until their own livers regenerate sufficiently to maintain crucial functions. A secondary goal of ALS is to sustain in a stable condition those patients whose livers cannot regenerate or are destroyed as in an ahepatic state until liver transplantation can be performed without serious complications. Glutamine plays not only as osmolyte but also as oxidative stress inducer in brain. Accumulation of glutamine in brain disrupts cell volume regulation and induces brain edema. Therefore removal efficacy of glutamine is crucial for ALS.

Methods: The present study involved 16 patients with fulminant hepatitis who were admitted to Showa University Fujigaoka Hospital between. Seven patients were acute type of fulminant hepatitis and nine patients were subacute type of fulminant hepatitis. All patients were placed on an ALS system that comprised plasma exchange and online hemodiafiltration. The effect of the ALS on various symptoms of fulminant hepatitis was evaluated, and the levels of glutamine in the patients' plasma samples and the discarded buffer were assayed using automatic analyser.

Results: 15 of the 16 patients regained full consciousness and 11 patients survived without liver transplantation. Two of the remaining five patients underwent liver transplantation and survived. Three patients died without organ donor although ALS sustained these patients in a favorable condition more than two weeks. The plasma glutamine levels were significantly reduced by artificial liver support. The estimated distribution volume of removed Gln ranged from 30 L to 60 L of plasma equivalent.

Conclusions: Plasma exchange in combination with online hemodiafiltration is a promising and effective method to maintain patients with fulminant hepatitis in a favorable condition. This treatment system thus provides more time for physicians to assess the indications for liver transplantation as well as giving the patient a greater chance of undergoing transplantation.

Abstract# P-315

Living Donor Liver Transplantation in Comparison to Cadaveric Liver Transplantation for Acute Liver Failure.

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Acute liver failure(ALF) carries a dismal prognosis with an overall mortality of 33% in spite of improved intensive care medical treatment. Liver transplantation may improve the prognosis of gravely ill patients with ALF. We reviewed 41 cases of liver transplantation in this institution since 1995 and compared the results of living donor and cadaveric liver transplantation. Methods: Retrospective review of 41 patients who underwent liver transplantation for ALF since 1995 was carried out. We compared living donor liver transplantation (LDLT) with cadaveric liver transplantation (CLT) for the treatment of ALF. These groups were analyzed according to Meld, Preoperative INR/PT, Creatinine, total bilirubin, cold ischemic time(CIT), warm ischemic time(WIT), grade 3-4 encephalopathy (Gr 3-4), ICU stay, ventilator day(Vent), postoperative dialysis (Dial) and outcome.

Results: Among 41 ALF, 3 due to Tylenol overdose, 6 due to other drugs, 4 due to autoimmune hepatitis, 2 due to viral, 1 due to acute Budd-chiari syndrome, 1 due to Wilson disease and 1 due to neonatal hemochromatosis. 3 of 6 LDLT were pediatric and 8 of 35 CLT were pediatric. Postoperative outcome for LDLT/CLT were: 1) icu stay days (17.6/8) 2) ventilator care days(4.5/3.8) 3) postop dialysis (2/11) 4) Death<3 months(1/5)and Death<1year(2/14).

Table 1-preoperative values

	cases	mean Meld	mean creatinin	mean PT	mean INR	total bilirubin	grade 3-4
total-	41	31.7	1.87	27.3		18.7	29/41
LDLT	6	24	1.18	22.5	2.7	20.3	5/6
CLT	35	33.1	2.02	26.7	2.6	19.1	24/35

Table2-intraoperative values

	mean CIT	mean WIT	mean blood loss	RBC units	FFP units	cryo units	intraop death
LDLT	38.8	33.3	1420 ml	5.4	8.2	10.6	0
CLT	406.2	39.2	4635 ml	16.9	7.2	17.2	2

Conclusion: Despite a significant improvement in intensive care management, most patients with ALF have poor prognosis. Liver transplantation is potentially the only option for the gravely ill patients with ALF. In spite of UNOS's Status 1 criteria for those patients Living donor liver transplantation seems to be an attractive choice due to shortage of cadaveric liver donor.

Abstract# P-316

Acute Hepatic Failure Due to Celecoxib Requiring Liver Transplantation.

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Introduction: Celecoxib is a selective cyclooxygenase-2 (COX-2) inhibitor widely used due to its efficacy and good safety profile. On the other hand, recent reports have described liver injuries due to them varying from acute hepatic failure to different degrees of transient cholestatic and/or hepatocellular injuries. We report a case of a 40 year old patient with an acute liver failure due to celecoxib and required a liver transplantation. Less than 5 cases are reported in the literature.

Clinical Case: A 40 year old man of South American origin with psoriasis without treatment, begins with a 14 day history of fatigue, loss of appetite, diffuse abdominal tenderness and vomits. The symptoms worsen and the patient and jaundice appears, for which the patients seeks medical attention. The patient refers a history of sporadic alcohol intake and NSAIDs as well as celecoxib recently for usual pains. The blood analysis showed acute renal insufficiency,hepatocellular damage with abnormal liver function tests with predominantly cytolytic pattern. Due to the progressive worsening of the patient's clinical and analytical situation, an OLT was performed 3 days after admission. The pathology of the native liver showed a submassive necrosis with coagulative degeneration compatible with toxicity for celecoxib. The postoperative course was uneventful.

The underlying mechanism for liver injury related to celecoxib is not well defined and in this case it seems as to be an idiosyncratic reaction rather than dose dependent since the patient hasn't taken celecoxib until recently. Conclusion: Although celecoxib is related to less side effects than NSAIDs especially referring to gastrointestinal symptoms,it may be associated to hepatotoxicity and acute liver failure requiring even an OLT as in this case.

Abstract# P-317

Rapid Virological Response in PBMC with an Increase of HCV-Specific IFN-γ Production Predisposes to SVR in Patients with HCV Recurrence after Liver Transplantation with Genotype 1 Undergoing Peg-IFNa2a Plus Ribavirin.

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Background: Viral load evaluation in plasma, after one month of treatment, represents one of the most important parameter to predict treatment response during interferon treatment in CHC. And in liver transplanted patients. It has been proved that HCV-RNA may be present in peripheral blood mononuclear cells (PBMCs) but few studies have been managed on viral load in PBMC during treatment in transplanted patients. Aim of the present study was to evaluate HCV-RNA in PBMC during pegylated-interferon plus ribavirin therapy and whether its clearance in PBMC may induce treatment response. Further we also analyzed the IFN-γ and IL-4 response of PBMC during therapy. Methods: we enrolled 20 liver transplanted patients (16M/4F) due to ESLD HCV related, genotype 1, undergoing antiviral treatment with Pegylated Interferon alpha 2a 180mcg weekly plus Ribavirin according to the weight. All patients were under CNI immunosuppressive schedule (9 on tacrolimus and 11 on Cyclosporine). In these patients we evaluated HCV-RNA in Plasma and PBMC (TaqMan RT-PCR), IFN-γ and IL-4 in ELISpot (stimulation with HCV core antigens; AID, Germany) at the following time points: T0 (before therapy); T1 (one month - RVR); T3 (three months - EVR), T12 (twelve months - ETR), T18 (six months after end of treatment - SVR). Results: We found that Rapid virological clearence of HCV-RNA in PBMC with a restored and improved HCV specific IFN-γ response was significant higher in those with SVR. Conclusion: Patients having RVR in PBMC with an improved Th1 network achieve a complete SVR whereas those having viral clearance only in plasma without a restored Th1 network have a relapse.

2452

Does Hypothermic Machine Perfusion (HMP) of Human Donor Livers Impact on Sinusoidal Endothelial Injury? A Feasibility Study Assessing Flow Parameters, Sterility and Sinusoidal Endothelial Ultrastructure

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Background: Hypothermic machine perfusion is better than conventional cold storage in kidney transplantation.
Large animal models suggest that HMP may be beneficial also for the liver but questions remain about perfusion mode (dual portal/arterial flow versus single flow) and hepatic vascular injury including endothelial dysfunction during HMP.
Methods: 16 human livers rejected for transplant by all UK centres with appropriate consent for research were randomised into 4 groups. Group1: 7 hours cold storage (CS) and one hour HMP through hepatic artery (HA) alone (n=4). Group2: 7 hours CS and 1 hour HMP through HA and portal vein (PV) (n=4). Group3: 7 hours CS and 1 hour HMP through PV alone (n=4). Group4: 8 hours CS. A pressure controlled prototype based on Lifeport Kidney Transporter was used (Organ Recovery Systems).Livers were perfused at 4 to 8 °C under sterile conditions using Belzer MPS. Perfusion parameters (pressure, flow, resistance and temperature) were recorded every 15 min. Perfusate for microbial culture and sensitivity were taken before and after HMP.
Electron microscopy of 3 liver biopsy samples taken before perfusion, were compared with 3 samples from adjacent areas after perfusion.
Results: Pre-set HA pressure of 30 mmHg and PV pressure of 7 mmHg were maintained throughout the perfusion. HA and PV flow ranged from 11 to 107 ml/min (average 59.5 ml/min) and 39 to 199 ml/min (average 96.2 ml/min) with no differences between groups. The same was true for resistance where HA and PV resistance ranged from 0.17 to 1.99 mmHg/ml/min (average 0.71) and 0.07 to 0.17 mmHg/ml/min, (average 0.08). Temperature was maintained between 4 and 8°C. No difference in sinusoidal endothelium ultrastructure was seen before and after machine perfusion, or between any of the groups.
Sterility was maintained throughout the HMP.
Conclusion: HMP of human livers did not produce evidence of sinusoidal endothelial injury. Single or dual perfusion modes did not impact on vascular resistance or flow. The results suggest that further studies into HMP on human livers are warranted.

