgery (*5, 17*). For this report, we evaluated posttransplantation changes in clinical and laboratory data related to ocular and CNS symptoms.

PATIENTS AND METHODS

Subjects

All FAP patients included in the study had a definite diagnosis of FAP on the basis of genetic investigations. The patients had a diagnosis

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[1] Department of Diagnostic Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.
[2] Department of Gastroenterology and Hepatology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.
[3] Department of Ophthalmology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.
[4] Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.
[5] Department of Transplantation, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan.
[6] Address correspondence to: Dr. Yukio Ando, Department of Diagnostic Medicine, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto 860–0881, Japan. E-mail: yukio@kaiju.medic.kumamoto-u.ac.jp.

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of FAP ATTR Val30Met (*18*) or FAP Tyr114Cys (*19*); there were 13 men and 12 women, aged 28 to 59 (average age 38.26) years (Table 1).

Ophthalmologic Examinations

Routine ophthalmologic examinations for the FAP patients who had had liver transplantations were performed before and every 1 to 6 months after transplantation. Patients were carefully evaluated for keratoconjunctivitis sicca, glaucoma, pupillary disorders, and vitreous opacity (*11*).

Analysis of the Vitreous Amyloid Sample

Amyloid fibrils were collected by centrifugation of the vitrectomized corpus vitreum from an FAP patient (no. 3 in Table 1). The fibrils were washed three times with 1 mL of saline and distilled water and were centrifuged at 9,000*g* for 5 minutes. The collected amyloid fibrils were ultrasonicated (Branson 1200, Shelton, CT) for 1 hour after addition of 20 μL of 6 M guanidine hydrochloride and 10 μL of 2.7 mM dithiothreitol (DTT). After ultrasonication, 500 μL of 50 mM Tris-HCl and 100 μL of polyclonal anti-TTR antibody (Dako, Dakopatts, Glostrup, Denmark) were added, and the sample was incubated overnight (final concentrations 225 mM guanidine hydrochloride and 0.06 mM DTT). The resulting precipitate was centrifuged at 9,000*g* for 5 minutes and was washed two times with 100 μL of saline and 100 μL of water, respectively, at 4°C. The precipitate was dissolved in 50 mL of 4% acetic acid and 4% acetonitrile in water, and the solution was passed through a 1,000 kDa centrifugal concentrator to separate the dissociated TTR from the antibody and to constitute a pass-through fraction. The centrifugal concentrators were washed with 3×100 μL with the same solution before use to clean the membrane surface (*20*).

Mass Spectrometry

The extracted amyloid samples were analyzed by using a Bruker Reflex mass spectrometer (Bruker Franzen Analytik GmbH, Bremen, Germany) operated at a wavelength of 337 nm. The best spectra of TTR were obtained at an ion accelerating voltage of 27.5 kV and a reflectron voltage of 30 kV. The spectra were calculated by using external calibration with $[M+H]^+$ ions produced from horse cytochrome c (m/z 12360.08) and horse myoglobin (m/z 16951.46). The matrix was a saturated solution of sinapinic acid in 1:2 acetonitrile:water containing 0.1% trifluoroacetic acid (TFA). The samples were deposited onto the sample probe assembly (*21*).

Two-Dimensional Electrophoresis

Before electrophoresis, an 11 cm Immobiline DryStrip gel (gel strip) (Pharmacia Biotech, Uppsala, Sweden) was hydrated overnight in the vitreous sample dissolved in 8 M urea, 1% Triton X, 65 mM DTT, 2% Pharmalyte (pH 4–7), and 0.025% bromphenol blue in a reswelling cassette (Pharmacia). Electrode wicks (Pharmacia) were oriented on the proper sides of the isoelectric focusing electrode strips (Pharmacia), and the gel strip was placed on an aligner (Pharmacia). Electrophoresis was carried out at 3,500 V for 5 hours (gradient mode, V×hours=15,000) at 15°C on a water-cooled Multiphor II (Pharmacia). After electrophoresis, the gel strip was soaked in 0.05 M Tris-HCl (pH 8.8), 6 M urea, 30% glycerol, and 2% sodium dodecyl sulphate (SDS) (i.e., equilibrating buffer) containing 50 mM DTT for 15 minutes, after which it was soaked in equilibrating buffer containing 135 mM iodoacetamide for 15 minutes. After the processes of reduction and alkylation, the gel strip was subjected to a second electrophoresis step (SDS-polyacrylamide gel electrophoresis). The strip was placed on a 10% acrylamide gel containing 0.375 M Tris-HCl (pH 8.8), 0.1% SDS, 0.03% ammonium persulphate, and 0.05% TEMED, and electrophoresis proceeded at 30 mA loading in a buffer consisting of 250 mM Tris base, 1.92 M glycine, and 1% SDS.

