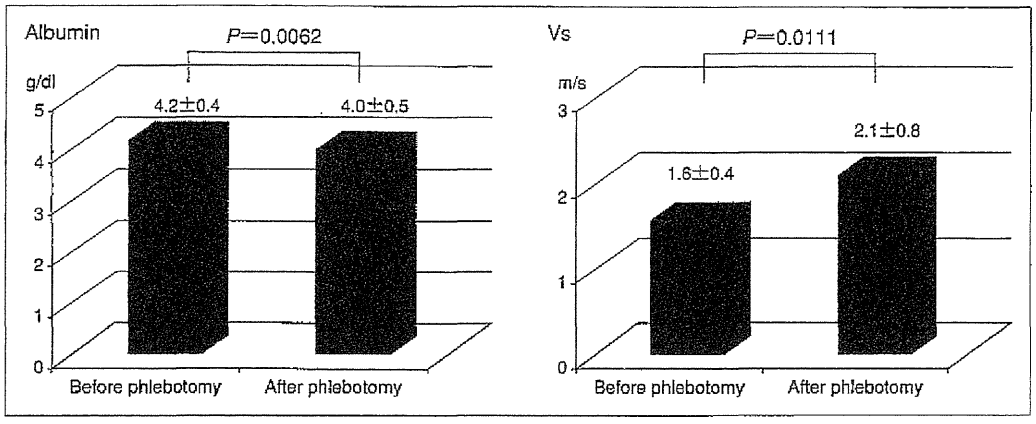

図 3-3　瀉血療法―前後のアルブミン値と肝硬度

等の精神神経系の副作用発症もIFN-α製剤に比べて少ないとされている．そのためIFN-β＋RBV併用療法は高齢者や血小板低値，うつ病・うつ傾向など，PEG-IFN＋RBVによる標準的な治療を断念するような症例にも治療が行える可能性がある[1)2)]．

今回の検討では，IFN-β＋RBV併用療法の目立った副作用としては貧血の進行のみであり，これはIFN-βよりもRBVの副作用という要素が強いと考えられる．IFN-β＋RBV併用療法の効果としては，transaminaseが有意な低下を示し，血小板低値例でも血小板数を維持して治療を継続できたという点である．しかし，genotype 2 型では少数ながら100％のSVRを得られたのに対して，1 型ではSVRは得られなかった．

これまでのIFN-β＋RBV併用療法に関する報告では，genotype 2 型ではSVR率は良好なものの[1)2)]，genotype 1 型では30％前後であり[3)4)]，telaprevirとの 3 剤併用療法など，さらなるSVR率向上の工夫が必要と考えられる．

門脈圧亢進症による脾機能亢進を伴うC型慢性肝炎～肝硬変では汎血球減少，特に血小板減少を有する症例が多い．このような症例に対して，脾摘あるいはPSEにより血小板を上昇させてからPEG-IFN療法やPEG-IFN＋RBV療法が行われる場合がある[5)6)]．

今回のわれわれの検討でも，脾摘/PSEにより血小板数と白血球数の有意な上昇がみられ，PEG-IFN＋RBV療法が可能となった．しかし，IL28B SNP(rs8099917)は全例TT(major)であったにもかかわらず，脾摘/PSE後のPEG-IFN＋RBV療法(1 例はPEG-IFN単独)のSVR率はgenotype 1 型で25％，2 型で 50％であり，決して高い治療効果とはいえないものであった．

これまでの報告でも，脾摘/PSE後のIFN療法やPEG-IFN＋RBV療法のSVR率は，特に 1 型・高ウイルス量の症例では20％台と概して低い．脾摘/PSEは重症感染症などの合併症の可能性もあるため，IFN治療を目的とした脾摘/PSEはgenotypeやIL28B SNPを考慮して治療効果が期待できる症例に限って行うべきであるという報告もある[6)]．今回の症例はIL28B SNP(rs8099917)は全例TT(major)であったにもかかわらず十分な治療効果は得られていないため，今後慎重に症例を選択する必要があると考えられた．

C型慢性肝炎では肝に鉄が過剰に蓄積している．肝細胞内で増加した遊離鉄が過酸化水素と反応してフリーラジカルを発生し，細胞障害を惹起する．そして細胞をアポトーシスへと導き組織障害をひき起こす．瀉血療法は，人工的に鉄欠乏性貧血状態を作ることで骨髄の造血を促進し，肝からの鉄の動員を高めてフリーラジカル産生を低下させることにより肝炎を沈静化する肝庇護療法である[7)]．

C型慢性肝炎に対する瀉血療法の有用性は，1994年Hayashiらにより世界で最初に報告された[8)]．その報告によると，IFN無効の 2 例を含むC型慢性肝炎10例に対して，血清フェリチン値

