表4　肝性脳症の昏睡度分類

Ⅰ	睡眠－覚醒リズムの逆転 多幸気分，時に抑鬱状態 だらしなく，気にとめない態度	retrospectiveにしか判定できない場合が多い (3-3-9度方式　1)
Ⅱ	指南力（時，場所）障害，物を取り違える（confusion） 異常行動（例：お金をまく，化粧品をゴミ箱に捨てるなど） 時に傾眠状態（普通の呼びかけで開眼し，会話ができる） 無礼な言動があったりするが，医師の指示に従う態度を見せる）	興奮状態がない 尿，便失禁がない 羽ばたき振戦あり (3-3-9度方式　2, 3, 10)
Ⅲ	しばしば興奮状態またはせん妄状態を伴い，反抗的態度を見せる 嗜眠状態（ほとんど眠っている） 外的刺激で開眼しうるが，医師の指示に従わない.または従えない（簡単な命令には応ずる）	羽ばたき振戦あり（患者の協力が得られる場合） 指南力は高度に障害 (3-3-9度方式　20, 30)
Ⅳ	昏睡（完全な意識の消失） 痛み刺激に反応する	刺激に対して払いのける動作，顔をしかめるなどが見られる (3-3-9度方式　100, 200)
Ⅴ	深昏睡 痛み刺激にもまったく反応しない	(3-3-9度方式　300)

高シトルリン血症など，尿素回路異常に伴う肝性脳症では，血液アンモニア濃度以外に緊急で測定可能な血液生化学検査での異常がないことから，血液アンモニア濃度の測定は肝性昏睡の診断に極めて有用である．

これらの検査を提出しつつ，瞳孔径，対光反射，麻痺の有無を時間をかけずに迅速に確認し，頭蓋内疾患の有無をチェックする．同時に神経学以外の徴候としての顔色，眼瞼結膜，呼吸状態，口臭を確認する．急性アルコール中毒によるアルコール臭や肝性口臭，農薬などの刺激臭，アーモンドのようなシアン化合物の臭いなどは判別可能なことがある．また，皮膚の湿潤，発汗なども確認する[1]．

2. 検査の進め方

脳の局所病変を疑わせる疾患は必ずCT検査が必要となり[2]，脳卒中であれば再破裂の防止や進行を防ぐ手段を専門医と連絡を取りつつ，専門機関へ至急搬送する．くも膜下出血，脳梗塞も適切な時間帯に適切な処置が行われることが必要である．肝性昏睡では劇症肝炎などで脳浮腫が出現している場合などの深昏睡ではCTにて所見があるが，肝硬変に伴う脳症では明らかな所見はない．採血は必ず必要であり，肝機能，腎機能，血糖，内分泌，電解質のチェックを行う．肝性脳症を含む代謝性脳障害の鑑別には採血による情報は重要となる．

6 肝性脳症の概念と分類

肝性脳症は劇症肝炎や肝硬変などの重篤な肝障害が原因で生ずる意識障害を中心とする精神神経症状である．肝性昏睡とほぼ同義語として用いられているが，これには指南力の低下あるいは異常行動などの軽度のものから刺激を加えてもまったく反応しない深昏睡まで幅がある．

肝性脳症の重症度は一般には昏睡度で判定することが多く，わが国では犬山シンポジウムの昏睡度の分類が用いられる（表4）．

1. 顕性脳症

肝性脳症は臨床経過や脳症の発症様式などにより急性型，慢性型，および特殊型に分類される．急性型は劇症肝炎に，慢性型は側副

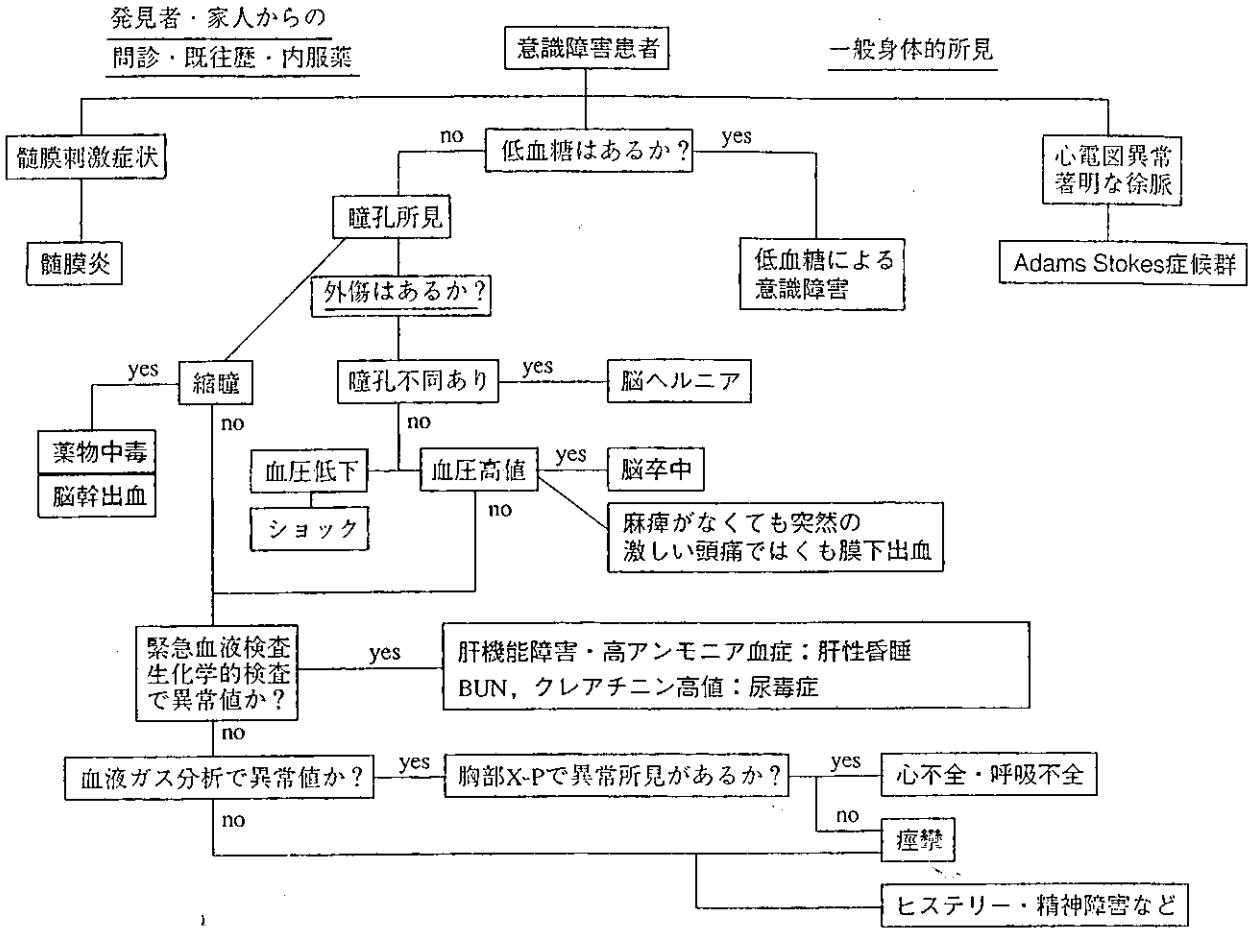

図1　意識障害の初期診断鑑別のポイント（文献1より改変）

血行路の発達した肝硬変におのおの代表され，さらに慢性型は門脈－大循環短絡の要因が強いタイプ（慢性再発型）と肝細胞障害の要因が強いタイプ（末期型）に分けられる[3]．特殊型の頻度は少ないが，先天性尿素サイクル酵素異常症ではシトルリン血症の頻度が高い．このタイプではアンモニアが肝性脳症の発生にもっとも強く関与していると考えられる．

最近，肝硬変がなく門脈大循環短絡のみで肝性脳症を発現する例や急性型といっても急性肝不全による肝性脳症なのか肝硬変で急性に脳症を発症したか区別が付きにくいといった問題点などが指摘され，欧米を中心として新しい肝性脳症分類が作成されている[4]（表5）．

2．潜在性肝性脳症

精神神経症状が明らかでなく，臨床的には肝性脳症とは認められない肝硬変に鋭敏で定量的な精神神経機能検査を行うことで精神神経機能の異常が指摘されることがあり，このような病態を潜在性肝性脳症と呼んでいる．これは脳症の重症度分類ではgrade 0にあたる部分に存在する概念である．このような潜在性肝性脳症は臨床的に動作能力や注意力の低下が指摘され，わが国の肝硬変例の半分が潜在性肝性脳症を呈すると仮定すると，その患者数は15万人程度と推定される．

潜在性肝性脳症を顕性脳症の前段階としてとらえるか否かについてはいまだ明確にされていないが，筆者ら[5]の検討では，初回に潜在性肝性脳症と診断されてから6カ月目

表5　新しい肝性脳症の分類（文献4より改変）

A型（Acute）	急性肝不全（劇症肝炎など）でみられる脳症
B型（Bypass）	門脈－大循環短絡による脳症で肝硬変などの肝疾患を伴わない
C型（Cirrhosis）	肝硬変と門脈圧亢進症／門脈－大循環短絡バイパスでみられる脳症
	●エピソード（間歇）型脳症
	●持続型脳症
	●ミニマル脳症（従来の潜在性肝性脳症）

表6　肝性昏睡の身体所見

1）肝性口臭	
2）黄疸	
3）腹水・浮腫	
4）肝濁音界縮小（劇症肝炎）	
5）脾腫・硬い肝臓（肝硬変）	
6）クモ状血管腫	（肝硬変）
手掌紅斑	（肝硬変）
女性化乳房	（肝硬変）
腹壁静脈怒張（Caput medusae）	（肝硬変）