The acrylamide gel was transferred onto polyvinylidene fluoride membrane and was subjected to Western blot analysis. Anti-human TTR antibody from rabbit (diluted in 1:1,000) and horseradish peroxidase-conjugated anti-rabbit antibody from goat (diluted 1:1000)

TABLE 1. Patients' characteristics

No	Age	Sex	Duration	Condition	Date	University	Other	Mutation
1	37	M	3	Full time	Feb, 1994	Sweden		Val30Met
2	40	M	4	Full time	Dec, 1994	Sweden		Val30Met
3	48	F	4	Full time	Jan, 1995	Sweden		Val30Met
4	41	M	5	Full time	Jul, 1995			Val30Met
5	54	F	5	Full time	Sep, 1995			Val30Met
6	41	F	4	Full time	Jan, 1996			Val30Met
7	36	F	1	Full time	Feb, 1996			Val30Met
8	51	M	5	Full time	Mar, 1996			Val30Met
9	30	M	2	Full time	May, 1997	Sweden	Domino	Val30Met
10	40	F	1	Full time	Nov, 1998	Sweden	Domino	Val30Met
11	33	M	2	Half time	Dec, 1998	Sweden		Val30Met
12	33	M	2	Full time	Dec, 1998	Sweden		Val30Met
13	35	M	2	Full time	Dec, 1998	Sweden		Val30Met
14	32	M	2	Full time	Jan, 1999	Kyushu Univ.	Partial LT	Val30Met
15	38	F	1	Full time	Jan, 1999	Kumamoto Univ.	Partial LT	Val30Met
16	61	M	1	Full time	Jul, 1999	Kyoto Univ.	Partial LT, Domino	Val30Met
17	52	F	4	Stay home	Jul, 1999	Kyushu Univ.	Partial LT, Domino	Tyr114Cys
18	37	F	2	Full time	Jul, 1999	Kumamoto Univ.	Partial LT	Tyr114Cys
19	34	M	1	Full time	Nov, 1999	Kumamoto Univ.	Partial LT	Val30Met
20	38	F	2	Full time	Nov, 2000	Australia		Val30Met
21	37	F	1	Half time	Nov, 2000	Australia		Val30Met
22	47	M	4	Full time	Dec, 2000	Kumamoto Univ.	Partial LT, Domino	Val30Met
23	35	F	3	Full time	Apr, 2001	Australia		Val30Met
24	40	M	3	Stay home	Sep, 2001	Kumamoto Univ.	Partial LT, Domino	Tyr114Cys
25	32	F	1	Full time	Dec, 2001	Kumamoto Univ.	Partial LT, Domino	Val30Met

The patients with clinical score (17) below 40 received liver transplantation.

Duration, duration after the onset of the disease; date, the transplant date; place, the transplant place; condition, daily activity; full time, works full time; half time, works half time; stay home, stay at home; LT, liver transplantation.

were used as the primary antibody and the secondary antibody, respectively. Electrogenerated chemiluminescence (Pharmacia) was adjusted according to the manual and mounted on the membrane for 1 minute, and radiographic film was exposed for 10 to 60 seconds. Chemiluminescence was quantified from scanner images by using Multi-Analyst version 1.0.2 (Bio-Rad, Marnes, France).