2553

Intensive Artificial Liver Support Systems as Perioperative Care in Liver Transplantation Improves Survival

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Introduction: The purpose of artificial liver support (ALS) is to sustain patients with fulminant hepatic failure (FHF) for long enough for the patient's liver to regenerate and regain its function. In cases where the liver cannot regenerate, ALS should support liver function until transplantation is successfully performed. If these liver support systems had the capability to sustain patients with FHF in a favorable condition, survival rates would be improved and the criteria for liver transplantation would be simpler and more accurate.
Method: Our study group of 159 patients comprised 90 cases of FH, 16 cases of late-onset hepatic failure (LOHF), and 53 cases of severe acute hepatitis (SAH). Immediately after the onset of hepatic coma, patients were placed on ALS involving plasma exchange and hemodiafiltration using huge volumes of buffer. Treatment for underlying hepatitis consisted of immunosuppressive therapy using a methylprednisolone pulse followed by withdrawal with continuous infusion of cyclosporin A. Antiviral treatment comprising interferon beta and/or a nucleic acid analogue.
Results: Of the 90 FH cases, 3 were the hyper-acute type and progressed to an ahepatic state. They were immediately placed on ALS, which sustained them in a good condition. One of the three patients subsequently underwent LDLTx and survived. Although the ALS system sustained the remained two in a favorable condition for more than two weeks, they died because an organ donor was not found. Of the remaining FH cases, 42 were FH acute type and 36 of the 42 patients survived under ALS. The remaining 45 patients were FH subacute type and 32 of these survived. They were placed on the ALS system and underwent treatment for underlying liver disease. Four of the remaining 13 patients underwent LDLTx and 2 survived. The survival rate of LOHF patients under the same treatment as FH subacute type was 50% (8/16). Of the 53 SAH patients, 51 survived (96%). After several session of ALS, 109 of 116 (94%) patients regained consciousness and the 2-week survival rate was 107 of 116 (92.2%). Brain edema was found in a few cases and was reversible by several sessions of ALS in most cases.
Conclusions: The Japanese treatment system for FH improved the prognosis of acute liver failure. The treatment system described in this study would sustain patients in good condition until the liver recovers or an adequate donor is found, and make perioperative management including organ sharing more appropriate.

2551

Imune-Modulatory Role of Artificial Liver Support System Using Huge Volume of Buffers

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Purpose: Fulminant hepatic failure is a clinical syndrome with various causal agents. The hepatitis C virus, hepatitis B virus, and autoimmunity can account for most etiologies of chronic hepatitis; however, 40% of fulminant hepatic failure cases are of indeterminate etiology in Japan. A clinical feature of hepatic failure of indeterminate etiology is sustained liver injury without spontaneous remission. The cellular and molecular mechanisms underlying fulminant hepatitis of indeterminate etiology are still not completely understood. In the present study, we analyzed cytokines in the serum and the discarded buffer of artificial liver support to clarify the immune-modulatory role of artificial liver support.

Method: The present study included 38 patients presenting with fulminant hepatic failure to our hospital in the last 5 years. They were placed on artificial liver support comprising plasma exchange and hemodiafiltration using huge volume of buffers. We examined them for all known hepatitis virus markers and autoantibodies. Autoimmune hepatitis was diagnosed by a recently proposed scoring system. Drug-induced hepatitis was diagnosed by a history of drug intake and a positive lymphocyte-stimulation test. Indeterminate hepatitis was diagnosed by negative results for: all other virus markers, the presence of autoantibodies, and a history of drug intake. Levels of 48 cytokines in the serum or the discarded buffer of artificial liver support were determined using Bio-Plex Pro cytokine kits.

Results: Etiologies of the patients were as follows: 7 with acute HBV infection, 6 with acute exacerbation of the HBV carrier, 2 with HAV, 3 with drugs, 9 with autoimmunity, and 11 with indeterminate etiology. Artificial liver support system removed significant amount of cytokines and reduced all of serum cytokines levels significantly. In patients with indeterminate etiology, IL-17 and IL-21 levels in the serum and the discarded buffer in deceased cases were significantly higher than that of survived cases.

Conclusion: The artificial liver support system has ability to ameliorate humoral condition removing huge amount of cytokines. This system is useful not only awaking patients but also improving cytokine storm. It also gives us an important clue to solve the underlying mechanism of sustained hepatocyte destruction in indeterminate etiology.

2447

Expression of Receptor Activator of Nuclear Factor-Kappa B Ligand in Leucocytes During Acute Kidney Rejection after Transplantation in Rats

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Introduction: Acute cellular rejection of the transplanted kidney is an important cause of impaired graft function. One of the basic characteristics of acute cellular rejection according to the latest Banff classification of renal allograft pathology is the presence of T lymphocytes in a large number in allograft tissue. Recent studies have shown the important role of T lymphocytes and macrophages in acute cellular rejection of kidney allograft. Osteoprotegerin (OPG), receptor activator of nuclear factor-kappa B (RANK) and RANK ligand (RANKL) are three relatively novel members of the tumor necrosis factor (TNF) superfamily. They have a crucial role in physiological and pathological bone metabolism but also in immunological processes. The aim of our study was to determine the expression of RANKL and RANK by T lymphocytes and macrophages in acute cellular kidney allograft rejection in rats.

Methods: The study included 15 male Wistar rats (3 months old; weight 250-300g) as recipients and 15 male DA rats (3 months old; weight 250-300g) as donors. Animals were sacrificed after 3 weeks and the transplanted kidney was extracted and processed for pathohistological analysis and immunoflorescence. The latest Banff classification of renal allograft pathology was used on all tissue samples by two experienced pathologists. In all tissue samples acute cellular rejection was proven. Acute cellular rejection kidney sections were examined by dual- labeled immunofluorescence to detect CD4, CD8 or CD68 (red) and RANK or RANKL (green) allowing clear definition of cells that co-express both (orange).

Results: Cytoplasmic and on surface granular RANKL immunoreaction on fluorescence microscopy of various intensities was detected in all tissue samples. RANKL positive expression was colocalized with CD4+ and CD8+T lymphocytes in acutely rejected kidney tissue. There was no association between CD4+ and CD8+T cells with RANK expression in any obtained sample. RANK but not RANKL, was expressed by infiltrating CD68 positive macrophages in the interstitium of kidney tissue.

Conclusion: RANK and RANKL are expressed by T lymphocytes and macrophages in acute cellular kidney rejection after transplantation in rats. They have a possible immunomodulatory role in acute cellular kidney rejection. Serum concentration of RANKL could be a marker for early detection of acute cellular kidney rejection.

<原　　著>

急性肝不全に対する on-line hemodiafiltration を用いた人工肝補助療法の確立

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要旨：我々は，急性肝不全に対して on-line hemodiafiltration を用いた人工肝補助療法を初めて臨床応用し，その成果を報告してきた．現時点で集積された全28例の脳症改善率，生存率，移植治療ブリッジユーズ，合併症，血液生化学検査等について再評価した．手技関連の合併症はなく，4.2 ± 0.5（mean±SD）回の治療後に25例（89.3％）が脳症から完全に回復し，覚醒までに必要な治療回数は開始時の意識状態と相関した（$P<0.001$）．転帰は，生存10例，肝外疾患死亡6例で，4例は移植治療を受け，ドナーが確保されなかった5例は最終的に肝不全で死亡された（救命率40％）．生存例と肝不全死亡例では，治療開始後の prothrombin time，direct bilirubin/total bilirubin ratio，血清アンモニア値の推移は大きく異なり，治療開始後の予後予測に有用と考えられた．On-line hemodiafiltration を用いた人工肝補助療法は急性肝不全時の脳症改善の第一選択肢に成り得ると考えられた．

索引用語：　肝不全　　人工肝補助療法　　on-line HDF　　劇症肝炎　　肝移植

はじめに

急性肝不全は重篤な病態で，わが国の劇症肝炎に対する保存的治療の救命率は，急性型で54％，亜急性型で24％である[1]．米国では急性肝不全症例の25％が肝移植治療を受け，救命率を劇的に上げているが，ドナー肝の迅速な確保は容易ではなく，44％の急性肝不全患者が移植治療にエントリーするものの，ドナーを待つ間（平均3日間）にその22.7％が死亡しており，成人死亡率は30％に上っている[2]．一方，全体の45％の患者は保存的に回復生存していることから，肝再生，もしくは移植までの信頼できる肝代償療法の確立は，救命率を大幅に改善することが期待される．

今までに様々な人工肝補助療法（artificial liver support；以下 ALS）が考案され，その機能が検証されて

きた[3)4)]．その多くは一定の有用性を認めているものの，確実に肝機能を代償できるかと言う点では，未だ確立されたものはない．わが国で主流の血漿交換療法と血液濾過透析のコンビネーション法[5]も良好な治療成績が報告されているが，覚醒能力は十分とは言い難い[6]．また，血液浄化に大量の市販置換液を準備する必要性と，交換作業を含めた手技の煩雑さという課題も残っている．これらの問題を解決するため，on-line hemodiafiltration（HDF）は，単純な回路，かつ低コストで絶大な浄化量を得られる腎代償療法として開発，使用されてきた[7]．

われわれは on-line HDF を急性肝不全の脳症改善目的で初めて臨床応用し[6]，その初期治療成績を報告してきた[8]．今回は，当院で on-line HDF を導入したすべての急性肝不全症例の治療成績を再評価し，ALS の標準化を目指して，施行法のポイントや施行期間の判断に示唆を与える検討を加えた．

方　　法

対象患者

On-line HDF を導入した2001年5月以降，当院の高度救命救急センターおよび消化器病センターに入院した一連の急性肝不全症例を対象とした．急性肝不全の診断は，発症後24週以内に進行した肝障害のうち，