までに23％（5/22例）の症例でII度以上の脳症を発症しており，潜在性肝性脳症と診断された症例の中に顕性脳症の前段階としてとらえられる症例がある．

7 肝性脳症の診断と鑑別診断

1. 診断

肝性脳症は肝機能異常，肝疾患の既往の有無，意識障害をはじめとする精神神経症状，高アンモニア血症，脳波異常，臨床検査成績などから他疾患を鑑別しつつ総合的になされる．ことに肝硬変に伴う肝性昏睡の場合，臨床徴候として，黄疸，浮腫・腹水や皮膚所見のクモ状血管腫，手掌紅斑，女性化乳房，腹壁静脈の怒張などがみられ，診断の参考となる（表6）．

II度以上の脳症では羽ばたき振戦（flapping tremor）が観察されることが多い．これはアステリキシス（asterixis）といわれ，上肢などを保持するときに出現する短時間の筋緊張の消失であり，両側の上肢を前方やや上方に挙上し，両手を手背方向にそらさせると誘発させやすい．舌を前方に突出させる動作を行っても誘発させることができる．しかし，IV度以上の狭義の昏睡状態に陥ると振戦は消失する．

肝性脳症では，昏睡の進行に伴って左右対称でびまん性の脳波の徐波化が見られ，昏睡の予知，重症度判定，治療効果の判定に有用である．また三相波もみられるが，昏睡II～III度での出現頻度が高い．昏睡IV度になると周波数，振幅は減少し平坦となる．

潜在性肝性脳症の診断は，統一した診断基準はないがWAIS（Wechsler式知能検査）などや，脳波などの神経学検査により行われる．具体的にはWAIS式知能検査の積木検査，符号検査や記号追跡試験の3項目が多く用いられるが，再現性を保つためには同一の検査条件で施行することが重要とされ，臨床で簡便に施行することには限界があった．最近筆者らはコンピュータを用いた簡便な精神神経機能検査を開発，実用化した．この検査ソフトは平成13年春から肝臓学会を通じ無償で供与，潜在性肝性脳症診断のスクリーニング法として使用が可能である[6]．

2. 鑑別診断

先行する肝疾患が明らかでない時や，肝硬変はあっても臨床検査成績などから肝性脳症と診断が確定できない時には，さらに検査を行い他の意識障害をきたす疾患との鑑別が必要である．中枢神経系疾患の鑑別には，脳CTスキャン，髄液所見など，糖尿病性ケトアシドーシスなどの鑑別には，血糖，尿中ケトン体，血液ガス所見，血清電解質などが有

表7　意識障害(二次性)をきたす代表的疾患の鑑別

●肝性昏睡
高アンモニア血症
高ビリルビン血症
AST，ALTの異常など
●糖尿病性昏睡
高血糖（低血糖）
血液ケトン体
代謝性アシドーシス
●尿毒症性昏睡
BUN＞80 mg/dl
高クレアチニン血症（＞10 mg/dl）
高カリウム血症
代謝性アシドーシス
●急性心筋梗塞
白血球上昇
CPK，AST，LDH上昇（発作12時間後より）
心電図所見（Q波，T波逆転：発作数時間後より）

BUN：Blood urea nitrogen（血中尿素窒素）
CPK：creatinine phosphokinase
（クレアチニンフォスフォキナーゼ）
LDH：lactate dehydrogenase（乳酸脱水素酵素）

用である．特に慢性肝疾患を有するアルコール依存症例では頭部外傷による硬膜下血腫やアルコール離脱症候群を伴うことがあり，肝性脳症との鑑別が重要となる(表7)．

8 おわりに

意識障害の原因は多岐にわたることから，その鑑別は重要であり，初期治療をはじめながら検査を進めていく必要がある．鑑別にあたっては発症の様式，既往歴，内服薬の有無などの情報が重要となる．検査を進めていく上ではCTにより脳内の器質的な疾患の有無の判断を，血液検査により肝性脳症を含む代謝性脳障害の有無を鑑別する．ことに血液アンモニア濃度の測定は肝性脳症の診断に有用となる．

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特集 呼気テストによる消化器疾患の病態診断

肝硬変における肝実質機能評価法としての^{13}C-フェニルアラニン呼気テストの有用性*

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Key Words : liver cirrhosis, ^{13}C-Phenylalanine, breath test, functional capacity of hepatocyte

はじめに

肝硬変は，慢性の肝細胞の障害により肝細胞の再生と結合組織の増生が生じ，びまん性に線維性隔壁に囲まれた再生結節(偽小葉)が形成された状態であり，臨床的には一つの独立した疾患ではなく，種々の原因によって生じる慢性・進行性肝疾患の終末像である．慢性肝疾患の肝実質機能(予備能)を評価することは治療の経過や予後の評価から重要であるが，定量法的な肝機能評価法として確立された方法はなくICG15分停滞率，ガラクトース負荷試験，アシアロシンチ検査，呼気テストなどが用いられている[1](表1)．

^{13}C呼気テストは炭素(^{12}C)の安定同位体である^{13}C標識化合物を投与することにより胃からの排出，小腸からの吸収，肝における代謝という過程を経て呼気中に$^{13}CO_2$が排出されることを利用した検査法である．肝機能検査として応用されている^{13}C呼気テストには，^{13}C-フェナセチン，^{13}C-アミノピリン，^{13}C-メタセチン，^{13}C-エセンザミド，^{13}C-カフェイン，^{13}C-フェニルアラニンなどがある[2]．本稿では，最近行われている呼気テストの^{13}C-フェニルアラニンの肝硬変における肝実質機能評価法としての有用性について述べる．

^{13}C-フェニルアラニン呼気テストの原理

肝臓は生体のアミノ酸代謝の中心的臓器であり，肝障害時にはアミノ酸代謝が障害されることが知られている．とくに芳香族アミノ酸であるフェニルアラニンとチロシンは主に肝臓で代謝され，その血漿濃度は肝細胞機能に依存する[3]．このためアミノ酸代謝の評価は外科手術の死亡率や罹病率の予測に有用との報告もある[4][5]．

フェニルアラニンは主に小腸上部に存在する担体輸送系により吸収され，そのほとんどが肝臓にあるフェニルアラニン水酸化酵素によりチロシンに変換される．ほ乳類ではこの過程が律速段階となり，フマル酸，アセト酢酸へと分解されCO_2が産生される(図1)．したがって，^{13}C-フェニルアラニン呼気テストで排出される$^{13}CO_2$排出量はこれらの代謝経路の総和である．

^{13}C-フェニルアラニン呼気テストの測定方法

^{13}C-フェニルアラニン呼気テストの検査方法は，トレーサーであるL-1-^{13}C-フェニルアラニンの投与量と測定方法により多少の差異がある．投与量にはIshiiら[6]による100mg/bodyを投与する方法と，10mg/kg/bodyを投与する方法[7]~[9]に大き

* Validity of ^{13}C-Phenylalanine breath test as a measurement of hepatocyte functional capacity in liver cirrhosis.

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表1 定量的な肝機能評価法

部位	基質	機能
細胞質	ガラクトース*	ガラクトシダーゼ(リン酸化)
	フェニルアラニン	水酸化
ミクロゾーム(チトクロームP450系)	アミノピリン	N-脱メチル化
	カフェイン	N-脱メチル化
	リグノカイン	N-脱メチル化
	アンチピリン	水酸化/脱メチル化
類洞側細胞膜の受容体	末端にガラクトースをもつ糖蛋白	アシアロ糖蛋白受容体

*低容量投与法により肝血流量を評価できる (文献[1]より一部改変)

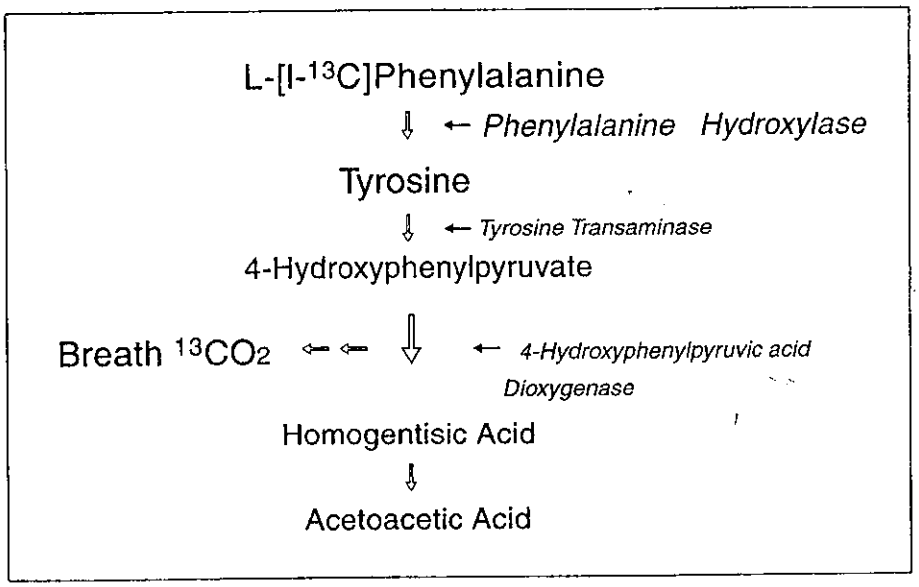

図1 L-[1-13C]-フェニルアラニンの代謝過程

く分けられる．われわれはIshiiらの方法に準じ検査前7時間以上の絶食後，早朝空腹時に呼気200mlを採取し，L-1-13C-フェニルアラニン100mgを精製水100mlに溶解し経口投与し，赤外分光光度計で計測している．