Neurologic Examinations

Routine neurologic examinations were performed before and every 1 to 6 months after liver transplantation. Several patients had lumbar puncture (nos. 1, 2, 3, 6, 7, 13, 17, 18, 21, and 24 in Table 1) and magnetic resonance imaging (MRI) studies (nos. 1, 3, 6, 13, 17, 18, 21, and 24 in Table 1) in addition to the clinical neurologic examinations before and after liver transplantation.

RESULTS

Ophthalmologic Examinations

Ophthalmologic examinations after liver transplantation revealed de novo pupillary disorders, including keratoconjunctivitis sicca, glaucoma, and vitreous opacity, in 3, 3, and 1 FAP patients, respectively (Table 2). A 46-year-old woman (no. 3 in Tables 1 and 2) underwent vitrectomy 5 years after liver transplantation, and matrix-assisted laser desorption-ionization/time-of-flight mass spectrometry (MALDI/TOF-MS) of the purified amyloid fibrils from the vitreous sample revealed that the variant TTR peak was predominant (peak labeled a in Figure 1A). The ratio was confirmed by two-dimensional electrophoresis (Fig. 1B), and the chemiluminescence intensity of the normal TTR and the variant TTR was 12% and 88%, respectively.

Neurologic Examinations

Neurologic examinations of patients with FAP ATTR Val30Met revealed no CNS disorders that appeared to be related to de novo leptomeningeal amyloidosis. Examination of two patients with FAP ATTR Tyr114Cys, however, showed the presence of CNS disorders after liver transplantation. Fifteen months after liver transplantation, a 50-year-old female patient with FAP ATTR Tyr114Cys (no. 17 in Table 1) experienced sudden numbness and muscle weakness in the right upper extremity and dysarthria that continued for approximately 5 hours. In a 38-year-old male patient with FAP ATTR Tyr114Cys (no. 24 in Table 1), transient depression and disorientation occurred 8 months after transplantation. In these two patients, cerebrospinal fluid (CSF) total protein and TTR levels increased after the surgery: CSF total protein levels before and 15 months after liver transplantation in the first patient were 235 mg/dL and 365 mg/dL, respectively; corresponding TTR levels were 1.6 mg/dL and 2.7 mg/dL. For the second patient, CSF total protein levels before and 8 months after the surgery were 123 mg/dL and 219 mg/dL, respectively; corresponding wild-type TTR and ATTR Tyr114Cys were 1.3 and 0.3 mg/dL and 1.8 and 0.4 mg/dL. In these two patients, de novo amyloid deposition in the leptomeninges below the cervical lesions was confirmed by MRI study (Fig. 2).

DISCUSSION

It has been widely accepted that liver transplantation can halt the worsening of systemic clinical symptoms of FAP. However, a few reports have appeared of vitreous opacity occurring in FAP patients after liver transplantation (*22*). In our 25 FAP patients who had liver transplantation, 7 (20%) patients and 2 (8%) patients showed new ocular changes and leptomeningeal amyloid deposits, respectively, after the surgery. These findings suggest that ocular tissues and CNS may have different amyloid formation mechanisms. Because the transplantation resulted in negligible serum levels of variant TTR in these patients and a cessation of the TTR supply from the liver to the ocular tissues and CSF (*8*), it was natural to consider that the amyloid deposits in both ocular tissues and leptomeninges may be induced by TTR produced by the retina and the choroid plexus (*9*, *10*). In fact, we reported that a significant amount of ATTR Val30Met was detected in the aqueous humor of FAP ATTR Val30Met patients receiving transplants (*23*). In addition, MALDI/TOF-MS and two-dimensional electrophoresis revealed that amyloid fibrils in the vitreous body from an FAP patient who had received a liver transplantation consisted predominantly of variant TTR (Fig. 1). This finding indicated that the variant TTR produced by the retina plays an important role in amyloid formation in the vitreous body (*22*). In our experience with FAP patients who have received a liver transplantation, the occurrence of ocular complaints increases yearly, and this problem may become more common in other types of FAP as well as in FAP ATTR Val30Met.