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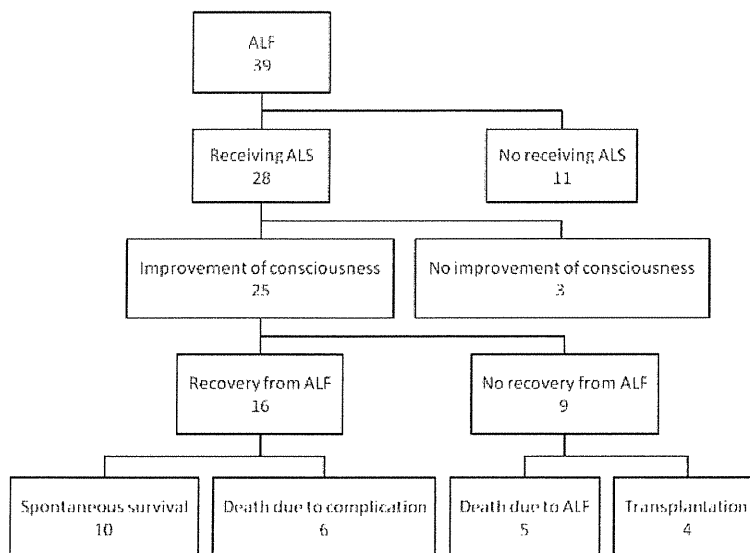<受付日2011年9月14日><採択日2011年12月4日>

Fig. 1　Flow of participating patients through each stage.
The number shows the number of patients. ALF, acute liver failure; ALS,
artificial liver support.

prothrombin time（PT）international normalized ratio
（INR）≧1.5，かつ，意識障害を呈するものとした．病
歴，身体所見，血液生化学所見，ウイルスマーカー，
画像所見も急性肝不全の診断に利用した．On-line HDF
の適応除外基準は，1）入院時に明らかな重症脳腫脹が
ある症例，2）本治療に文書同意の得られない症例，3）
原疾患に対する治療により集中治療室入室時に明らか
に病態の改善を認める症例，とした．重症脳腫脹とは
脳 computed tomography（CT）所見における，脳溝の
著しい狭小化あるいは消失，皮髄境界の不鮮明化，び
まん性の density 低下を満たすもので，それぞれ1名以
上の肝臓専門医，脳外科専門医，放射線科専門医の診
断によった．

　2001年5月から2011年3月の間に39例の急性肝不
全症例が入院し，除外基準に従って11例が除外となっ
た（Fig. 1）．そのうち3例は入院時に重症脳腫脹と多臓
器不全を呈し，入院1日，2日，4日後にそれぞれ死亡
した．残りの8例（心不全7例，脱水によるショック
1例）は原疾患に対する治療が奏功し，集中治療室入室
後に速やかな改善傾向を認め，全例が ALS なしで肝不
全を離脱した．On-line HDF を導入した28例の内訳は，
男性17例，女性11例で，平均年齢は46.2歳（21-72）
であった．成因の内訳は hepatitis B virus（HBV）急性
感染11例，HBV キャリアの急性増悪5例，薬物性3
例，アルコール性2例，成因不明5例，鬱血性（循環

不全），悪性腫瘍の肝浸潤が各1例であった．発症から
入院までは平均 14.3±3.4日（3-60），発症から脳症出現
までは平均 16.1±6.7日（3-90）であった．肝性脳症の
診断と評価は the working party on studies in hepatic
encephalopathy[9]の推奨に従って the West Haven crite-
ria of altered mental state[10]と Glasgow Coma Scale を用
い，複数の肝臓専門医によってなされた．ALS 開始時
の昏睡度は Stage 2；6例，3；12例，4；10例であった．
全例が stage 2以上の脳症であり，対象症例は，発症か
ら脳症出現までそれぞれ65日，90日を要した2例を除
いて，難治性の肝・胆道疾患に関する調査研究班から
最近発表された基準に照らすと，昏睡型急性肝不全に
相当すると考えられた[11]．アルコール性の2症例も，回
復後には明らかな肝予備能の低下はなく画像所見など
も総合的に勘案して，この基準[11]に概ね合致すると考え
られた．ALS 開始時の検査所見は asparatate aminotrans-
ferase（AST）2156.9±702.0 IU/L，総ビリルビン値 12.72±
1.46 mg/dL，direct bilirubin/total bilirubin ratio（DT
比）0.59±0.04，PT-INR 3.31±0.41，血清アンモニア値
213.5±34.0 μg/dL であった．

人工肝補助療法

　on-line HDF

　血管アクセスデバイスは，内頸静脈アプローチを第
一選択として，ダブルルーメンカテーテル（Vas-Cath[®]，
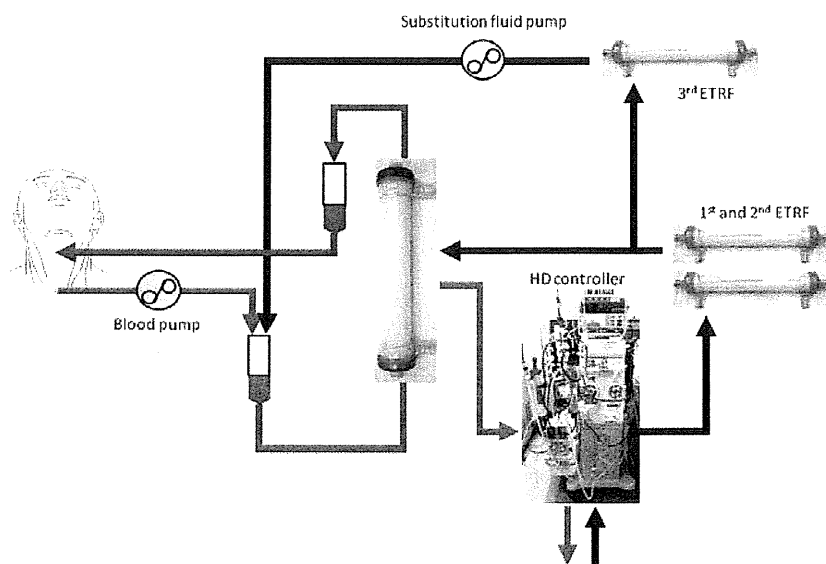Niagara[®]；Bard, Salt Lake City, UT, USA）を中心静脈

Fig. 2 The circuit of on-line hemodiafiltration with pre dilution.
Sterile substitution fluid produced on-line from the dialysate through three ultrafilters was infused pre-filter with a substitution fluid pump. HD, hemodialysis; ETRF, endtoxin retentive filter.

に挿入した. On-line HDF の詳細は既報に詳しいが[12)13)], 我々のシステムでは, 従来の HDF が透析濾過後の血液回路内に市販の無菌重炭酸バッファーを投与する (後希釈) のに対し, 透析濾過前の血液回路内に限外濾過法で透析液より連続的に作成した置換液を投与する(前希釈)のが最大の特徴である. 透析液(AK-ソリタ®FL もしくはカーボスター®L; 味の素製薬, 東京) は2つのエンドトキシン捕捉フィルター(EF-01, polyester-polymer alloy [PEPA] 膜; 日機装, 東京) を経て作成され, そのおよそ半量を別回路で第3のエンドトキシン捕捉フィルター (FLX-18GW, PEPA 膜; 日機装, 東京) を通し, ポンプを用いて脱血側に置換液として前希釈投与した(Fig. 2). 置換液側に使用する回路は市販 の on-line HDF 用 ポ ン プ・回 路 (PRS-12, NV-A300PA; 日機装, 東京)を使用した. 濾過透析側の回路は, NV-Y888PC(血液回路, 日機装, 東京), 個人用多用途透析装置(DBG-02, 日機装, 東京)を使用した. ダイアライザーは APS-15E(1.5 m²ポリスルホン膜; 旭化成クラレメディカル, 東京)で, 機能区分 V 型に区分される高い透過性を有する高性能膜である. 小分子のみならず低分子蛋白の高い除去性能を有する. 開始時の設定は, 血液流量(QB) =350 mL/min, 透析液流量 (QD) =350 mL/min, 置換液流量 (QS) =350 mL/min で, 覚醒を得るまでは体液量の3倍(実体重[g] ×
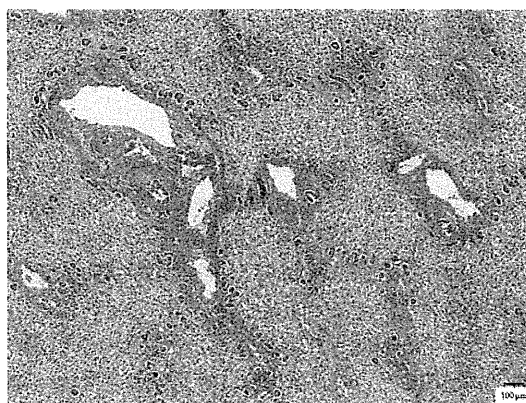

Fig. 5 Photomicrograph of histopathological specimen.
Histopathological specimen (hematoxylin and eosin staining) obtained from the patient who died of liver failure revealed absence of hepatocytes and destruction of normal structure and inflammatory cell infiltration. Portal venous areas are close each other and hyperplasia of the small bile ducts is seen.