測定方法には質量分析法と赤外分光光度計を用いた方法があり，従来より$^{13}CO_2$などの安定同位体は質量分析法で測定されていた．質量分析法はもっとも精度が高いが，高価であること，手技が煩雑であること，時間がかかることなどの難点もあり，現在簡便に測定できる方法として赤外分光光度計を用いた方法が普及してきている．

^{13}C-フェニルアラニン呼気テストの肝機能検査結果

呼気テストの主なパラメーターはΔ‰，^{13}C排出速度(%^{13}C dose/h)，^{13}C累積回収率(^{13}C-cumulative %dose)である[10](表2)．以下に現在まで得られている当科および諸家の成績を示す．

1．肝硬変ではピーク到達時間が遅延し，ピーク値が低くなる

健常者ではL-1-^{13}C-フェニルアラニン100mgの経口投与後15分に^{13}C排出量が高いピークを呈しすみやかに下降するが，肝硬変ではピーク到達時間が遅延し，ピーク値が低くなる傾向を示す．この傾向は肝硬変が重症(Child-Pugh分類)になるにつれて顕著になり，Child Cではピークが明らかでなくなる[6][11]．図2に当科の肝硬変20例と健常者9例の経時的変化を示したが同様の結果であった．またIshiiら[7]は肝硬変症例2例の呼気テストを経時的に観察し，2年の経過で，ピーク値，^{13}C累積回収率は次第に低下することを報告した．

表2 ^{13}C呼気テストに用いられるパラメーター

1. $\Delta^{13}C=\delta^{13}C$値変化量(‰)
   $\Delta^{13}C$：$=(\delta^{13}C)_t-(\delta^{13}C)_0$
   $(\delta^{13}C)_t$：試薬投与後t時間での$\delta^{13}C$値(‰)
   $(\delta^{13}C)_0$：試薬投与後t時間での$\delta^{13}C$値(‰)
2. ^{13}C排出速度(^{13}C-%dose/hr)
   投与量(Dose)に対する^{13}Cの単位時間あたりの排出量
   ^{13}C排出速度＝^{13}C排出量(mmol/hr)/^{13}C投与量(mmol)
3. ^{13}C累積回収率(^{13}C-cumulative %dose)
   排出速度を曲線を時間で積分して，曲線下面積(area under curve：AUC)を計算して求める

(文献[9]より引用)

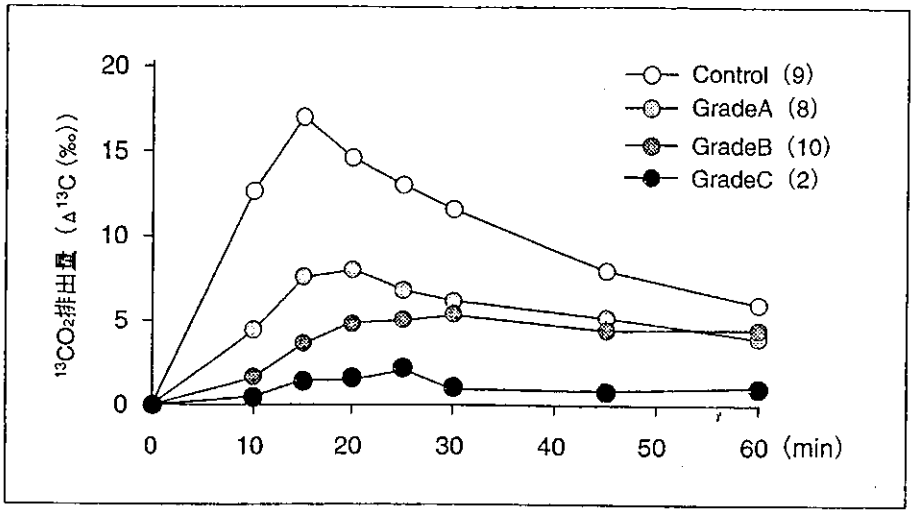

図2 ^{13}C-フェニルアラニン呼気テストの経時的変化

表3 ^{13}C-フェニルアラニン呼気テストにおける^{13}C累積回収率と各種血液生化学検査成績との関連

報告者	投与量	測定法	正の相関	負の相関
Burkeら[11]，1997	100mg/body	重量分析法	Alb, PT	T.Bil, Child-Pughスコア
Ishiiら[6]，2001	100mg/body	赤外分光法	Alb, TC, ChE, HPT, Plt., Fischer比	T.Bil
Kobayashi[13]ら，2001	100mg/body	赤外分光法	Alb, TC, ChE, PT, Plt.	T.Bil

Alb：アルブミン，TC：総コレステロール，ChE：コリンエステラーゼ，PT：プロトロンビン時間，HPT：ヘパプラスチンテスト，Plt：血小板数，T.Bil：総ビリルビン

2. 肝の重症度を示す血液生化学検査成績との関連

報告者により多少の差異はあるが，各種血液生化学検査成績と^{13}C累積回収率の関連を表3に示す．血清アルブミン，プロトロンビン時間，ヘパプラスチンテスト，コリンエステラーゼ，総コレステロール，総ビリルビンなど肝の重症度を示す検査成績と有意な相関が認められる[6][12][13]．さらにBurkeら[12]はChild-Pughスコアと有意の負の相関が認められることを報告している．当科の検討でもプロトロンビン時間，ヘパプラスチンテスト，総コレステロールと正の相関を，総ビリルビンと負の相関を認めた．

3. ICG15分停滞率との関連(図3)

ICG15分停滞率は肝予備能検査として認められている方法であり，肝切除のcriteriaにも取り上げられている．Kobayashiら[13]は，ICG15分停滞率をICG<10%，ICG 10～20%，ICG>20%と3群に分類し，30分の^{13}C排出速度および^{13}C累積回収率との関連を調べ，3群間に有意の差を認めることから呼気テストの有用性を報告している．

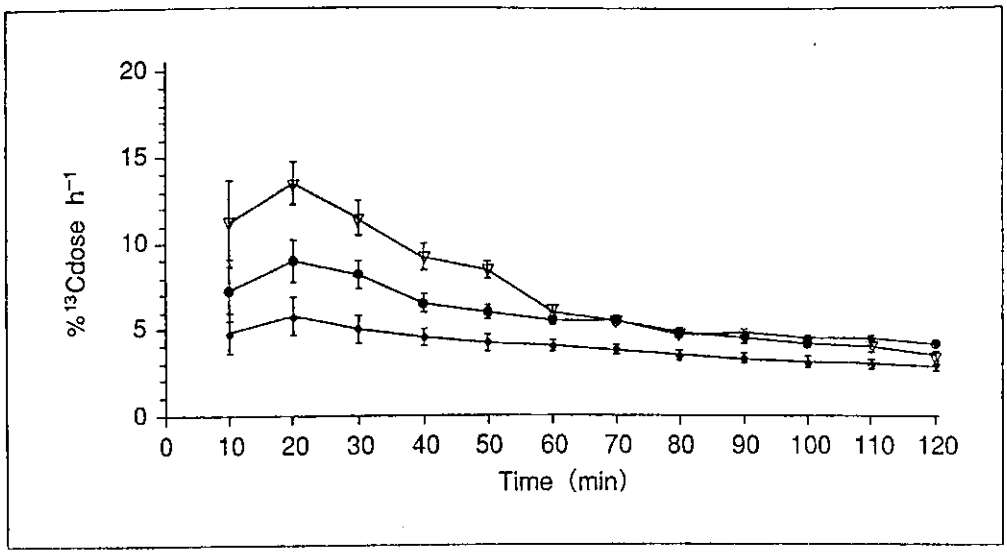

図3 L-[1-^{13}C]-フェニルアラニン100mg経口投与後の^{13}C排出量曲線
GroupⅠ(ICG R15<10%，n=14；▽)，GroupⅡ(ICG R15 10~20%，n=14；●)，GroupⅢ(ICG R15>20%，n=14；◆)．データーはmean±SEMで表示．(文献[13]より一部改変)

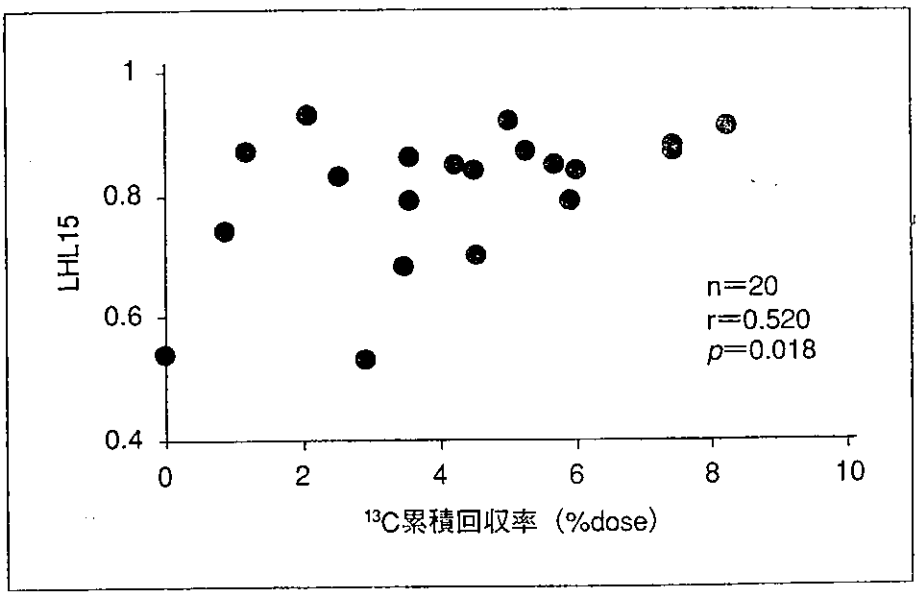

図4 ^{13}C-フェニルアラニン呼気テストとICG15分停滞率の関連

4．アシアロシンチとの関連

アシアロシンチグラフィーは，^{99m}TC-GSAが肝細胞膜のアシアロ糖蛋白受容体に特異的に結合することを利用した検査方法であり，特にLHL15は肝の実質機能を評価する方法として有用であることが報告[14]されている．今回^{13}C累積回収率とアシアロシンチ検査(LHL15)との関連をみると図4に示すように有意の正の相関(r=0.52，p=0.018)がみられた．