TABLE 2. Changes in ocular manifestations

No.	Age	Sex	Point of mutation	Before				After			
				P	K	G	V	P	K	G	V
1	35	M	Val30Met	−	−	−	−	−	−	−	−
2	38	M	Val30Met	−	+	−	−	+	+	−	−
3	46	F	Val30Met	−	−	−	−	+	−	+	+
5	52	F	Val30Met	−	+	−	−	+	−	+	+
8	49	M	Val30Met	−	+	−	−	−	+	−	−
10	38	F	Val30Met	−	+	−	−	−	+	−	−
12	33	M	Val30Met	−	−	−	−	−	−	−	−
13	31	F	Val30Met	−	−	−	−	−	−	+	−
15	38	F	Val30Met	−	+	−	−	−	+	−	−
16	61	M	Val30Met	+	+	−	−	++	+	−	−
17	50	F	Tyr114Cys	+	−	+	+	+	−	++	+
18	35	F	Try114Cys	−	−	−	+	−	−	−	++
25	31	F	Val30Met	−	+	−	−	−	+	−	−

P, papillary disorders; K, keratoconjunctivitis sicca; G, glaucoma; V, vitreous opacity.

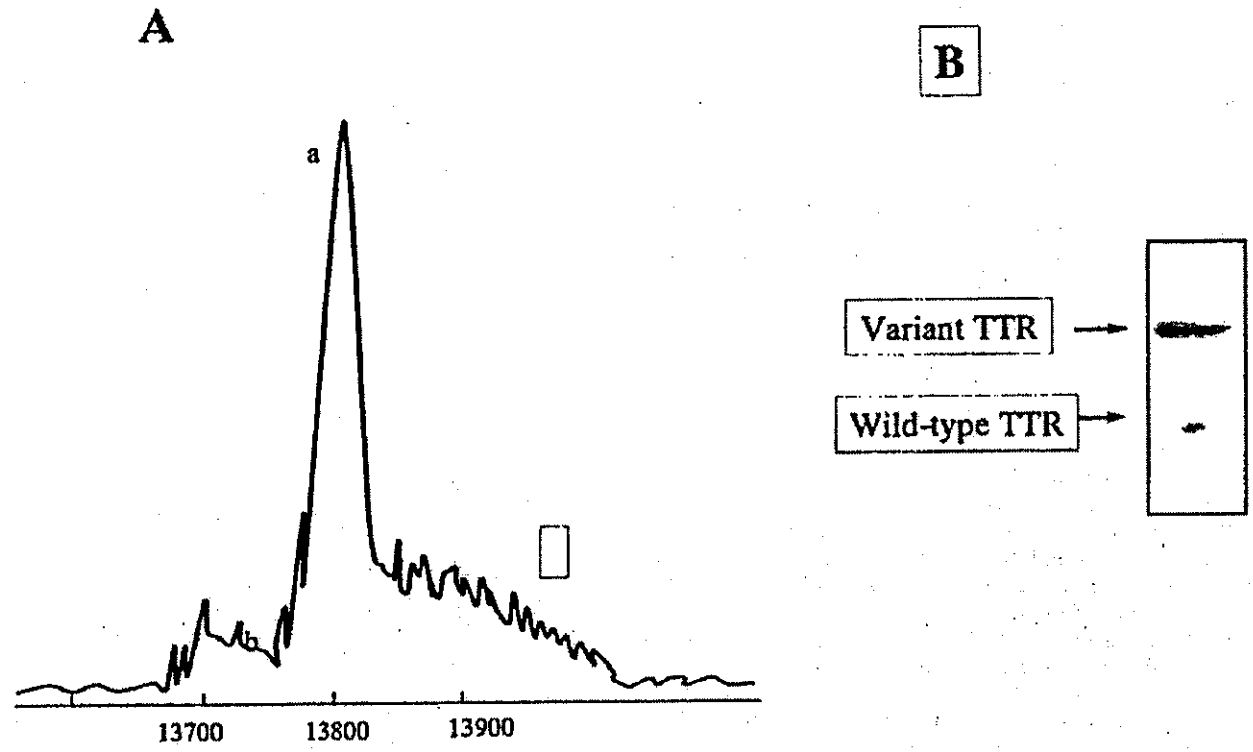

FIGURE 1. **Transthyretin (TTR) in the vitreous body. The vitrectomized sample obtained from a 46-year-old female patient with familial amyloidotic polyneuropathy (FAP) amyloidogenic (A)TTR Val30Met was analyzed by matrix-assisted laser desorption-ionization/time-of-flight mass spectrometry (MALDI/TOF-MS) (A) and two-dimensional electrophoresis (B).**