0.6×3 mL)を一回浄化量とした. 透析液は血清ナトリウム値が 140 mEq/L 未満とならないように主に血液ガス分析のナトリウム濃度を指標に適宜調整し, 概ね 142–154 mEq/L の範囲で使用した. 現在われわれが使用している透析装置は透析液の濃度プログラムが3種類ま
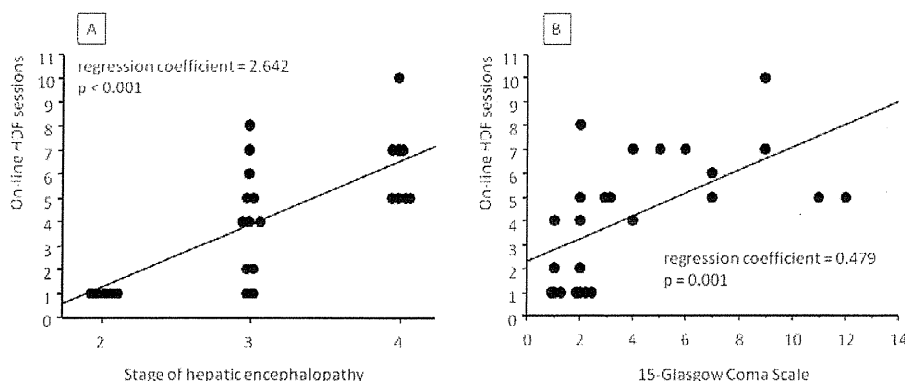
Fig. 3 Correlation between the degree of encephalopathy and the number of sessions of on-line hemodiafiltration to recovery of consciousness.
Significant correlation was observed between (A) stage of hepatic encephalopathy [10] and (B) Glasgow Coma Scale at the start of on-line hemodiafiltration and the number of sessions of on-line hemodiafiltration from the start of the treatment to recovery of consciousness. HDF, hemodiafiltration.

で設定可能なため，プリセット値であればボタン操作のみで変更できる．良好な覚醒が得られれば，1回浄化量を3分の2に減じ，この設定で意識清明が維持されるならば，隔日施行に移行し，さらに施行間隔を広げてALSなしで意識清明，PT-INR＜2.0が維持されればALS離脱とした．

　血漿交換療法

　血漿交換療法は凝固能の代償を主たる目的とし，PT-INRが2.0を超える場合にon-line HDFと併用した．原則として，1回に40単位の新鮮凍結血漿を使用し，治療翌日のPT-INRが2.0を超える場合に再度同量を用いた血漿交換を併用した．PT-INRが2.0未満の場合は血漿交換をスキップし，1日当たり8単位程度の新鮮凍結血漿の輸血を行った．新鮮凍結血漿輸血の際は，過剰な容量負荷を避けるために同分量を総輸液量より減量とした．

　評価

　1次エンドポイントは脳症の改善割合とそれに要した治療回数とし，2次エンドポイントとして，救命率，移植治療へのブリッジ，および合併症発生率を評価した．また，移植なしで生存した患者と肝不全死亡患者については，血液生化学検査の推移より治療開始後の予後予測が可能かどうかを検討した．

　統計解析

　データは平均値±標準誤差として表示した．カテゴリカルデータの比較にはχ²検定を，連続データの比較にはMann-Whitney U検定を用いた．On-line HDF

開始時の脳症の程度，年齢，AST値，総ビリルビン値，PT値，アンモニア値が，意識回復までに必要なon-line HDF施行回数に関連していたかどうかの検討には単回帰分析を用いた．移植なしで生存した患者群と肝不全死亡患者群における連続データ推移の群間比較には線形混合効果モデルを用いた．すべてのP値は両側検定で0.05未満を統計的有意とした．

結　　果

　On-line HDFの総施行回数は333回，症例平均12.7±1.9回 (2-47) で，一回の平均施行時間は6.6±0.1時間 (200-780分)，血液浄化量の平均は115.0±1.9 L/回(46.8-229.0) であった．血漿交換療法は20例に平均5.3±1.0回 (1-17) 施行された．ALS施行期間は平均14.5±2.4日 (2-55) であった．治療中のダイアライザーの血液凝固等による交換は4症例で9回 (2.7%) 必要であった．On-line HDF施行中の治療手技に関わる感染性合併症，出血性合併症，治療関連の凝固障害はみられなかった．また，緊急例を除き，すべて日勤時間帯で施行可能であった．

　On-line HDFを開始した28例中25例はほぼ意識清明 (GCS 15点) の状態まで脳症の改善が得られ (覚醒率89.3%)，治療期間中は安定した覚醒が維持された(Fig. 1)．覚醒の得られた25例において，3回のon-line HDF後にはアンモニア値は全例で200 µg/dL以下となり，平均4.2±0.5回 (1-10) のon-line HDFの後に意識はほぼ清明となった．意識改善までに必要なon-line HDF
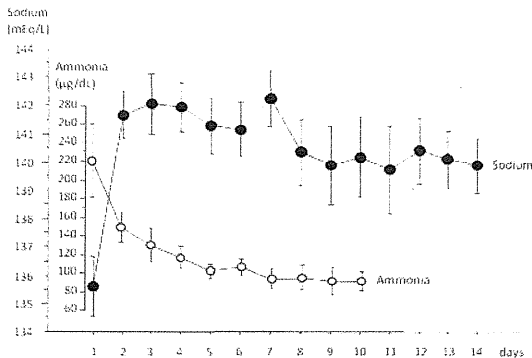
Fig. 4 The changes of the serum sodium levels during first 14 days after the start of artificial liver support.

Closed circle shows the changes of the serum sodium levels in 22 patients in whom we adjusted the sodium concentration of dialysate to 142-154 mEq/L to maintain the serum sodium level at 140 mEq/L or more. The serum sodium levels increased rapidly after first session of artificial liver support, and it were kept at 140 mEq/L or more during first 7 days. The serum ammonia levels decreased to less than 200 μg/dL after first session of artificial liver support (open circle). Data are expressed as mean± SE.

回数は，開始時の年齢，AST 値，総ビリルビン値，PT 値，アンモニア値とは有意な相関はなく，意識状態と相関した（P＜0.001）（Fig. 3）．治療期間中，特に急性期の，血清ナトリウム値は 140 mEq/L 以上にコントロールされていた（Fig. 4）．25 例中の 11 例が，肝性脳症による高度の意識障害を理由に，治療前あるいは治療開始早期に経口気管挿管管理となったが，酸素化障害を理由に人工呼吸器補助の延長を要した 1 例を除き，全例で意識の改善後に抜管された．抜管した 10 例のうち 9 例は抜管 1.4 日後（0-4）には経口摂取を開始したが，1 例は嚥下障害で経口摂取開始は ALS 終了後となった．3 例で脳症の改善が得られなかったが，このうち 2 例は治療開始翌日に脳ヘルニアに進展し，深昏睡からの回復傾向もなく，各々 1 回および 5 回の on-line HDF 施行後に ALS 中止となり，その後死亡された．残りの 1 例は，アンモニア値は良くコントロールされたが安定した覚醒状態に至らず，家族の治療継続希望なく 10 回の on-line HDF 後に ALS 中止となり，その後死亡された．

On-line HDF で覚醒の得られた 25 例の最終転帰は，保存治療生存 10 例，移植 4 例，肝外疾患死亡 6 例（呼吸不全，白血病，心不全，肺炎 2 例，出血性十二指腸

潰瘍），肝不全死 5 例であった（救命率 40%）（Fig. 1）．保存治療生存例および肝外疾患死亡例は，それぞれ 12.8± 3.9 回（6-47）と 10.5±2.8 回（2-21）の on-line HDF 施行後に全例で肝不全状態を離脱した（ALS 離脱）．ALS 離脱とならない 9 例中 4 例はドナーが確保されたため，移植チームに引き継がれ移植手術が施行された．移植例の ALS 開始から移植実施施設転院までの期間は 14.8± 5.5 日（8-31）であり，転院直前まで 13.5±5.3 回（7-29）の on-line HDF を施行し ALS を継続することで良好な全身状態を保つことが可能であった．ドナーが確保されなかった 5 例のうち 1 例は家族からの on-line HDF に対する同意撤回があり，早期に ALS 中止となり（5 日間），3 日後に死亡した．残りの 4 例は，21.0±7.0 日（13-42）の治療期間中に 16.8±3.5 回（11-27）の on-line HDF と 11.3±1.9 回（9-17）の血漿交換療法を施行されて，ALS 中は意識清明で過ごすことが可能であった．ALS 中止後 2-4 日で死亡された．最終的な肝臓容積（CT による推定 3 例，解剖所見 1 例）は 375.0±31.5 g（332-467）と著明な肝萎縮を認めた．剖検が得られた症例では病理学的に正常肝組織の残存を認めなかった（Fig. 5）．

保存治療生存群（10 例）と肝不全死群（5 例）の比較では，年齢，性別，成因，入院時期，脳症出現時期，昏睡度，AST 値，PT 値，総ビリルビン値，DT 比，アンモニア値のいずれも ALS 開始時に有意差はなかったが（Table 1，Fig. 6-8），線形混合効果モデルを用いた開始後の PT 値（Fig. 6）（p＝0.012），DT 比（Fig. 7）（p＜0.001），アンモニア値（Fig. 8）（p＜0.001）の推移は，有意に群間差を認めた．PT 値と DT 比は，それぞれ治療開始 2 日，6 日以降は有意に生存群で良好であった（Fig. 6, 7）．

考　察

On-line HDF を用いた ALS は急性肝不全症例に安全に施行可能で，血漿交換療法との併用で，肝再生あるいは移植治療までのブリッジユーズとして有用であった．本法の優れた点として 1）大量の置換液を用いることで肝性脳症の原因とされる中分子量物質の除去効率が極めて高いこと，2）透析液から置換液を作成することでコストが抑えられること，3）前希釈法を用いることで，ダイアライザー劣化や血液凝固による治療中の回路交換の頻度が低く，施行手技が簡便となったこと，があげられる．肝不全死群 5 例の肝機能は，肝容積，剖検組織所見から廃絶していたものと推定されるが，

Table 1 Comparative results of participating patients who survived hepatic failure without transplantation and the patients who died of liver failure.