5．肝の線維化，フェニルアラニン水酸化酵素活性との関連

Ishiiら[8][9]は肝臓の線維化と呼気テストの結果との関連や肝組織中のフェニルアラニン水酸化酵素活性の変化が呼気テストにどのように反映されるかについて詳細に検討した．その結果，^{13}C排出速度や^{13}C累積回収率は肝の線維化の進行に伴い低値を示し，^{13}C排出速度45分値は肝臓の線維化を，^{13}C排出速度30分値はフェニルアラニン水酸化酵素活性をモニターする指標となることを報告している．

ま と め

これまで報告されている成績をもとに肝硬変における^{13}C-フェニルアラニン呼気テストの定量

的肝細胞機能評価の有用性を示した．^{13}C-フェニルアラニン呼気テストは頻回の採血などの侵襲的処置を要しないこと，放射線の被曝もないこと，リアルタイムに繰り返し評価できることなど多くの利点を有するが，今後の検討課題として①トレーサーとして用いられるL-1-^{13}C-フェニルアラニンの至適投与量はいくらか(100mg/bodyか10mg/kg/bodyなのか)，②適切な測定間隔，測定時間の検討，あるいはワンポイントの測定で肝実質機能を評価することが可能か，③肝臓以外の検査結果に影響を及ぼす因子の検討(胃排泄能の遅延や吸収障害など)，④肝実質機能(予備能)の評価としての有用性を検証するためのprospective studyなどがあげられる．

^{13}C標識化合物による呼気テストの適応は，肝機能検査のみならず消化吸収検査，消化性潰瘍における*Helicobactor pyroli*の存在診断，有害物質の曝露に関するスクリーニング，薬物投与量決定のためのモニターなど多岐にわたる可能性がある．今後^{13}C呼気テストが消化器疾患の診断，治療等の効果判定に広く用いられるようになることを期待したい．

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Brain glutamine and glutamate levels in patients with liver cirrhosis: assessed by 3.0-T MRS

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Abstract

Background/Objectives: Magnetic resonance spectroscopic (MRS) studies have revealed abnormal metabolism in the brain of patients with liver cirrhosis, including an increase in total brain glutamine (Gln) and glutamate (Glu) levels (Glx). However, with conventional MRS techniques, it was difficult to separate the Glx signals. Using a high-magnetic field MR equipment and a newly developed data processing method, we attempted to separate the Glx signals on an MRS. *Subjects and Methods:* Twenty-three patients with liver cirrhosis and 11 healthy adults were enrolled in this study. After designating a region of interest in the occipital lobe gray matter of each subject, ^{1}H(proton)-MRS was performed using 3.0-T MR equipment. *Results:* MRS conducted using the 3.0-T MR equipment allowed Gln signals in the brain to be distinguished from the Glx signals. The brain signal intensity of Gln was found to be significantly higher in the liver cirrhosis group (0.658 ± 0.23) than in the control group (0.473 ± 0.08) ($P < 0.05$). Neither the Glu nor the Gln signal intensity showed any correlation with the blood ammonia level. *Conclusion:* High-magnetic field MRS allowed us to separate the Glx signals in the brain and revealed that the increase in the total brain Glu and Gln levels in patients is solely attributable to an increase in the level of Gln.

Keywords: Liver cirrhosis; Magnetic resonance spectroscopy; Astrocyte; Glutamate; Glutamine

1. Introduction

Hepatic encephalopathy is a complication of liver cirrhosis, which describes a spectrum of neuropsychiatric abnormalities seen in these patients, including disturbances of consciousness. In cases of chronic liver failure, as prevails in cases of uncompensated-liver cirrhosis, the nervous system is exposed to certain toxic substances such as ammonia for prolonged periods of time, resulting in abnormalities of neurotransmitters, receptors, as well as in the blood–brain barrier. As a result, the sensitivity of these patients to toxic substances is increased, which hastens the onset of hepatic encephalopathy. The brain under this condition is called a "hypersensitive brain" [1].

In recent years, close attention has been paid to the dysfunction of astrocytes associated with elevated brain levels of toxic substances because it has been suggested that this may be involved in the pathogenesis of hepatic encephalopathy [2]. According to one theory, the degradation of toxic substances such as ammonia in the brain results in elevation of the glutamine (Gln) level in astrocytes, which causes both swelling and dysfunction of these cells [2]. In relation to hypotheses explaining the pathogenesis of hepatic encephalopathy, the following sequence of metabolic events were postulated from the results of spectroscopic studies. As results of the increase in ammonia level, the synthesis of glutamine (Gln) from glutamate (Glu) intensifies and Gln accumulates in the astrocytes. Astrocytes are the only cells in the CNS having Gln synthesis pathway, which is the main path of ammonia transformation. A previous magnetic resonance spectroscopic (MRS) study revealed that the level of Gln in the brain was high even in cases of liver cirrhosis not complicated by apparent encephalopathy [3]. However, in MRS using the conventional 1.5-T equipment, it was difficult to distinguish between glutamine (Gln) and glutamate (Glu) signals, and the total combined Gln and Glu level in the brain was used as an indicator of the increased Gln levels [3]. Thus, it was difficult to determine by conventional

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MRS if the elevation of the total Gln and Glu levels was attributable to elevation of the Gln level alone, or rather an elevation of both the Gln and the Glu levels. Therefore, using high-magnetic field MR equipment with high resolution, we attempted to distinguish the Gln from the Glu signals and analyzed the relationship between the brain Gln and Glu levels to various blood biochemical data in patients with liver cirrhosis.

2. Subjects and methods

2.1. Subjects

The subjects of the study were 23 liver cirrhosis patients with no history of brain disease, who were inpatients at the First Department of Internal Medicine, Iwate Medical University School of Medicine (Table 1). There were 15 males and 8 females, with a mean age of 61 years (mean ± S.D. = 60.7 ± 10.8 years). The diagnosis of liver cirrhosis was confirmed by a general evaluation of the findings on diagnostic imaging (abdominal ultrasonography, CT, etc.), histological examination, and blood biochemical data. The liver cirrhosis was etiologically related to excessive alcohol consumption in one case, viral infection in 20 cases (HCV in 14, HBV in 2, and HCV + alcohol in 4 cases), and was of unknown etiology in 2 cases. The condition was judged to be child-pugh class A in 2 cases, class B in 18 cases, and class C in 3 cases. Eleven healthy adults without a history of liver disease and free of organic brain disease served as controls (nine males and two females; mean age ± S.D., 38.5 ± 11 years).

2.2. Methods

The equipment used for the MRS was a Signa Horizon LX-VH/i 3.0-T (GE Medical Systems). A cubic region of interest (ROI) with the dimensions of 20 mm × 20 mm × 20 mm was designated in the occipital lobe gray matter of each subject and the ^{1}H(proton)-MR spectrum of this region was measured by the point-resolved spectroscopy (PRESS) sequence method (Fig. 1). Measurements of MRS were performed under overnight fasting condition. The measured parameters were as follows: repetition time (TR), 2000 ms; echo time (TE), 60 ms; and number of summations (NS), 192. To quantify the levels of *N*-acetylaspartic acid (*N*-AA), glutamate (Glu), glutamine (Gln), and creatine (Crn), phantom solutions of each of these substances at a known level of concentration were prepared in advance and used for measuring spectral signal intensities. The concentration of each phantom solution was adjusted with a phosphate buffer (pH 7.4) to a final concentration of 5.0 mm.

Table 1
Characteristics of the cirrhotic patients and a control group

Characteristic	Controls	Liver cirrhosis
Number	11	23
Gender male	9	15
Height (cm)	169.7 ± 4.1	161.1 ± 8.9
Weight (kg)	66.4 ± 9.2	60.6 ± 10.6
BMI	23.2 ± 3.1	23.2 ± 2.7
Female	2	8
Height (cm)	160.0 ± 0.0	147.3 ± 6.0
Weight (kg)	55.5 ± 4.5	50.8 ± 8.5
BMI	21.5 ± 1.5	23.4 ± 3.3
Age (yr)	38.5 ± 11.0	60.7 ± 10.8
Child-Pugh classification		
Grade A	–	2
Grade B	–	18
Grade C	–	3

Age, height, weight, BMI represent: mean ± S.D.

Since it is difficult to measure absolute levels of metabolites by non-invasive means in MRS, the levels of Gln and Glu were determined as molar ratios relative to the internal standard (*N*-AA level) [2].