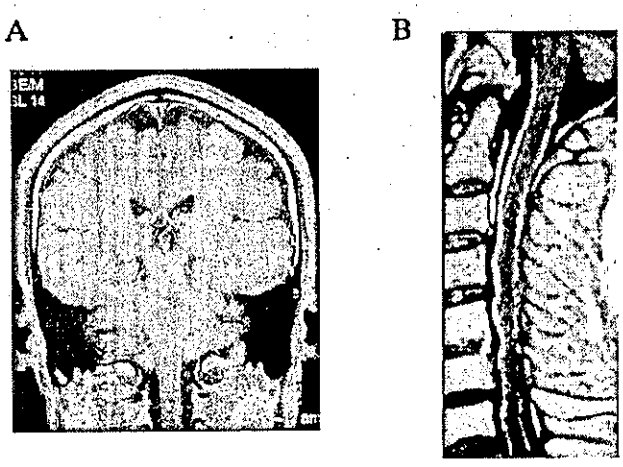

FIGURE 2. **Changes in the leptomeninges as evidenced by magnetic resonance imaging (MRI) before and after liver transplantation. Gadlinium enhanced MRI (T_1) before (A) and 1.5 years after liver transplantation (B) in a 50-year-old female FAP patient (no. 17).**

Our study demonstrated that liver transplantation could not prevent posttransplantation leptomeningeal amyloid deposition. It has been reported that amyloid deposition in the leptomeninges is often recognized in autopsied materials from patients with FAP ATTR Val30Met, and a few FAP patients have shown amyloid angiopathy and CNS disorders (*24–26*). As for amyloid formation in the CNS, little is known about the role of TTR produced by the choroid plexus (*27, 28*). The TTR may play an important role in CSF and CNS because TTR is the second-most abundant protein in the CSF, and it may be indispensable for metabolism in the brain or CSF.

In our 22 FAP ATTR Val30Met patients, no CNS disorders were observed, and TTR levels in the CSF before liver transplantation did not change after the surgery (data not shown). MRI studies of seven FAP ATTR Val30Met patients revealed no amyloid deposition in the leptomeninges after transplantation. In contrast, FAP ATTR Tyr114Cys patients showed de novo amyloid deposition after the transplantation, which was confirmed by MRI. In addition, one of the patients had a transient ischemia attack 1.5 years after liver transplantation. Recently, we reported a different amyloid formation mechanism in ATTR Tyr114Cys (*29*), which may facilitate leptomeningeal amyloid deposits more than in ATTR Val30Met.

Although FAP ATTR Tyr114Cys is classified as an oculoleptomeningeal amyloidosis (*13*), there is a possibility that long-term follow-up after liver transplantation may show that leptomeningeal amyloidosis and other unknown symptoms may also become serious consequences of FAP ATTR Val30Met as well as other types of FAP. Before the use of liver transplantation as treatment of FAP, the life span of patients with FAP ATTR Val30Met was usually approximately 10 years after the onset of the disease. It is possible that leptomeningeal amyloidosis may not show itself as a

serious problem in such a short time period. However, most FAP patients now undergo liver transplantation and live much longer. In this situation, ATTR produced from the choroid plexus and retina may induce ocular amyloidosis and leptomeningeal amyloidosis in FAP ATTR Val30Met as well as in other types of FAP.

It has been well documented that cardiac amyloid deposits progressed even after liver transplantation because wild-type TTR participated in amyloid fibril formation, mainly in non-FAP ATTR Val30Met-type patients (*30, 31*). In addition, as reported here, ocular manifestations such as vitreous opacity and glaucoma restrict the daily life of patients. Because these ocular disorders can be addressed surgically, precise and long-term follow-up evaluations for ocular manifestations before and after liver transplantation are needed to ensure a good quality of life for these FAP patients. With regard to leptomeningeal amyloidosis, we cannot predict when and what types of clinical manifestations may occur and become serious problems in FAP ATTR Val30Met. In FAP ATTR Tyr114 Cys, leptomeningeal amyloidosis does have clinical importance, as demonstrated in this report. To answer these questions, we must continue radiologic and neurologic follow-up over the long term.