		Spontaneous survival (n = 10)	Died of ALF (n = 5)	p-Value
Age		43.9 ± 2.9	55.2 ± 7.5	0.065
Gender (M/F)		7/3	5/0	0.465
Etiology	HBV acute	6	3	0.199
	HBV carrier	0	2	
	alcohol	2	0	
	Unidentified	2	0	
Onset to admission (days)		5.9 ± 0.7	8.2 ± 2.4	0.534
Onset to encephalopathy (days)		7.0 ± 0.6	11.8 ± 4.0	0.951
Glasgow Coma Scale		10.5 ± 1.3	10.2 ± 1.7	0.849
Highest AST (IU/L)		8969.9 ± 2654.7	3332.2 ± 1228.1	0.221

Spontaneous survival; survival without transplantation, ALF, acute liver failure; HBV, hepatitis B virus; AST, asparatate aminotransferase; Highest AST, Highest values that could be recorded during illness. Data are expressed as mean ± SE.
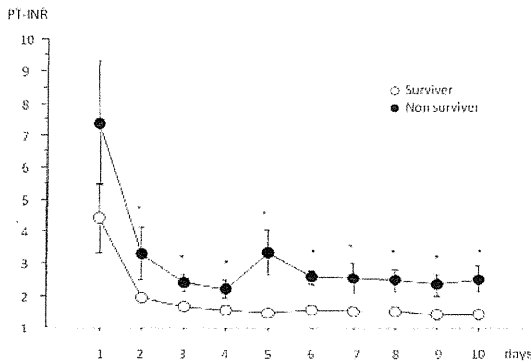

Fig. 6 The changes of PT-INR during first 10 days after the start of artificial liver support.
The open circle and closed circle shows the values of the patients who survived hepatic failure without transplantation (n = 10) and the patients who died of liver failure (n = 5), respectively. The PT-INR decreased rapidly and were kept almost 1.5 in patients who survived hepatic failure without transplantation. In patients who died of liver failure, it could not be below 2.0 with well scheduled plasma exchange. Data are expressed as mean ± SE. P = 0.012 by linear mixed effects models between the two groups. *Mann-Whitney U-test, P < 0.05. PT-INR, prothrombin time-international normalized ratio.

本法はこれらの患者においても治療中止まで，意識清明で生命を保証することができた．本法は従来のHDFよりも安価で，回路交換の頻度も少なく，確実に肝機

能を代償できることから，ALSの第一選択と成り得ると思われる．

　肝性脳症の種々の成因のうち[14]，アンモニアは代表的な脳症起因物質であり[15]，かつ脳浮腫を惹起し，動脈血アンモニア濃度200μg/dL以上は急性期の主たる死因である脳ヘルニアの危険因子と報告されている[16]．しかし，アンモニアを除去可能な血液透析（hemodialysis; HD）[17)18]の脳症治療効果は不十分で[19]，主たる原因物質は中分子領域に想定されるに至っている[20]．また，最近では，肝不全が惹起する炎症反応の関与を指摘し，炎症性メディエータ制御の必要性を示唆する意見もある[21]．現時点で急性肝不全における血液浄化療法の目的は，脳浮腫増悪因子であるアンモニアと中枢神経系毒性の可能性が高い中分子物質の除去，炎症性メディエータのコントロールが挙げられる．

　血漿交換療法，小分子量物質除去に効果的なHD，中分子量物質除去に効果的な血液濾過（hemofiltration; HF）による脳症治療効果は，それぞれ37.5％，40％，78％とされ[19]，いずれの治療法でも単独では不十分である．HDFはHD，HFの利点を併せ持ち[22)~24]，わが国では90年代後半から急性肝不全に対して導入されてきた．血漿交換療法とのコンビネーション法は現在では主たるALSと位置付けられている[5)6)25)26]．HDFを持続的に，かつ，通常のcontinuous hemodiafiltration（CHDF）より多いQDとQSを用いて行うhigh flow-volume CHDFも選択肢のひとつとなっている[27)28]．覚
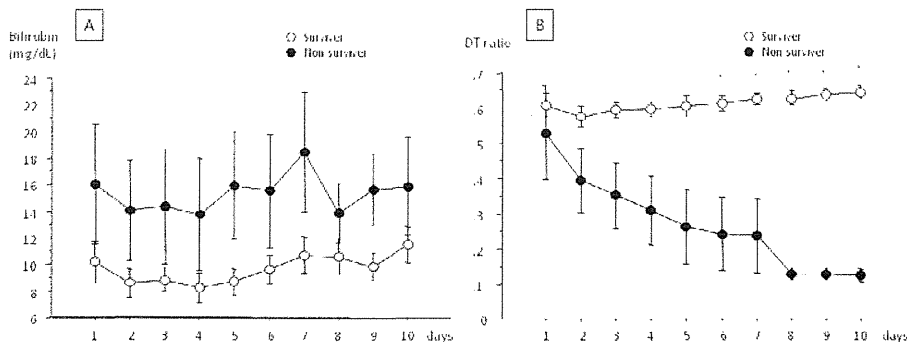
Fig. 7 The changes of the serum bilirubin levels and DT ratio during first 10 days after the start of artificial liver support.

The open circle and closed circle shows the values of the patients who survived hepatic failure without transplantation (n=10) and the patients who died of liver failure (n=5), respectively. The serum bilirubin levels increased gradually in patients who survived hepatic failure without transplantation. In patients who died of liver failure, it shows no remarkable changes in first 10 days after the start of the treatment (A). The DT ratio increased gradually in patients who survived hepatic failure without transplantation. In patients who died of liver failure, it decreased remarkably (B). Data are expressed as mean ± SE. P<0.001 by linear mixed effects models between the two groups in the changes of DT ratio. *Mann-Whitney U-test, P<0.05. DT ratio, direct bilirubin/total bilirubin ratio.
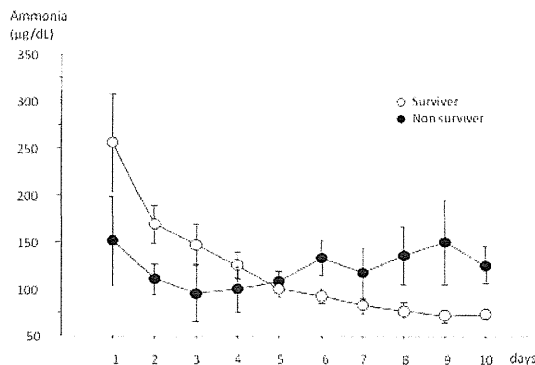

Fig. 8 The changes of the serum ammonia levels during first 10 days after the start of artificial liver support.

The open circle and closed circle shows the values of the patients who survived hepatic failure without transplantation (n=10) and the patients who died of liver failure (n=5), respectively. The serum ammonia levels of the patients who survived hepatic failure without transplantation decreased to less than 100 μg/dL on the 7th day after the start of the treatment with a constant tendency. The serum ammonia levels increased gradually in almost patients who died of liver failure. Data are expressed as mean ± SE. P<0.001 by linear mixed effects models between the two groups.

醒率は 50-97.0％ で[5)6)25)27)29]，極めて良好な成績を報告する施設もある一方で，兼坂らは，血漿交換療法と HDF を施行したウイルス性劇症肝炎 32 例中の 7 例（21.9％）は，肝性脳症 3 度より改善しなかったと報告している[25]．後希釈法を採用する HDF は前希釈法よりも濾過効率が高い半面，ダイアライザー劣化や血液凝固により回路交換が必要となることが多く，治療時間の延長がしばしば必要となる．また，製品化された無菌重炭酸バッファー（Sublood®-BSG；1292 円/2020 mL，扶桑薬品，大阪）が置換液量分必要となるなど，高コストで交換作業も煩雑である．このため慣れた血液浄化担当スタッフが必要でかつ彼らの血液汚染事故のリスクが低くない．

一方，on-line HDF は置換液が透析液（カーボスター®L；2500 円/340 L，味の素製薬，東京）から準備されるので，安価で大量かつ簡便に供給することができ[7]，前希釈法を用いるため回路交換の必要性がほとんどない．われわれの現システムで通常の血液透析に加わるエクストラコストは補液系回路（NV-A300PA；1150 円/セット）と 3 本目のエンドトキシン捕捉フィルタ（FLX-18GW；1760 円/本）程度である．一般的な HDF の置換液量が 30-40 L/回であるのに対して，われわれのシステムでは 6 時間足らずで 120 L を置換することが

可能である. HD との比較では, その安全性と中分子物質の除去効率で優越性が報告されている[30][31]. さらに心血管安定性[32][33], エリスロポイエチン製剤の必要量の減少[32][34][35], 免疫反応の改善[36] も報告されている. これらはそれぞれ抑制的に働く物質の絶大な除去性能によると考えられている. 急性肝不全症例における高い覚醒率も, 従来法を凌駕する置換量に起因すると推測される. CHDF において透析量の強化は覚醒率に有益と報告されているが[27], 本法の客観的根拠については自験データを持っていない. 井上らは on-line HDF において置換液量とグルタミン除去量がよく相関することを報告し, 脳浮腫発生抑制や覚醒効果に寄与する可能性を示している[37]. さらに, 透析液のナトリウム濃度調整が容易であることも, 急性肝不全の一般的な電解質異常である低ナトリウム血症の是正によって脳ヘルニアによる死亡を避けるために重要である[38].

透析液から置換液を作成するため, 透析液の清浄化とそのモニタリングが重要となる. わが国の水質基準では少なくとも2つのエンドトキシン捕捉フィルターを使うことを推奨しているが[39], 第1のフィルター通過後の透析液の品質はヨーロッパおよび米国の Pharmacopoeia 基準を満たすと報告され[40], 第2のフィルター通過後の透析液はオートクレーブ処理された等張食塩液のそれと同等の清浄度である[41]. われわれは置換液をサンプルにエンドトキシン測定を行い陰性であることを確認し, これまで治療関連を疑う感染性合併症は経験していない. 本法を施行する場合は, 患者関係者に十分な情報提供の上, 文書による同意を取得している.