Previous reports on measurements using MRS have suggested that Glu can be quantified on the basis of the spectra yielded at two different echo times [4,5]. Therefore, in the present study, we attempted to measure the Glu intensity at various TEs, using a 3.0-T MR device. Separation of the Glu from the Gln signals was the best when the TE was 60 ms. Fig. 2 shows the brain MR spectra of individual subjects, as well as the spectra of the reference materials used for the quantification of *N*-AA, Gln, and Glu. In this figure, (A) represents the spectrum of the brain in a case of liver cirrhosis, (B) represents the *N*-AA and Crn spectra of the reference solution, (C) shows the Glu and Crn spectra of the reference solution, and (D) indicates the Gln and Crn spectra of the reference solution. Each substance showed its own unique spectrum. The main signals of Glu and Gln were recorded at between 2.0 and 2.5 ppm.

Separate quantifications of brain Glu and Gln were performed by the computerized processing of the data (transferred from the MR equipment to the computer) using the GRAMS/32 (Galactic Industries, Corp. Salem, NH, USA) program and Excel (Microsoft Corp., Tokyo, Japan). The procedure for this newly developed method of separate quantification of Glu and Gln is shown below (Fig. 3). First, the *N*-AA spectrum (b) was subtracted from the subject's spectrum (a) to yield the amount of *N*-AA. On the differential spectrum (c), the amount of *N*-AA can be determined based on the point at which the signal of the methyl group of *N*-AA (2 ppm) disappears. In the second step, the Glu spectrum (d) was subtracted from the differential spectrum (c) obtained in the first step to yield the amount of Glu. The Glu signal at a point near 2.3 ppm was used as an indicator for this step (e). In the third step, the Gln spectrum (f) was subtracted from the differential spectrum obtained in the second step (e) to yield the amount of Gln. The Gln signal in the vicinity of 2.4 ppm served as an indicator for this step (g).

In each subject, the blood biochemical parameters, including the venous blood level of ammonia (μg/dl:enzyme assay) and serum albumin (g/dl), the serum prothrombin

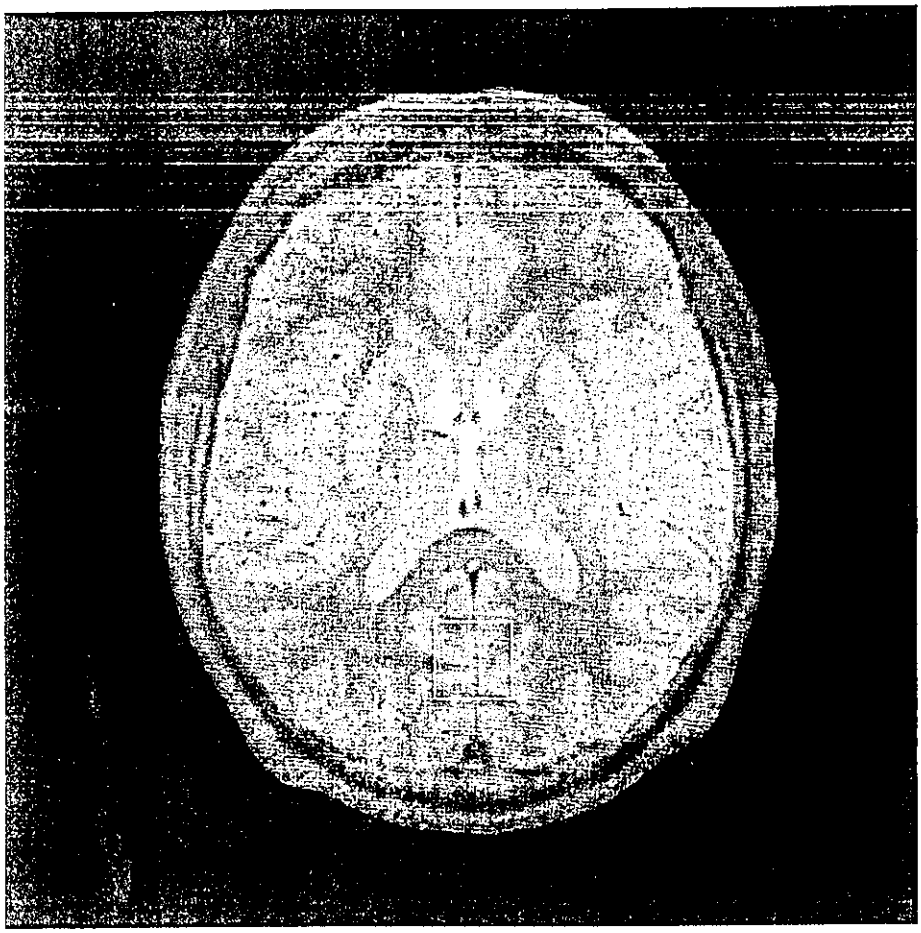

Fig. 1. The region of interest (ROI) in brain MRS. A cubic region of interest (ROI) with the dimension of 20 mm × 20 mm × 20 mm was designated in the occipital lobe grey matter of each subject in brain MRS.

time (%), the serum total bilirubin level (mg/dl), and the plasma free amino acids including Gln (μmol/l:HPLC) and Glu (μmol/l:HPLC), were also measured.

2.3. Statistical analysis

Values were expressed in mean ± S.D. Student's *t*-test and Mann–Whitney's *U*-test were used for the comparison of parameters between the two groups. Fisher's PSLD was used for testing correlations. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Brain glutamine and glutamate levels compared with healthy controls

The molar ratio of the total amount of Glu and Gln to *N*-AA, i.e., (Glu + Gln)/*N*-AA, was significantly higher in the liver cirrhosis group (1.305 ± 0.33) than in the control group (1.046 ± 0.11) ($P < 0.05$) (Table 2). The molar ratio of brain Gln to *N*-AA (Gln/*N*-AA) was also significantly higher in the cirrhosis group (0.658 ± 0.23) than in the control group (0.473 ± 0.08) ($P < 0.05$). On the other hand, the molar ratio of brain Glu to *N*-AA (Glu/*N*-AA) did not differ significantly between the control group (0.573 ± 0.06) and the liver cirrhosis group (0.648 ± 0.15). None of these molar ratios showed any significant correlation with the level of severity of liver disease.

3.2. Relationship to the blood biochemical data

The venous blood ammonia level showed no significant correlation with the brain Gln or Glu signal intensity (Table 3). No significant correlation was observed between these two parameters even when the cirrhosis group was subdivided into the high-ammonia level group (over 60 μg/dl; Gln/*N*-AA 0.656 ± 0.27) and the normal-ammonia level group (less than 60 μg/dl; Gln/*N*-AA 0.659 ± 0.19).

There was no significant relationship between the serum albumin level, prothrombin time, or the serum total bilirubin level and the brain Gln or Glu signal intensity. In regard to the relationship between plasma free amino acid levels

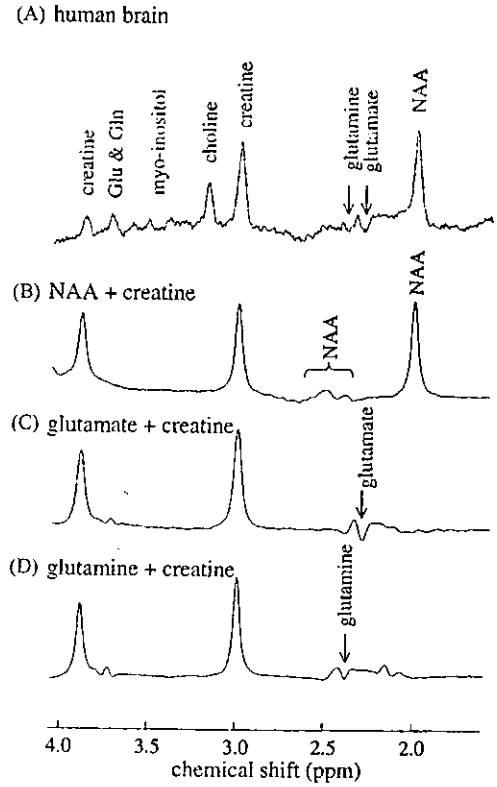

Fig. 2. ^{1}H-MR spectra of the subjects' brains and reference solutions. A 10-Hz filter was used to obtain the spectra of the solutions, so as to compare these with the spectra in vivo. Measurements were conducted by the PRESS method (TR 2000 ms, TE 60 ms, NS 192). Phantom solutions were measured by the PRESS method at ROI with dimensions of 20 mm × 20 mm × 20 mm, TR of 3000 ms, TE of 60 ms and NS of 64. (A) ^{1}H-MR spectra of the brains of patients with liver cirrhosis. An ROI with the dimensions of 20 mm × 20 mm × 20 mm was designated in the occipital lobe gray matter. (B) Reference spectra for quantification of NAA. NAA and creatine were dissolved in phosphate buffer (pH 7.4), to obtain a 5.0 mm solution of each. (C) Reference spectra for quantification of glutamate. Glutamate and creatine were dissolved in phosphate buffer (pH 7.4), to obtain a 5.0 mm solution of each. (D) Reference spectra for quantification of glutamine. Glutamine and creatine were dissolved in phosphate buffer (pH 7.4), to obtain a 5.0 mm solution of each.

and the Gln and Glu signal intensities, it was found that the latter exhibited no significant correlation with the Fischer's ratio, aromatic amino acid (AAA) levels, plasma Glu levels, or plasma Gln levels.

Table 2
MRS findings in liver cirrhosis and controls

	Gln + Glu/*N*-AA (molar ratio)	Gln/*N*-AA (molar ratio)	Glu/*N*-AA (molar ratio)
Contorols (11)	1.046 ± 0.11	0.473 ± 0.08	0.573 ± 0.06
Liver cirrhosis (23)	1.305 ± 0.33[a]	0.658 ± 0.23[a]	0.648 ± 0.15
Child-Pugh classification			
Grade A (2)	1.566 ± 0.40	0.937 ± 0.26	0.629 ± 0.15
Grade B (18)	1.272 ± 0.35	0.620 ± 0.23	0.652 ± 0.17
Grade C (3)	1.330 ± 0.07	0.697 ± 0.12	0.634 ± 0.08

[a] Control vs. liver cirrhosis ($P < 0.05$).