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Early graft failure due to a veno-occlusive disease after a pediatric living donor liver transplantation

Izaki T, Inomata Y, Asonuma K, Okajima H, Ohshiro H, Ueno M, Hamamoto R, Iyama K, Tanaka K. Early graft failure due to a veno-occlusive disease after a pediatric living donor liver transplantation. Pediatr Transplantation 2004: 8: 301–304. © 2004 Blackwell Munksgaard

Abstract: A 10-month-old boy with biliary atresia after Kasai procedure underwent a living donor liver transplantation (LDLT). Five days after the LDLT, high fever and increased ascites followed by poor bile drainage was accompanied by elevation of serum liver enzymes. Liver biopsy showed occlusion of the central veins by fibro-edematous endothelium and submassive necrosis of the parenchyma. Veno-occlusive disease (VOD) was suspected, and re-LDLT was urgently performed because of deterioration of hepatic failure. There are few cases of VOD after liver transplantation and this is the first one in an infant after LDLT.

Toshiya Izaki[1], Yukihiro Inomata[1], Katsuhiro Asonuma[1], Hideaki Okajima[1], Hajime Ohshiro[1], Mikako Ueno[1], Rieko Hamamoto[1], Ken-ichi Iyama[2] and Koichi Tanaka[3]

[1]Department of Pediatric Surgery, [2]Pathology, Kumamoto University Hospital, [3]Department of Transplantation and Immunology, Kyoto University, Japan

Key words: veno-occlusive disease – complication – rejection – living donor liver transplantation

Yukihiro Inomata, Department of Pediatric Surgery, Kumamoto University, Honjo 1-1-1, Kumamoto 860-8556 Japan
Tel.: 81-96-373-5616
Fax: 81-96-373-5616
E-mail: yino@fc.kuh.kumamoto-u.ac.jp

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VOD of the liver was first described by Bras et al. (1). In the transplantation field, most cases of VOD have been reported after preconditioning treatment for bone marrow transplantation, or immunosuppression associated with renal transplantation (2, 3). Clinical symptoms like jaundice, body weight gain or ascites, and painful hepatomegaly, have been the diagnostic clues. A limited number of cases with VOD after liver transplantation have been reported, and the outcome is quite poor (4). Conversely, the incidence of early graft failure is quite low after LDLT except for technical or infectious complications. We present a case of infantile VOD, which occurred only 5 days after LDLT, and the patient survivor with a re-LDLT.

Abbreviations: LDLT, living donor liver transplantation; VOD, veno-occlusive disease; UW, University of Wisconsin; MPD, methylpredonisolone; CDC, complement dependent cytotoxicity; ALT, alanine amino-transferase; AST, aspartate transaminase; Tbil, total bilirubin.

Case report

A LDLT was performed in a 10-month-old boy following a failed Kasai procedure. The donor was his ABO-identical father. Preoperative lymphocyte cross-match using the CDC method was negative. Donor surgery was uneventful. The left lateral segment graft was 240 g in weight. Graft weight per recipient's body weight ratio was 3.9%. The graft was preserved in the UW solution until transplanted orthotopically, with the usual technique (5). The hepatic artery was reconstructed by a microsurgical technique. The cold and the warm ischemia time were 54 and 34 min, respectively. After the completion of vascular reconstruction, doppler ultrasonography (US) comfirmed excellent flow in the hepatic vein, portal vein, and hepatic artery. A transanastomotic biliary stent was passed through the Roux-en-Y loop via a Witzel-type enterostomy and conducted to the outside of the abdomen. Good blood flow in all reconstructed vessels was again confirmed just before and after the closure of the abdomen. Operation time was 8 h and

Table 1. Correlation of lab data, tacrolimus level, fever, and volume of drained ascites in the post-operative days