意識覚醒までの on-line HDF の必要回数と, 開始時の意識障害の重症度がよく相関することより, 開始時の脳症の程度で覚醒までの治療必要回数を予測できる可能性がある. 最重症の脳症患者では10回程度の治療を要することがある一方, 軽度の患者が5回以上の治療後も覚醒しない場合は, 浮腫または出血の有無を評価するために脳 CT などの検索を考慮すべきと考える. また, 本研究から治療開始2-6日程度で PT 値, DT 比, アンモニア値の推移から予後の推定が可能と考えられ, 移植治療のエントリーや肝補助療法継続の適応に重要な情報を与える. われわれは, 移植治療の希望なく肝再生徴候もない症例においては, 概ね2週間程度で ALS 継続の是非を検討している.

On-line HDF の優れた除去性能は[32]~[36], 溶質除去の選択性としばしば相反する. われわれの経験では, 一回の治療でアルブミンは3.9-8.8 g が除去された（未発表成績）. 本法は既知の有益蛋白のみならず, 未知の肝再生促進因子も除去する可能性を持ち, ALS が肝再生を遅れさせる可能性も指摘されていることから[42], 現時点では覚醒状態の維持に必要な置換液量に留めるなど過度の浄化を避ける配慮が必要と思われる.

ALS 施行例の生存率は HDF や high flow-volume CHDF では 30-55.2% と報告され[25][26][28][29][43], 本研究でも移植症例を含めて50%で全国平均と同等である[1]. 生存群と肝不全死群の PT 値や DT 比に差異が認められるとおり, 本法は肝機能を完全に代償するものではない. ALS の回数や時間を増加させることは, 凝固能やアンモニア値に良い効果をもたらすと推測されるが, その合併症やコストは無視できない. 現時点では肝再生, もしくは移植までの短期間の対症療法と考えられる. 脳死下移植治療が増加すれば, 確実なブリッジデバイスとして救命率向上につながることが期待される.

欧米では, the MARS® device (Molecular Adsorbent Recirculating System; Gambro, Stockholm, Sweden), Prometheus® (Fresenius Medical Care, Bad Homburg, Germany), the BioLogic-DT™ (HemoCleanse, Lafayette, Indiana, USA) の3種の ALS と, the HepatoAssist™ device (developed by Arbios Systems, Allendale, New Jersey, USA), the ELAD® (Extracorporeal Liver Assist Device; Vital Therapies, San Diego, California, USA) の2種のバイオ肝臓が肝補助療法の主流となっている. これらの肝補助療法と, 標準的治療の randomized controlled trials (RCT) を対象とした2003年のメタ分析では, 急性肝不全を対象とした5つの RCT のうち, 脳症改善効果が有意だったのは1件のみで, メタ解析では肝移植治療へのブリッジにも有用ではなかった (relative risk [RR] 0.87, 95% confidence interval [CI] 0.73-1.05) (acute on chronic liver failure との mixed cases)[44]. 2011年のメタ分析では, 3件の RCT を対象とし, 個々の報告はいずれも有意差を見出せなかったものの, 若干の救命率改善を報告している (RR 0.70, 95% CI 0.49-1.00)[45]. しかしながら, 移植治療のブリッジユーズの検証はなく, 解析できる1件の RCT においては脳症改善効果も明らかではなかった[46]. また, 2件の RCT では PT 値の改善も認めなかった[46][47]. いずれの RCT においても, 比較された標準的治療とは ALS なしのカナマイシンやラクツロース, 抗菌薬の投与などを指しており, これらの治療との比較で優越性に汲々としている状況である. わが国の ALS を標準化し, その有用性について多数例の検証をもって世界に情報を

発信するのはわれわれの急務と思われる.

　本研究は小規模で，この結果が多様な急性肝不全症例において一般化することができるかは今後も検証が必要であり，厚生労働省研究班を中心とした有効性評価も始まっている. On-line HDF 用として認可された使用機器を用いて，2008 年に示された新たな水質基準[48]に準拠した標準的施行方法が検証されていくことと思う. 本法が世界の標準的 ALS となり，有用な肝再生治療が確立されるまでの命綱になることを切に期待したい.

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Establishment of artificial liver support by on-line hemodiafiltration for patients with acute liver failure

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Background: We introduced a new artificial liver support (ALS), on-line hemodiafiltration, in patients with acute liver failure (ALF).

Methods: This case series study was conducted from May 2001 to March 2011. Twenty-eight patients were treated with ALS including daily on-line hemodiafiltration and plasma exchange.

Results: After 4.2 ± 0.5 (mean $\pm$ SD) sessions, 89.3% of patients completely recovered from encephalopathy and maintained consciousness until discontinuation of ALS. Significant correlation was observed between the degree of encephalopathy and number of sessions required for recovery of consciousness. Ten patients fully recovered, 6 died of complications of ALF except brain edema, and 4 received liver transplantation, 5 died without transplantation after discontinuation of ALS. The changes of prothrombin time, direct bilirubin/total bilirubin ratio, and serum ammonia level shows good information for prognosis.

Conclusions: On-line hemodiafiltration was effective for the management of encephalopathy in patients with ALF.

Key words:　　liver failure　　　artificial liver support　　　on-line HDF　　　fulminant hepatitis
　　　　　　　　liver transplantation

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Original Article

ADAMTS13 activity may predict the cumulative survival of patients with liver cirrhosis in comparison with the Child-Turcotte-Pugh score and the Model for End-Stage Liver Disease score

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Aim: Decreased plasma ADAMTS13 activity (ADAMTS13:AC) results in accumulation of unusually large von Willebrand factor multimers and platelet thrombi formation. Our aim was to evaluate whether ADAMTS13:AC is a prognostic marker in patients with liver cirrhosis.

Methods: Plasma ADAMTS13:AC and its related parameters were examined in 108 cirrhotic patients.

Results: ADAMTS13:AC decreased as the severity of liver disease increased (means: controls 100%, Child A-cirrhotics 79%, Child B-cirrhotics 63%, and Child C-cirrhotics 31%). ADAMTS13:AC markedly decreased in the cirrhotics with hepatorenal syndrome, refractory ascites and hepatic encephalopathy. The cumulative survival time was the shortest (median: 4.5 months) in the cirrhotics with severe to moderate ADAMTS13:AC deficiency (<3–25%), followed by those with mild ADAMTS13:AC deficiency (25–50%), and was the longest in those with normal activity (>50%). In contrast, based on the Child-Turcotte-Pugh (CTP) score, Child C-

cirrhotics had the worst survival, but the survival probabilities did not differ between Child A and B cirrhotics. Based on the Model for End-Stage Liver Disease (MELD) score, the survival was the worst for the cirrhotics in the fourth quartile, but it was not different among cirrhotics in the first three quartiles. Cox proportional-hazards regression analysis showed that ADAMTS13:AC and serum albumin were independent factors affecting the survival.

Conclusions: ADAMTS13:AC concomitantly decreases as the functional liver capacity decreases. This activity may be a useful prognostic marker that is equal or superior to the CTP score and the MELD score to predict not only the short-term prognosis but also the long-term survival of the cirrhotic patients.

Key words: ADAMTS13 activity, Child-Turcotte-Pugh score, liver cirrhosis, Model for End-Stage Liver Disease score, prognosis

INTRODUCTION

ONCE PATIENTS WITH liver cirrhosis (LC) develop a decompensated condition, the risk of early mortality sharply increases.[1] Any patient with LC is at risk for specific life threatening complications such as variceal bleeding, sepsis, hepatorenal syndrome, and hepatopul-

monary syndrome. Many studies examined the factors that predict the survival of patients with LC.[1–7] The Child score was originally designed to assess the prognosis of cirrhotic patients undergoing surgical treatment for portal hypertension in 1964,[2] and thereafter its modified form, the Child-Turcotte-Pugh (CTP) score,[3] has been widely used to prognosticate the patients with LC.[1] However, this score includes some subjective components and does not estimate other factors, such as renal dysfunction and pulmonary dysfunction, that are commonly associated with decompensated cirrhosis.[2,3] Furthermore, the CTP score is not always sufficient, particularly when predicting the short-term prognosis of

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patients.[4] The Model for End-Stage Liver Disease (MELD) score was designed to assess the prognosis of cirrhotics who receive transjugular intrahepatic porto-systemic shunt (TIPS), and has been used as a disease severity index and a new liver organ allocation system for liver transplantation since 2002.[5] However, the main causes of death, such as variceal bleeding, ascites, hepatorenal syndrome and hepatopulmonary syndrome, in the advanced cirrhotics are not included in the MELD score.[6] Patients with advanced liver diseases tend to bleed because of reduced plasma levels of several clotting factors and thrombocytopenia, but they also exhibit thrombotic complications.[7] Portal or hepatic vein thrombosis is often observed in advanced cirrhosis,[8,9] and microthrombi formation was found in one or multiple organs in half of the autopsied cirrhotics.[10] This hypercoagulable state may not only affect hepatic parenchymal extinction, the acceleration of liver fibrosis, and disease progression but also influence other organs and potentially lead to multi-organ failure.

ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) is a metallo-proteinase that specifically cleaves the multimeric von Willebrand factor (VWF) between the Tyr1605 and Met1606 residues in the A2 domain.[11,12] ADAMTS13 deficiency, caused either by mutations in the *ADAMTS13* gene[11-14] or by inhibitory autoantibodies against ADAMTS13,[15,16] results in accumulation of "unusually large" VWF multimers (UL-VWFM) in the plasma. This accumulation leads to platelet clumping and/or thrombi under high shear stress and subsequent microcirculatory disturbances. ADAMTS13 is produced exclusively in the hepatic stellate cells (HSC),[17] although platelets,[18] vascular endothelial cells,[19] and kidney podocytes[20] have been implicated as ADAMTS13-producing cells. The plasma levels of von Willebrand factor antigen (VWF: Ag), the substrate for ADAMTS13, substantially increases as the liver disease progresses,[21,22] and thrombocytopenia is commonly seen in patients with advanced LC.[23-25] Previous studies revealed a significant reduction in the ADAMTS13 activity (ADAMTS13:AC) in advanced LC,[26,27] while the ADAMTS13 activity was unchanged in another study.[28] Subsequently, we demonstrated that both the plasma ADAMTS13 activity and antigen levels decreased as the severity of cirrhosis increased, and an imbalance between the decreased ADAMTS13:AC and the increased levels of its substrate may reflect a state that predisposes the patients with advanced LC to platelet thrombus formation.[29] In addition, we have shown

that ADAMTS13:AC is reduced in the patients with hepatic veno-occlusive disease,[30] alcoholic hepatitis,[31] and those undergoing living-donor-related liver transplantation.[32] Thus, ADAMTS13:AC likely decreases as the functional liver capacity also declines in advanced liver diseases.

In this study, we investigated the relationship between ADAMTS13:AC and the prognosis of patients with LC, and examined whether the ADAMTS13:AC is a useful prognostic factor for cirrhotic patients as compared to the CTP score and the MELD score.

METHODS

Patients

I N THIS STUDY, we examined a total of 108 LC patients, including one patient with thrombotic thrombocytopenic purpura (TTP).[33] Patients with a known history of coagulopathies, platelet disorders, or liver transplantation at basal evaluation were excluded. The origin of liver disease was hepatitis C virus (HCV) in 67 patients, hepatitis B virus (HBV) in 16, alcohol abuse in 10, primary biliary cirrhosis (PBC) in four, and cryptogenic in 11. Cirrhosis was diagnosed based on physical findings and laboratory tests, and in many cases was confirmed by histological criteria. The cirrhotic patients were classified into subgroups, according to the CTP score (Table 1), the quartiles of the MELD risk score (RS) (first quartile, RS: 0–3; second quartile, RS: 4–7; third quartile, RS: 8–13; and fourth quartile, RS: 14–43) (Table 2), or the ADAMTS13:AC (severe to moderate deficiency: <3–25% of the healthy control, mild deficiency: 25–50%, and the normal range: >50%) (Table 3). The MELD RS was calculated according to the following formula: $RS = 3.8 \times \log_e$ (bilirubin mg/dL) + $11.2 \times \log_e$ (INR) + $9.6 \times \log_e$ (creatinine mg/dL) + $6.4 \times$ (cause of cirrhosis) in which the value for the cause of cirrhosis was 0 for an alcoholic or cholestatic etiology and one for viral or other etiologies.[5] The spleen volume was determined by computed tomography (CT) scanning.[34] The diagnosis of ascites, spontaneous bacterial peritonitis (SBP), and hepatorenal syndrome was made according to previously described criteria.[35] Hepatic encephalopathy grade II or higher was evaluated by the classification of Trey *et al.*[36] Endoscopic signs of an impending variceal rupture were classified according to the criteria of the Japanese Research Society for Portal Hypertension.[37] The Japan Integrated Staging (JIS) score was obtained by adding the tumor stage score of hepato-

Table 1 Clinical data of the patients with liver cirrhosis according to the Child-Turcotte-Pugh (CTP) score

	Child A (n = 35)	Child B (n = 33)	Child C (n = 40)
Age (years)	66.4 ± 7.8	63.6 ± 8.3	64.7 ± 15.1
Sex (male/female)	25/10	17/16	22/18
Cause of liver disease			
HCV/HBV/Alcohol/PBC/Cryptogenic	24/4/4/0/3	20/7/2/0/4	23/5/4/4/4
Child–Pugh score	5.5 ± 0.5	7.9 ± 1.0**	11.4 ± 1.5**,***
MELD score	6 ± 5	9 ± 5*	16 ± 8**,****
Platelet count (×10⁴/mm³)	9.6 ± 4.6	6.9 ± 2.4*	5.9 ± 3.6*
Spleen volume (mm³)	323 ± 181	399 ± 250	551 ± 243**,***
Ascites (−/easily mobilized/refractory)	0	21/10/2	8/6/26
Spontaneous bacterial peritonitis	0	0	10*,***
Hepatorenal syndrome (+)	0	0	10*,***
Encephalopathy (+)	0	9*	32**,****
Esophageal varices (−/mild/severe)†	10/12/13	3/7/23*	3/6/31**
Each incidence (−/mild/severe)†	29%/34%/37%	9%/21%/70%*	7%/15%/78%**
Hepatocellular carcinoma (+)	22	16	19
JIS score‡	1.4 ± 0.9	2.8 ± 1.0**	3.7 ± 1.1**,***
Portal thrombosis	0	3	3
Outcome (alive/died)	30/5	27/6	9/31
Cause of death			
Hepatocellular carcinoma	4	5	17
Hepatic failure	0	0	7
Hepatorenal syndrome	0	0	6
Gastrointestinal bleeding	0	1	0
Thrombotic thrombocytopenic purpura	0	0	1
Acute myocardial infarction	1	0	0

*P < 0.01 and **P < 0.001 vs. cirrhotics with Child A, respectively. ***P < 0.01 and ****P < 0.001 vs. cirrhotics with Child B, respectively.
†Mild or severe esophageal varices indicate lesions without or with endoscopic signs of impending variceal rupture, respectively.
‡The Japan Integrated Staging score obtained via the summation of Child–Pugh score and tumor stage score.[38]
The data are expressed as mean ± standard deviation (SD).
HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis.

cellular carcinoma (HCC) and the CTP score.[38] The portal thrombosis was examined by Doppler-ultrasonography (US). Once patients were discharged, they were carefully followed up by conventional liver function tests and tumor markers including α-fetoprotein and des-γ-carboxy prothrombin every month as outpatients until death, which was the primary end-point to estimate survival probabilities by the CTP score, the MELD score and ADAMTS13:AC. Patients with liver transplantation were, therefore, excluded. Image diagnosis by US, dynamic enhanced CT, or dynamic enhanced magnetic resonance imaging (MRI) was performed every 3 months to evaluate the presence or absence of HCC or ascites. All subjects provided informed consent to participate in the study. The study protocol was approved by the Nara Medical University Hospital Ethics Committee.

Determination of ADAMTS13:AC, VWF: Ag, VWF: RCo, UL-VWFMs and ADAMTS13:INH

Blood samples were taken from patients at the time of admission or during their hospital stay. These samples were stored in plastic tubes containing 1/10th the volume of 3.8% sodium citrate. Platelet-poor plasma was prepared by centrifuging at 3000 g at 4°C for 15 min and stored in aliquots at −80°C until analysis. For seven patients with LC, a second plasma sample was taken between days 120 and 630 (mean: 345 days) during their second hospitalization because of hepatic encephalopathy (three patients), ascites augmentation (three patients), and variceal rupture (one patient). Plasma ADAMTS13:AC was determined by the classic VWFM assay[39,40] and the sensitive chromogenic enzyme-linked immunosorbent assay (ELISA, ADAMTS13-act-

Table 2 Clinical data of the patients with liver cirrhosis according to the Model for End-Stage Liver Disease (MELD) score

	First quartile ($n = 12$)	Second quartile ($n = 30$)	Third quartile ($n = 33$)	Fourth quartile ($n = 33$)
Age (years)	63.8 ± 7.9	68.0 ± 8.8	65.1 ± 10.4	66.7 ± 9.5
Sex (male/female)	6/6	16/14	21/12	24/9
Cause of liver disease				
HCV/HBV/Alcohol/PBC/Cryptogenic	6/2/3/0/1	21/3/4/1/1	20/7/0/3/3	20/4/3/0/6
Child–Pugh score	6.6 ± 1.7	6.8 ± 2.0	7.9 ± 1.9***	11.1 ± 2.5**,****,******
ADMTS13 activity (%)	69 ± 25	74 ± 34	68 ± 29	32 ± 21**,****,******
MELD score	2 ± 3	6 ± 1**	10 ± 2**,****	19 ± 6**,****,******
Platelet count ($\times10^4$/mm^3)	9.2 ± 6.0	10.7 ± 8.7	7.8 ± 4.2	6.8 ± 4.1*,***
Spleen volume (mm^3)	285 ± 75	397 ± 146	386 ± 99	567 ± 162*,***,*****
Ascites (−/easily mobilized/refractory)	11/1/0	24/4/2	24/5/4	5/6/22**,****,******
Spontaneous bacterial peritonitis	0	1	1	8****,******
Hepatorenal syndrome (+)	0	1	0	9*,****,******
Encephalopathy (−/+)	1	4	9	27**,****,******
Esophageal varices (−/mild/severe)†	4/3/5	6/9/15	4/9/20	2/4/27
Each incidence (−/mild/severe)†	33%/25%/42%	20%/30%/50%	12%/27%/61%	6%/12%/82%**,*****
Hepatocellular carcinoma (+)	5	16	16	20
JIS score‡	1.8 ± 1.3	1.7 ± 1.0	2.2 ± 1.2	3.6 ± 1.3*,****,******
Portal thrombosis	0	3	2	1
Outcome (alive/died)	9/3	25/5	24/9	8/25
Cause of death				
Hepatocellular carcinoma	0	3	6	17
Hepatic failure	1	1	3	2
Hepatorenal syndrome	0	1	0	5
Gastrointestinal bleeding	1	0	0	0
Thrombotic thrombocytopenic purpura	0	0	0	1
Acute myocardial infarction	1	0	0	0

*$P < 0.05$ and **$P < 0.001$ vs. cirrhotics with the first quartile, respectively. ***$P < 0.05$ and ****$P < 0.001$ vs. cirrhotics with the second quartile, respectively.
*****$P < 0.05$ and ******$P < 0.001$ vs. cirrhotics with the third quartile, respectively.
†Mild or severe esophageal varices indicate lesions without or with endoscopic signs of impending variceal rupture, respectively.
‡The Japan Integrated Staging score obtained via the summation of Child–Pugh score and tumor stage score.[38]
The data are expressed as mean ± standard deviation (SD).
HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis.