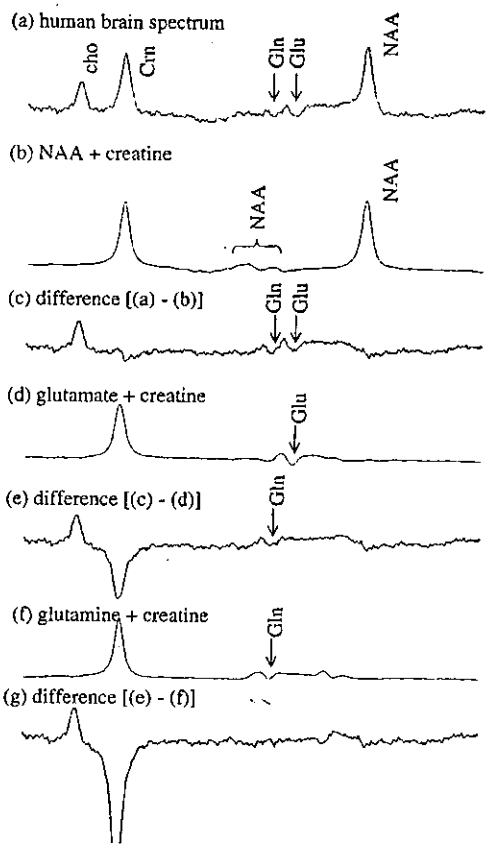

Fig. 3. Method used for quantifying glutamine and glutamate. (a) ^{1}H-MR spectra of the brains of patients with liver cirrhosis; (b) reference spectrum for quantification of NAA; (c) differential spectrum (a)–(b); (d) reference spectrum for quantification of glutamate; (e) differential spectrum (c)–(d); (f) reference spectrum for quantification of glutamine; (g) differential spectrum (e)–(f).

Table 3
Correlation matrix of MR findings and biochemical data

	Gln/*N*-AA		Glu/naa	
	r	P	r	P
B-NH3	0.063	0.778	0.079	0.721
T-Bil	0.276	0.290	0.172	0.516
Albumin	0.191	0.388	0.010	0.971
PT(%)	0.023	0.918	0.097	0.715
Fischer's ratio	0.028	0.941	0.171	0.463
AAA	0.293	0.200	0.005	0.983
Glutamate (blood plasma)	0.300	0.146	0.187	0.423
Glutamine (blood plasma)	−0.120	0.609	0.183	0.432

B-NH3: blood ammonia; PT: prothrombin time; AAA: aromatic amino acids; Fischer's ratio: branched chain amino acids/AAA.

4. Discussion

In a previous study, an increase in the brain Glx signal intensity was demonstrated in patients with liver cirrhosis [3]. The present study shows that this increase is attributable to an increase in the signal intensity of Gln alone.

Hepatic encephalopathy refers to a spectrum of neuropsychiatric abnormalities, including disturbances of consciousness, and occurs in association with severe liver disease, including fulminant hepatic failure and liver cirrhosis. The severity of this condition ranges widely, from mild cases detectable only by quantitative neuropsychiatric functional testing [6], to deep coma with absent response to stimulation.

Recently, close attention has been paid to swelling of astrocytes as a possible cause of hepatic encephalopathy. Astrocytes constitute the only compartment of the brain in which ammonia is detoxified by biosynthesis of Gln. A recent ^{1}H-MRS study revealed that the balance of astrocyte volume is lost in patients with chronic hepatic encephalopathy [2]. When the cells swell up due to elevation in the osmotic pressure, myo-inositol, which is a component of astrocytes and serves as an organic electrolyte [7], is released from the cells. In cases of hepatic encephalopathy, the brain Glx signal intensity, as measured by ^{1}H-MRS, is increased, while that of myo-inositol is markedly decreased. These changes have been reported to be correlated with the severity of coma seen in patients with hepatic encephalopathy [8]. It has been proposed that in cases of hepatic encephalopathy, the Glx levels in the astrocytes rise to degrade the excessive ammonia, resulting in swelling of the astrocytes.

Substances or factors known to cause swelling of astrocytes, besides ammonia, include hyponatremia, several neurotransmitters, TNF-α, and benzodiazepines [9]. It is thought that as the Glx-level rises, myo-inositol is released, and the resultant swelling of astrocytes induces gliopathy (compromised glial cell dysfunction), which is associated with disturbed communication between the astrocytes and neurons.

Previous studies in vitro have shown that the swelling of astrocytes is associated with effects such as activation of MAP-kinase [9], up-regulation of peripheral-type benzodiazepine receptors [10], changes in the protein phosphorylation-Ca^{2+} equilibrium [11], and changes of cellular pH [12]. It has also been shown experimentally that swelling of astrocytes alkalizes intracellular granules and thus affects the functions of cell receptors [3]. In our previous ^{1}H-MRS study of patients of liver cirrhosis who did not have any apparent hepatic encephalopathy, we found that the myo-inositol signal intensity was low and the Glx level was high, similar to the findings in cases with hepatic encephalopathy, and that these parameters were correlated with the severity of the liver disease. This suggests that brain metabolism is already affected in cases of liver cirrhosis even before the appearance of signs of hepatic encephalopathy [13]. The concentrations of brain GLN and GLU were evaluated using biopsied human brain tissue. Rothman et al. [4] reported that the gray matter concentration of GLU was 7.8 ± 4.0 μmol/g and that of GLN was 4.1 ± 2.0 μmol/g. They reported that the gray matter concentrations of GLU were larger than that of GLN in healthy controls. We also clarified that the intensity of GLU is larger than that of GLN in healthy controls. In the cirrhotic group, however, the intensity of GLU is smaller than that of GLN and it is estimated that there were abnormalities in the metabolic pathway of GLU and GLN in cirrhotic patients.

With the conventional–technique of MRS, for which a 1.5-T MR device is used, while an increase in the Glx signal intensity in the brain could be detected, increased Gln levels in situ could not be discerned (a hypothesis proposed by Haussinger et al. [12]). In the present study, we conducted MRS using a high-magnetic field and high-resolution MR equipment, and analyzed the data for known concentration levels of *N*-AA, Crn, Gln, and Glu by a new data processing method, and adequate echo time. This study demonstrated that the increased Glx signal intensity in the brain previously observed in cases of liver cirrhosis is attributable to an increase in the Gln alone. We have thus endorsed the validity of the theory of abnormal brain metabolism in situ.

In the present study, the Gln signal intensity did not exhibit any correlation with the venous blood ammonia level or indeed any of the other blood biochemical parameters tested. The blood samples for quantification of ammonia were collected in the early morning before breakfast on the day on which MRS was performed, and there was no significant time lag between the blood sampling and MRS. However, it remains unknown whether or not the blood ammonia levels have any immediate effects on the Gln level in the glial cells. It also seems likely that chronic exposure of cells to blood ammonia may be involved in the increased brain Gln level. This is, however, a still untested hypothesis. In the present study, venous blood was used for measuring the blood ammonia level. However, the venous blood ammonia level may not always be correlated with the brain Gln level, when one considers the removal of ammonia in the peripheral muscle. Therefore, it may be necessary to study the relationship between the arterial blood ammonia level and the brain Gln level. In the present study, the mean age differed between the control group and the liver cirrhosis group. However, in the cirrhosis group, no effects of aging on the Gln or Glu signal intensity were noted (data not shown). There were several reports about the effect of age on brain metabolite concentrations. Chang et al. [14] reported that concentrations of creatine, choline and myo-inositol increased with age. In contrast, there was a relatively stable concentration of Glx measured by a 1.5-T MRS in 36 normal healthy volunteers (19–78 years).

It, therefore, appears unlikely that the difference in age between the two groups served as a significant confounding factor in the study. According to the previous reports [2,15,16], abnormal metabolism of Glx and myo-inositol were detected in various brain regions (basal ganglia, temporal lobe, occipital lobe, etc.) in patients with liver cirrhosis complicated by hepatic encephalopathy. In the present study, only one ROI was designated in the occipital lobe when performing MRS. It would therefore be desirable to examine other areas of the brain also for the presence or absence of similar changes.

It has recently been reported that abnormal metabolism of Glx and myo-inositol in the brain can be reversed by treatment. Haseler et al. [17] examined the changes in the Glx and myo-inositol levels before and after administration of a synthetic bisaccharide (lactulose, 60 ml/day for 7 days), in comparison with those in an untreated group. Their study revealed a 15% decrease in the Glx and a 29% increase in the myo-inositol levels in the lactulose-treated group, while no such change was observed in the untreated group. It has also been reported that these abnormalities could be reduced by liver transplantation [18]. These findings suggest that measurements using MRS may also be useful for evaluating the responses to treatment. Measurements using MRS may also be useful for the early detection of the subclinical hepatic encephalopathy, which can only be detected by quantitative neuropsychiatric function tests. From our data, the main reason for the increase of Glx intensity was the increase of GLN in the brain. However, the level of GLU also increased following the increase of the GLN intensity in patients with liver cirrhosis. Thus, the clinical significance of the separation of Glx is to be able to evaluate the effect of the treatment or the clinical condition for cirrhotics from the balance of GLU and GLN intensities.