POD	AST (IU/L)	ALT (IU/L)	Tbil. (MG/DL)	Platelet ($\times 10^4/mm^3$)	Tac level (ng/mL)	BT max. (C)	Ascites (mL/day)
1	712	1240	5.6	12.0	2.5	39.9	356
2	279	879	4.0	12.1	13.9	36.6	341
3	464	1614	4.1	12.1	8.2	37.0	181
4	124	995	3.9	9.1	6.3	37.9	584
5	51	502	6.1	8.4	3.4	38.6	554
6	167	451	6.7	8.9	3.0	39.2	689
7	590	881	6.5	1.9	3.6	38.0	812
8	909	1064	13.4	5.4	7.5	37.8	931

33 min. Immunosuppressants consisted of oral tacrolimus and low dose steroids. Biopsy of the donor liver at the time of transplantation (zero biopsy) did not show any specific pathology.

Post-operatively, the peak transaminase (ALT) level was over 1600 IU/L on day 3 (Table 1). This peak ALT was unusually high in comparison with other age-matched cases in our experience, but the exact cause was unknown. The patient was awake and extubated the day after the transplantation. He was clinically stable until 5 days when he developed a high fever, with increased volume of drained ascites and a pulmonary effusion. The trough level of tacrolimus was 13.9 ng/mL on day 3. Considering the high transaminase level reflecting the poor metabolic function of the liver, we transiently stopped. The level decreased to 3.4 ng/mL on day 5, when high fever was initially noted. We did not re-start the tacrolimus because we thought the patient was infected, although a focus was not apparent. From the evening of day 6, we re-started tacrolimus because the transaminase level which had decreased started to increase, although the patient still had a high fever. From day 7, color of the drained bile became pale. Hepatic blood circulation frequently checked by doppler US did not show any abnormality either of the hepatic artery or the hepatic vein. However, the portal vein flow decreased on day 7. The graft blood flow was excellent and any infection focus was not defined by repeated pan-culture of various specimens, including arterial blood. At this time, we assumed that the series of the events was because of an acute cellular rejection that occurred under the low level of tacrolimus. Steroid pulse therapy by bolus injection of MPD was started on day 7 without biopsy confirmation. On day 8, thrombocytopenia and a rapid increase of the transaminase level with deterioration of consciousness were recognized. We did a liver biopsy on day 8 and performed an exchange transfusion to improve the level of consciousness and coagulation function. Liver biopsy findings were available on day 9 and showed centrilobular hemorrhagic necrosis (Fig. 1), fibro-edematous thickening of intima of the central veins (Fig. 2), associated with moderate cellular infiltration (Fig. 1) and endothelitis of the portal area. On day 9, the patient became comatose with poor coagulation function. Urgent re-LDLT from his blood type compatible mother was performed, and the patient subsequently recovered uneventfully. Explanted liver was 310 g (1.7 times larger than the original graft weight), and more than 90% was histologically necrotic. All three reconstructed vessels were macroscopically patent without any sign of thrombosis. Correlating the clinical and histological findings, we diagnosed that the event

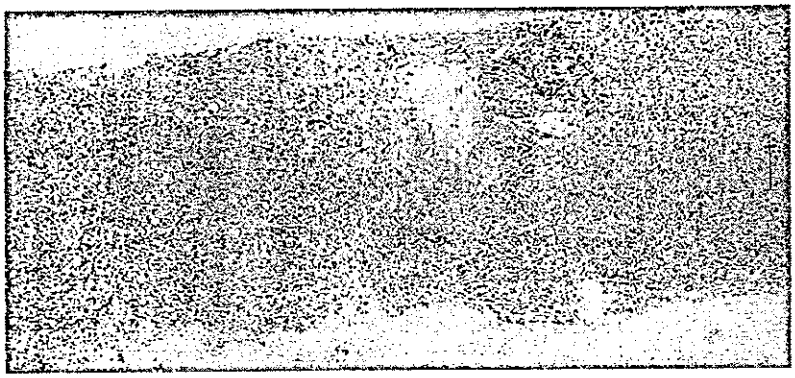

Fig. 1. Cell infiltration in the portal area and hemorrhagic necrosis of the graft (needle biopsy on day 8, H&E, ×100).