Table 3 Clinical data of the patients with liver cirrhosis according to the ADAMTS13 activity

	Normal range (>50%) (n = 56)	Mild deficiency (25–50%) (n = 27)	Severe to moderate deficiency (<25%) (n = 25)
Age (years)	64.9 ± 10.8	66.8 ± 7.6	67.8 ± 9.4
Sex (male/female)	32/24	15/12	20/5
Cause of liver disease			
HCV/HBV/Alcohol/PBC/Cryptogenic	34/9/7/2/4	19/3/1/0/4	14/4/2/2/3
ADMTS13 activity (%)	83 ± 22	43 ± 12**	20 ± 18**,****
Child–Pugh score	7.0 ± 1.9	9.5 ± 3.0**	10.7 ± 2.3**
MELD score	7 ± 5	13 ± 9**	16 ± 6**
Platelet count (×10⁴/mm³)	9.5 ± 5.6	8.2 ± 7.9	6.5 ± 3.2*
Spleen volume (mm³)	324 ± 85	559 ± 104**	534 ± 198**
Ascites (–/easily mobilized/refractory)	46/8/2	11/4/12**	7/4/14**
Spontaneous bacterial peritonitis	0	2*	8**,***
Hepatorenal syndrome (+)	0	2*	8**,***
Encephalopathy (–/+)	6	13**	22**,***
Esophageal varices (–/mild/severe)†	9/18/29	5/4/18	2/3/20
Each incidence (–/mild/severe)†	16%/32%/52%	19%/15%/66%	8%/12%/80%
Hepatocellular carcinoma (+)	27	15	15
JIS score‡	1.9 ± 1.3	2.5 ± 1.5	3.6 ± 0.9**,***
Portal thrombosis	0	3	3
Outcome (alive/died)	45/11	15/12	6/19
Cause of death			
Hepatocellular carcinoma	6	7	13
Hepatic failure	3	3	1
Hepatorenal syndrome	0	2	4
Gastrointestinal bleeding	1	0	0
Thrombotic thrombocytopenic purpura	0	0	1
Acute myocardial infarction	1	0	0

*$P < 0.05$ and **$P < 0.001$ vs. cirrhotics with >50% of ADAMTS13 activity, respectively. ***$P < 0.05$ and ****$P < 0.001$ vs. cirrhotics with 25% to 50% of ADAMTS13, respectively.
†Mild or severe esophageal varices indicate lesions without or with endoscopic signs of impending variceal rupture, respectively.
‡The Japan Integrated Staging score obtained via the summation of Child–Pugh score and tumor stage score.[38]
The data are expressed as mean ± standard deviation (SD).
HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis.

ELISA: Kainos Laboratories Inc., Tokyo, Japan).[41] The normal values were 102 ± 23% in the VWFM assay[39] and 99 ± 22% in the act-ELISA.[41] Plasma VWF: Ag was measured using a rabbit polyclonal sandwich ELISA (Dako, Denmark), and its normal level was 100 ± 53% (n = 60, age: 20–39 years). VWF ristocetin cofactor activity (VWF: RCo) was determined as previously described,[42] and its normal value was 100 ± 15%. In 49 LC patients with lower ADAMTS13:AC (<50% of the normal control), the plasma UL-VWFMs were analyzed by a vertical sodium dodecyl sulfate (SDS)–1.0% agarose gel electrophoresis system[43] and evaluated using NIH image J. ADAMTS13 inhibitor (ADAMTS13: INH) was evaluated using plasma that was heat-inactivated at 56°C for 30 min.[15,16] One Bethesda unit

(BU) of inhibitor was defined as the amount of plasma that reduces ADAMTS13:AC to 50% of the control,[44] and its titer was considered significant at >0.5 BU/mL.

Statistical analysis

The differences among cirrhotics with the CTP score, the MELD score, and the ADAMTS13:AC, and healthy subjects were analyzed with the Kruskal–Wallis rank test before pair-wise comparisons (Tables 1–4). If the Kruskal–Wallis rank test showed significant differences in each parameter among groups, pairwise comparison in each parameter was evaluated by Mann–Whitney U-test for continuous variables. The χ^2-test was used for

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Table 4 The plasma values of ADAMTS13 activity and its related parameters

Variable	Liver cirrhosis			Healthy subjects (n = 60)
	Child A (n = 35)	Child B (n = 33)	Child C (n = 40)	
ADAMTS13:AC (%) (VWFM assay)	79 ± 25**	63 ± 34**·***	31 ± 22**·****·******	102 ± 23
ADAMTS13:AC (%) (ELISA)	80 ± 24**	65 ± 31**·***	40 ± 22**·****·******	99 ± 22
VWF: Ag (%)	320 ± 174**	436 ± 267**·***	486 ± 254**·****	100 ± 53
VWF: RCo (%)	186 ± 137*	198 ± 172*	227 ± 187*	100 ± 15
VWF: RCo/VWF: Ag ratio	0.63 ± 0.49**	0.50 ± 0.46**	0.51 ± 0.40**	1.1 ± 0.42
VWF: RCo/ADAMTS13 ratio	1.6 ± 1.7**	5.0 ± 5.7**·***	16.8 ± 28.2**·****·*****	0.9 ± 0.2
VWFM patterns† (degraded/normal/unusually-large)	2/0/0	8***/5***/0	6/20****·*****/8***·*****	
Inhibitor against ADAMTS13† (number of positive cases)	1	9***	19****	absent

*P < 0.05 and **P < 0.001 vs. healthy subjects, respectively. ***P < 0.05, and ****P < 0.001 vs. cirrhotics with Child A, respectively. *****P < 0.05 and ******P < 0.001 vs. cirrhotics with Child B, respectively.
†The VWFM patterns and ADAMTS13 inhibitor were analyzed in 49 cirrhotic patients with lower ADAMTS13:AC (less than 50% of the healthy control).
The data are expressed as mean ± standard deviation (SD).
ADAMTS13: AC, ADAMTS13 activity; ELISA, enzyme-linked immunosorbent assay; VWF: Ag, von Willebrand factor antigen; VWF: RCo, von Willebrand factor ristocetin cofactor activity; VWFM, von Willebrand factor multimer.

categorical data. The differences in the ADAMTS13:AC and CTP scores obtained by the sequential study in identical patients were estimated by Wilcoxon signed-ranks test. Correlations were calculated by the Spearman rank test. The analyses were carried out using Statview statistical software (version 5.0; SAS Institute Inc., Cary, NC, USA). The Kaplan–Meier analysis was used to evaluate the prognosis of cirrhotic patients according to the degree of the CTP score, the MELD score and plasma ADAMTS13:AC (Figs 1–3) by the log-rank test using StatMate IV for Windows (AT0484, Advanced Technology for Medicine & Science, Tokyo, Japan). Cox proportional-hazard model was used to evaluate the hazard ratio of each class in the CTP score, the MELD score, and the ADAMTS13:AC (Table 5). In addition, Cox proportional-hazards regression analysis was applied to determine independent prognostic markers including the ADAMTS13:AC. The following eight variables were analyzed for potential covariates to predict the survival at the time of sample collection: age, sex, ADAMTS13:AC, albumin, total bilirubin, prothrombin time, blood ammonia, and platelet count. Although continuous variables without data conversion were used at first, some multivariate analyses could not be performed because of the multi-collinearity among parameters, and then, several variables were transformed into categorical data consisting of two or three simple ordinal numbers to obtain each hazard ratio (Table 6).

To further compare the overall accuracy of these three models including the CTP score, the MELD score, and the ADAMTS13:AC, the areas under the curves (AUCs) were determined by receiver operating characteristic (ROC) curves for 1-year and 2-year survival (Fig. 4). The
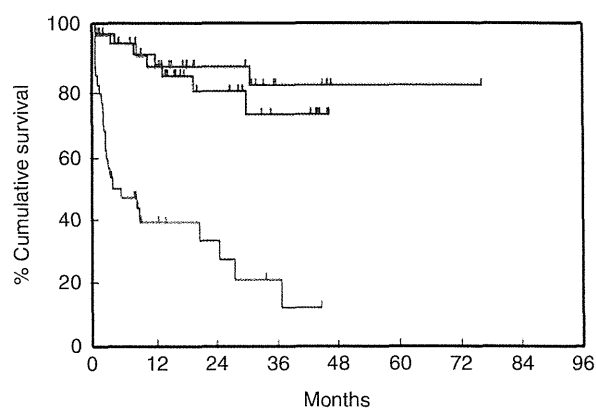

Figure 1 The cumulative survival rate of 108 patients with liver cirrhosis according to the Child classification. Child C patients had worse survival than Child A and B patients, but the survival probabilities were not different between Child A and Child B patients (Log rank test among the three groups, P < 0.0001; Child C vs. Child A, P < 0.0001; Child C vs. Child B, P < 0.0001). The red, blue, and green lines indicate cirrhotic patients with Child A, Child B, and Child C, respectively.