In brief, high-magnetic-field MRS revealed that the increase in the total brain Glu and Gln levels, previously observed as a sign of abnormal brain metabolism associated with liver cirrhosis, is attributable to an increase in the level of Gln alone. The study results also suggested that the brain Gln level shows no significant correlation with the venous blood ammonia level. In the future, it would be desirable to conduct a chronological study of the changes in the Gln levels in the brain in cases of liver cirrhosis under follow-up, with or without active treatment.

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Development of quantitative neuropsychological tests for diagnosis of subclinical hepatic encephalopathy in liver cirrhosis patients and establishment of diagnostic criteria—multicenter collaborative study in Japanese

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Abstract

At present, there are no generally accepted diagnostic criteria or methods for subclinical hepatic encephalopathy (SHE) associated with liver cirrhosis. We therefore developed an easily conducted computer-aided quantitative neuropsychiatric function test system for use in routine medical practice. We established normal values in healthy Japanese subjects and determined differences between healthy persons and liver cirrhosis patients without clinical encephalopathy in a multi-center clinical trial. The test system consists of eight tests: number connection tests A and B, a figure position test, a digit symbol test, a block design test, and reaction time tests A, B and C. The test results were affected by age, but not by gender or facility. No learning effect was noted. The results were therefore reported by 5-year quartile ranges and differences were evaluated between 542 healthy subjects and 292 cirrhotic patients. When the cut-off value was set at the 10th/90th percentile of the results in healthy subjects, the results of each of the 8 tests were abnormal in about 25% of cirrhotic patients, and at least 1 of the 8 tests gave values greater than the 10th/90th percentile cut-off value in 58.2% of the 292 liver cirrhosis patients. SHE patients were thought to be

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included in these 58.2% of patients. The developed test makes it possible to quantitatively assess neuropsychiatric function, and the results obtained can be used as a basis for the diagnosis of SHE.

Keywords: Liver cirrhosis; Subclinical encephalopathy; Neuropsychological tests; Computer-aided test system

1. Introduction

Subclinical hepatic encephalopathy (SHE), which gives abnormal results to sensitive quantitative neuropsychiatric function tests without showing any abnormal physical findings, is seen in 30–84% of liver cirrhosis patients. The importance of SHE is being increasingly recognized as it may interfere with the activities of daily living [1–14]. Recently, Ferenci et al. [15] presented new proposals regarding the definition and classification of hepatic encephalopathy and the degree of associated coma. They named SHE "minimal hepatic encephalopathy (mHE)" as low-grade hepatic encephalopathy and emphasized its importance.

SHE includes pre-clinical hepatic encephalopathy and is associated with no clinical symptoms or signs of the degree of coma being grade I or 0 according to the conventional grading system. There are no standard diagnostic criteria, and it is diagnosed at individual medical institutions by their own diagnostic methods based on the paper–pencil test and electrophysiological tests, including EEG [3]. However, none of these tests is satisfactory in terms of ease in operation or reproducibility, and the need for development of a simpler quantitative test has been pointed out.

Since SHE is associated with a reduction in performance cognition, a combination of multiple neuropsychiatric function tests designed to assess performance cognition is used for its diagnosis. Recently, Kircheis et al. reported that the critical flicker-frequency (CFF) test alone makes it possible to easily diagnose SHE in liver cirrhosis [16]. However, because results of the CFF are normal in about 40% of SHE patients diagnosed by conventional neuropsychiatric function tests, the CFF has limitations as a diagnostic method although it is a simple, easy-to-use method [17].

We developed a computer-aided simple neuropsychiatric test system consisting of eight tests that can be easily conducted on outpatients using a touch panel. In order to evaluate the utility of this test system, we first conducted a pilot study in healthy subjects and identified factors that influence the results of the test. This was followed by a multiple cooperative study designed to establish standards for neuropsychiatric functions in healthy subjects and liver cirrhosis patients. The criteria for the SHE diagnosis were established based on the findings obtained.

2. Materials and methods

The test system consisted of the following eight tests and was designed to assess psychomotor, attention, memory and special functions: number connection tests A and B (NCT-A and B), figure position test (FPT), digit symbol test (DST), block design test (BDT), and reaction time tests A, B and C (RTT-A, B and C) [10–13,18,19]. The system was simplified so that two-dimensional operations using a computer were possible. All tests can be completed in about 20 min, including the time needed for practice and operation guide.

2.1. Neuropsychological (NP) test system

Software was developed by Otsuka Pharmaceutical Co., Ltd., Kokuyo Co., Ltd., and ISB Co., Ltd. Hardware consisted of a personal computer (OS: window3.1, ThinkPad 365X, IBM) and a 33 cm size touch panel connected to the PC (GUNZE Access Vision AV4833FT, Gunze Ltd., Tokyo, Japan).

2.2. Number connection tests (NCTs)

Two tests were conducted, NCT-A, in which the time needed to serially connect figures from 1 to 20 on the touch panel was determined (time limit: 60 s), and NCT-B, in which the time needed to connect figures from 1 to 10 and 10 Japanese characters was determined (time limit: 180 s).

2.3. Figure position test (FPT)

Subjects were asked to remember the shape and position of 2–4 figures displayed on the panel for 15 s. They were asked to return each figure to its original position after randomly moving them on the panel, and the time needed to complete this task was determined (time limit: 90 s). This test was developed after the tactual performance test.

2.4. Digit symbol test (DST)

Nine different symbols were displayed on the panel in 60 s, and subjects were asked to select a digit corresponding to each symbol on the panel. The number of correct answers was determined (maximum number of questions: 40).

2.5. Block design test (BDT)

Six different cards were displayed on the panel, and the time needed to complete the same design as that displayed on the panel was determined (time limit: 60 s).

2.6. Reaction time tests (RTTs)

Three types of RTT were conducted: RTT-A, in which the reaction time needed by subjects to press an enter key after the color of a circle on the panel changed from white to red; RTT-B, in which subjects were asked to press the enter key when the color changed from white, blue or yellow to red; and RTT-C, in which subjects were asked to press the enter key when a combination of white, blue and yellow changed to that of yellow and red.

2.7. Construction of the NP test system (pilot study)

A pilot study was conducted at three university hospitals in Japan between June 1996 and March 1997. One hundred and twelve healthy subjects under no medical treatment, who were almost evenly divided into 3 age brackets (40–49, 50–59, and 60–69), were enrolled. Informed consent was obtained from all volunteers after providing thorough information on the objectives and method of the study. One of the objectives of this pilot study was to determine if the test system could be operated without any problem. In addition, the correlation between the test results and factors that were thought to influence them (age, gender and facility) was evaluated. The tests were conducted three times by repeating the tests 1 and 7 days after the first tests in order to determine the presence or absence of learning effects (effects of familiarization with the tests). The tests were always conducted in an independent test room between 10 a.m. and 4 p.m. in order to minimize the environmental effects.

2.8. Establishment of normal ranges and estimation of cut-off values

Cut-off values were estimated in order to establish the normal ranges and extract a population showing abnormal neuropsychological function test results. The following number of subjects, aged between 40 and 69, were enrolled at 14 university hospitals between June 1996 and March 1997: 328 patients with hepatic cirrhosis and 550 healthy subjects (not included in the 112 enrolled in the pilot study). Healthy subjects under no medical treatment and without hepatic disease were enrolled. Hepatic cirrhosis was diagnosed based on liver biopsy, imaging diagnosis, or clinical diagnosis combining objective findings and hematological tests. Those with any of the following were excluded: previous neurological focal episode or other neurologic illness, history of psychiatric illness, history of consumption of psychotropic drugs, and clinical hepatic encephalopathy (≤grade II). Those under treatment with lactulose, poorly absorbed antibiotics or branched-chain amino acid to deal with hyperammonemia were enrolled if they could tolerate the tests.

Informed consent was obtained from all subjects after thoroughly explaining the objectives, methods and other relevant details of the study.

3. Statistical analysis

In data analyses, subjects were grouped by age at 5-year intervals since findings obtained in the pilot study showed that age affects analytical results when they are grouped at 10-year intervals. Subjects in each age bracket were classified into the healthy subject group and the cirrhotic patient group. Differences between the two groups were shown by the quartile range. The upper and lower 10, 20 and 30 percentiles were estimated for each test item in healthy subjects in order to evaluate cut-off values as percentages of upper and lower test values obtained in cirrhotic patients.

Test values were handled as mentioned below. Namely, the actual time needed to complete the task was reported in NCT-A and NCT-B. The number of correct answers were reported in the DST. The mean effective test time, i.e. the test time spent for correct operations divided by the number of correct operations, was reported for the FPT, BDT, RTT-A, -B and -C.

A subcommittee was created to establish a data handling policy. It excluded all "outliers due to apparent errors in operation" (data from subjects who did not completely understand the operation procedures and those who undertook the tests under apparently abnormal physical conditions due to night duties or a lack of sleep) from analysis. All data from subjects younger than 40 and those aged 70 or older were excluded from analysis as a deviation from the protocol.

4. Results

4.1. Construction of the NP test system

Results obtained from the 112 healthy subjects showed no significant differences in the test results by gender or facility or any significant learning effects on the test results due to repetition of the tests by each subject. However, the test results were found to be affected by age as is typically shown by the results of NCT-B shown in Fig. 1. As is evident from this figure, the test time clearly increased with age in healthy subjects when they were divided into groups at 10-year intervals.