が10 ng/ml未満になるまで1～2週間に1回200 gあるいは400 gの瀉血を継続したところ，ALT値が全例で有意に低下し，半数では正常化したことが示されている．また，Pipernoらは12例のIFN無効例に対する瀉血によりALTの有意な低下を報告し，瀉血療法がIFN無効例に対して有用な対症療法である可能性を示した[9]．その後C型慢性肝炎に対する瀉血療法の効果が次々と報告され，2004年にはYanoらが3か月間のrandomized controlled trialにより瀉血療法の有効性を示した[10]．これらの報告を踏まえて，2006年にはIFN治療や他の肝庇護療法に抵抗性のあるC型慢性肝炎の治療法として，瀉血療法は本邦で保険適用されることとなった．

今回のわれわれの検討では，瀉血によるフェリチン，血清鉄の低下に伴い，AST, ALT, γ-GTPの有意な低下を認め，また肝細胞癌の腫瘍マーカーであるAFPとPIVKA-IIは低下する傾向を示した．その一方で，アルブミン値が有意に低下し，ARFIにより測定した肝硬度は有意に上昇していた．これらの結果から，瀉血療法は肝炎の沈静化や発癌抑制には一定の効果が期待できる半面，肝予備能の低下や肝線維化の進展には注意が必要であると考えられた．

瀉血療法の長期成績として，Yanoらは組織学的検討で炎症所見，線維化ともに改善したと報告しており[11]，Katoらの報告でも除鉄療法による肝線維化の有意な改善を示している[12]．また，Katoらは瀉血療法を受けた35例(新犬山分類で線維化ステージF2またはF3)について，瀉血療法を受けなかった症例を対象に肝発癌率を解析したところ，累積発癌率は瀉血群で有意に低率であったと報告している[12]．さらに，徐鉄療法中の平均血清フェリチン値を20 ng/ml未満に抑えることにより肝発癌が抑制されるとも報告している[13]．

今回の検討で腫瘍マーカーが低下傾向にあったことは瀉血療法の発癌抑制効果を反映している可能性がある．一方，ARFIにより測定される肝硬度は，C型慢性肝炎における肝線維化の非侵襲的診断に有用とされるが[14]，その肝硬度が有意に上昇していることは，瀉血療法による肝線維化の改善という過去の報告とは異なる結果となっている．また，肝予備能の指標となる血清アルブミン値が有意に低下していたことも，肝の組織学的改善を認めたという報告とは矛盾する結果である．今回はretrospectiveな検討で，瀉血療法の期間が短い症例や瀉血量の少ない症例も多く含まれていることが過去の報告と異なる結果につながった可能性も否定できない．しかし，瀉血療法はHCVが駆除されていない状況下での肝庇護療法であり，やはり肝予備能の低下や肝線維化の進展には十分な注意が必要であることは間違いないと考えられる．現在瀉血療法で維持されている症例にも近い将来DAAs併用による新規治療が適用できる可能性が高く，HCV駆除による肝線維化や予備能の改善が期待される．

おわりに

C型肝炎治療困難例では，症例を適切に選択した上で瀉血療法，IFN-β療法，脾摘後PEG-IFN療法により病状の維持や改善が期待でき，今後の新規治療につないでいけると考えられた．

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C型肝硬変に合併した多発肝MALTリンパ腫の1例

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＜症例報告＞

C型肝硬変に合併した多発肝MALTリンパ腫の1例

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要旨：症例は70歳女性，C型肝硬変にて近医通院中に肝腫瘍を認め，肝細胞癌（HCC）が疑われ当科紹介となった．US上，肝S8，S3，S7に境界不明瞭な低エコー結節を認め，ソナゾイド造影USでは同部位が早期に染影され後血管相でdefect像を示した．CTでは肝S8に約5 cm，S3に約3 cm，S6に約2.5 cm大の境界不明瞭な淡い低吸収域を認め，動脈相で淡く濃染され平衡相でやや低吸収域を示した．EOB-MRIではこれらの病変を含め，肝両葉に早期相で染影され肝細胞相で淡い低信号域を示す多発性の腫瘤性病変を認めた．S3の腫瘍内には門脈の腫瘍内貫通像を認めた．PET-CTでは，肝両葉に多数の異常集積を認めた．HCCとしては非典型的であり，IL-2Rの上昇もみられたため肝腫瘍生検を施行．病理所見にてMALTリンパ腫と診断され，化学療法が導入された．肝MALTリンパ腫は稀な疾患であり，C型肝硬変を背景とした肝内多発病変である点，治療前に組織診断を行い化学療法導入が可能になった点で貴重な症例と考え報告する．

索引用語：肝MALTリンパ腫　C型肝硬変　肝腫瘍生検　PET-CT　H.Pylori

はじめに

MALTリンパ腫は粘膜関連リンパ組織（MALT）に発生する低悪性度の悪性リンパ腫であり，胃に好発し，H.Pyloriによる慢性胃炎との関連が有名である．肝原発の悪性リンパ腫は稀で，そのほとんどはdiffuse large B-cell lymphomaであり，肝原発悪性リンパ腫におけるMALTリンパ腫の頻度は低い．今回，C型肝硬変を背景として肝内多発病変を呈し，治療前に肝腫瘍生検にて組織診断を行い，化学療法導入が可能になった症例を経験したため，文献的考察とともに報告する．