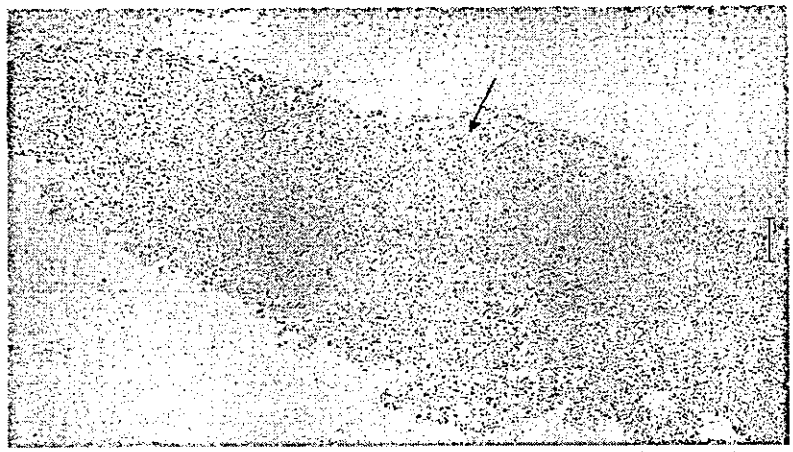

Fig. 2. Fibro-edematous thickening of the intima of a central vein (arrow) (needle biopsy on day 8, H&E, ×100).

was VOD associated with moderate acute cellular rejection.

Discussion

One review article mentions that about 1.9% of patients with cadaveric liver transplantation suffer from VOD at various times post-transplant (4). In another older report, 43% of the patients after liver transplantation had hepatic venular stenosis with immunosuppression using azathioprine, even though part of those episodes were transient (6). The cause and pathophysiology of VOD after liver transplantation is not resolved. Azathioprine has been cited as one of the causative agents for VOD after renal transplantation or liver transplantation (3, 6). However, this drug cannot be the only one cause because not all the VOD was reversible after discontinuation of the drug (4). In the present case, azathioprine was not used. Weigel et al. reported that azathioprine may contribute to the endothelial injury (7). Sebagh et al. suspected that VOD results from an immunological phenomenon (4). In this case, acute cellular rejection triggered by the attenuation of immunosuppression may have precipitated VOD. In the present case, needle biopsy specimen showed pathological findings compatible VOD, associated with acute cellular rejection. Clinically, the transaminase level increased with the attenuation or discontinuation of immunosuppression. This suggests the possibility of a contribution of immunological factors.

In LDLT, it is quite rare to see early graft failure except in cases of incompatible ABO-blood type matching, thrombosis of reconstructed vessels, or marked mismatch of the graft size. Acute cellular rejection can occur within a week after LRLT, but usually is treated with enhanced maintenance immunosuppression or steroid pulse therapy except the cases of incompatible matching. In the present case, the patient had no pre-formed reactive antibody. Therefore, hyperacute rejection is not likely. Most of the reported cases of VOD were diagnosed by liver biopsy and there were few cases of VOD diagnosed clinically. At the early phase after transplantation, it is difficult to diagnose VOD without pathological findings. In the present case, the first diagnostic clue was inflammation, increase of ascites and abnormal coagulation function. These were nonspecific, but the diagnosis of VOD should be considered with such symptoms even after LDLT.

In reported cases of VOD after liver transplantation, the age of the recipients ranged from 5 to 63 yr. Onset of VOD ranged from 11 to 3972 days after transplantation (4). So far, our case was the youngest recipient with the early onset of VOD. The survival rate of the VOD after liver transplantation has been reported to be very poor (4). Re-transplantation was the only way to save the patient in our case. If the diagnosis of VOD was possible before completion of the graft failure, anticoagulation therapy might be one treatment option, although the effectiveness is not approved (8).

In conclusion, VOD should be included as one of the causes of the early graft failure after LDLT. It can be complicated by acute rejection, and early suspicion may be effective for the successful treatment.

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