4.2. Establishment of normal ranges and estimation of cut-off values

The NP test system was conducted in a total of 834 subjects, including 542 healthy subjects and 292 liver cirrhosis patients. However, 8 healthy subjects and 37 liver cirrhosis patients were excluded due to violations of the protocol in terms of the age. The backgrounds of healthy subjects and cirrhotic patients are shown in Table 1. Relatively old males were predominant among cirrhotic patients ($P < 0.0001$). The severity of liver cirrhosis was mild in most patients according to the child classification system, and the cause was viral in

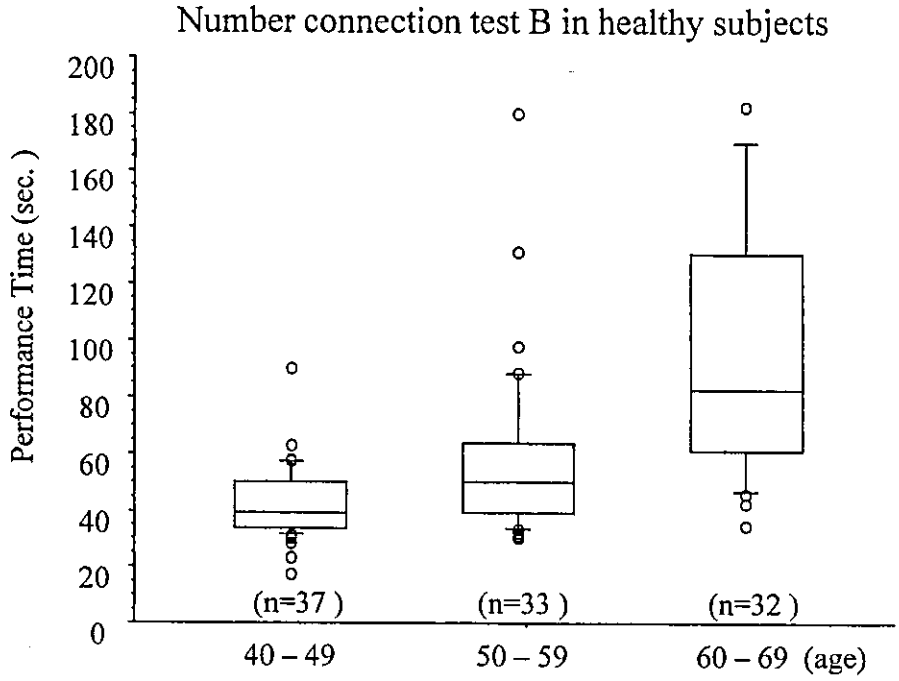

Fig. 1. Results of number connection test B in healthy subjects are indicated with horizontal bars. The vertical bars indicate the range and the horizontal boundaries of boxes represent the first and third quartiles.

the great majority of patients (81%). Overall, 131 cirrhotic patients (45.0%) were receiving at least 1 drug for hyperammonemia: 74 (25.3%) lactulose, 107 (36.6%) BCAA, and 9 (3.1%) poorly absorbed antibiotics.

The number of subjects analyzed with respect to each test item ranged between 517 and 542 healthy subjects (95.4–100%) and between 273 and 290 cirrhotic patients (93.5–99.3%). Because the pilot study showed that the test re-

Table 1
Clinical and laboratory characteristics of cirrhotic patients and healthy subjects

Items	Cirrhotic patients, *n* (%)	Healthy subjects, *n* (%)	*P*-value
Total	292	542	
Sex			
Male	191 (65.4)	275 (50.7)	
Female	101 (34.6)	267 (49.3)	<0.0001[a]
Age (years)			
40–44	14 (4.8)	98 (18.1)	
45–49	27 (9.2)	112 (20.7)	
50–54	33 (11.3)	109 (20.1)	
55–59	54 (18.5)	99 (18.3)	
60–64	82 (28.1)	79 (14.6)	
65–69	82 (28.1)	45 (8.3)	
Mean ± S.D.	59.0 ± 7.3	52.6 ± 7.9	<0.0001[b]
T-bilirubin (mg/dL) (mean ± S.D.)	1.6 ± 1.9		
ALT (IU/L) (mean ± S.D.)	70.1 ± 50.0		
Child classification			
A	130 (44.5)		
B	134 (45.9)		
C	28 (9.6)		
Etiology			
Virus	237 (82.2)		
Alcohol	39 (13.4)		
Others	16 (5.5)		

[a] Chi-square test.
[b] Student's *t*-test.

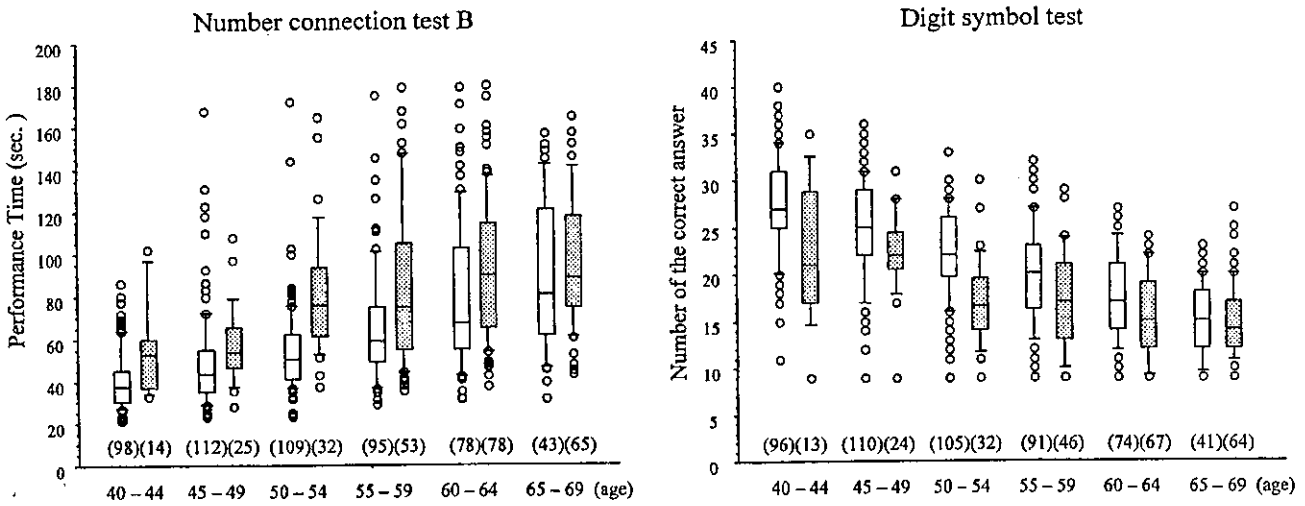

Fig. 2. Results of number connection test B and digit symbol test in healthy subjects and cirrhotic patients are indicated with horizontal bars. The vertical bars indicate the range and the horizontal boundaries of boxes represent the first and third quartiles: (□) healthy subjects; (▨) cirrhotic patients (*n*).

sults and their standard deviations become increasingly variable with age (data not shown), test data were compiled at 5-year age intervals and shown by the quartile range (box and whisker plots). Results of NCT-B and DST are shown in Fig. 2 as typical results obtained. Test results obtained from both healthy subjects and cirrhotic patients showed clear age-related changes, with the test time increasing and the number of correct answers decreasing with age. The test time increased and the number of correct answers decreased in cirrhotic patients compared to healthy subjects regardless of age up to 65. Among those aged 65 or older, however, there were no significant differences between healthy subjects and cirrhotic patients. Results of all tests showed similar effects of aging as well as the differences between healthy subjects and cirrhotic patients.

Data were missing from 11 healthy subjects (2%) for DST and 1 healthy volunteer (0.2%) for all of the 7 other tests, while among hepatic cirrhosis patients, 7 (2.4%) gave no data for NCT-A, 25 (8.6%) for NCT-B, 19 (6.5%) for FPT, 46 (15.8%) for DST, 6 (2%) for BDT, and 11 (3.8%) for RT-A, B and C. DST data were missing from the largest number of subjects in both the healthy volunteer and hepatic cirrhosis patient groups.

Cut-off values were determined based on the upper and lower 10th, 20th and 30th percentiles, which are regarded as outliers in healthy subjects, and were set at the upper and lower 10th percentile due to great variability because of the effects of aging on standard deviations. The percentage of liver cirrhosis patients who were regarded as giving abnormal values based on the 10th/90th percentile cut-off value in healthy subjects and the test results obtained at 5-year intervals ranged between 9 and 47% for NCT-B and 21.0% overall and between 2 and 44% for DST and 18.7% overall. The percentage of liver cirrhosis patients who gave abnormal values in the eight tests varied according to the age bracket and ranged between 10 and 25% (Table 2). Of the 292 cirrhotic patients, 170 (58.2%) showed deviations from the 10th percentile cut-off value with respect to at least 1 test item.

5. Discussion

We developed a computer-aided quantitative neuropsychological test system in order to facilitate the diagnosis of SHE associated with liver cirrhosis.

Results obtained with this test system showed apparent differences between healthy subjects and liver cirrhosis patients without clinical hepatic encephalopathy (grade 0 or 1). Both healthy subjects and liver cirrhosis patients showed increases in the test time and decreases in the number of correct answers with age, and it was evident that the results of the neuropsychological tests need to be analyzed at 5-year age intervals. The results of the present study thus indicated that major shortfalls of the this test method are the great variability of the test results in both healthy subjects and liver cirrhosis patients and increases in the test time with age even in healthy subjects. The test method seemed to be able to differentiate healthy subjects from liver cirrhosis patients and attribute the difference to subclinical encephalopathy if they are younger than 65. In older patients, however, discrimination seemed to be impossible and the test method seemed to have its own limitation.

Although the tests were not repeated in the same patients, it was thought that they could be properly conducted in liver cirrhosis patients as well as in healthy subjects because data were missing from only a few patients.

Among various factors with possible effects on the test results, blood ammonia levels affected the test results, but the severity of hepatic dysfunction did not. Test results obtained in the present study were not significantly different