症　例

症例：70歳　女性．

主訴：肝腫瘍精査目的．

既往歴：高血圧症，C型肝硬変．

生活歴：喫煙歴なし，飲酒歴なし．

現病歴：C型肝硬変にて近医通院中，腹部USにて肝S3とS8に肝腫瘍を認め，肝細胞癌が疑われ当科紹介受診となった．

入院時身体所見：特記すべきことなし．眼球結膜に黄染なし，表在リンパ節を触知しない．腹部は平坦，軟で，肝を触知しない．

入院時血液検査所見：肝硬変に伴い，Plt 4.3×10⁴/mm³と低下を認める他，T.Bil 1.5 mg/dl，AST 91 IU/L，ALT 60 IU/Lと軽度上昇を認めるが，その他の血液一般・生化学検査に異常所見なし．HCV-RNAは6.6 logIU/ml，HBs抗原，HBc抗体は陰性であった．腫瘍マーカーは，AFP 77.1 ng/ml，AFPレクチン分画L3 10.8%，CEA 11.3 ng/ml，IL-2R 2243 U/mlと上昇を認め，PIVKA-II 13 mAU/ml，CA19-9 0.4 U/mlは正常範囲内であった（Table 1）．

腹部US所見：Bモードでは肝S8，S3，S7に境界不明瞭な低エコー結節を認め，ソナゾイド造影USでは，同部位が早期に周囲肝よりも強く染影され，後血管相

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Table 1　Laboratory data on admission

Hematology		Coagulation	
WBC	2500 (/mm³)	PT	87 (%)
Baso	1		
Eosino	3	Biochemistry	
Mono	3	TP	7.0 (g/dl)
Lympho	24	Alb	3.7 (g/dl)
Neutro	68	T-Bil	1.5 (mg/dl)
RBC	3.54×10^6 (/mm³)	D-Bil	0.7 (mg/dl)
Hb	11.2 (g/dl)	AST	91 (IU/l)
Ht	32.8 (%)	ALT	60 (IU/l)
PLT	4.3×10^4 (/mm³)	ALP	221 (IU/l)
		LD	228 (IU/l)
Serological tests		γ-GTP	28 (IU/l)
HBs-Ag	−	ChE	183 (IU/l)
HBc-Ab	−	AMY	220 (IU/l)
HCV-Ab	+	CPK	85 (IU/l)
HCV-RNA	6.6 (logIU/ml)	TG	156 (mg/l)
HCV group	1	T-Chol	113 (mg/dl)
CRP	0.3> (mg/dl)	BUN	14.9 (mg/dl)
Tumor marker		Cre	0.63 (mg/dl)
AFP	77.1 (ng/ml)	NH3	100 (U/l)
AFP-L3	10.8 (%)		
PIVKA-II	13 (mAU/ml)		
CEA	11.3 (ng/ml)		
CA19-9	0.4 (U/ml)		
IL-2R	2243 (U/ml)		

では周囲肝に比し，造影の減弱を認めた（Fig. 1）．

腹部CT所見：単純CTでは，肝S8に約5 cm，S3に約3 cm，S6に約2.5 cm大の境界不明瞭な淡い低吸収域腫瘤を認め，動脈相では淡く染影され，平衡相では周囲肝よりやや低吸収域を示した（Fig. 2）．

Gd-EOB-MRI所見：CT，USで指摘された病変を含め，肝両葉に境界不明瞭で，早期相で染影され，肝細胞相で淡い低信号域を示す腫瘤性病変が散見された．これらの病変は，T1強調像ではごく軽度の低信号～等信号域を示し，T2強調像では軽度高信号域を示した．また，S3の腫瘍内には門脈の腫瘍内貫通像が認められた（Fig. 3）．

以上の所見より，HCC，炎症性偽腫瘍，転移性肝腫瘍，肝内胆管癌などを鑑別に挙げ，PET-CTを施行した．

PET-CT所見：肝両葉に多数の異常集積を認め，縦隔リンパ節，肺門リンパ節にも各々10 mm大の集積亢進を認めた．その他の部位に異常集積は認められなかった（Fig. 4）．

上部消化管内視鏡検査：食道静脈瘤（Lm，F1，Cb，RC1（CRS），Lg（-）），慢性胃炎を認めたが，腫瘤性病変は認めなかった．生検病理にてH.Pylori陽性であった（Fig. 5）．

以上から，画像的に腫瘍内門脈貫通像などHCCとしては非典型的な所見を認め，PET-CTにて縦隔肺門リンパ節の集積亢進を認め，また，血清IL-2Rの上昇も認め，悪性リンパ腫などの悪性疾患が疑われたため，肝腫瘍生検を施行した．なお，血小板低値であり，縦隔肺門リンパ節からの生検は施行しなかった．

病理組織学的所見：HE染色では，比較的小型～中型で不整な核と比較的淡明で豊富な細胞質を持つ異型性の低いリンパ球，centrocyte-like cellの集簇性増生を認め，悪性リンパ腫と考えられた．Hepatocyte染色では，異型リンパ球が肝細胞を破壊しながら浸潤する像を認めた．サイトケラチンAE1/AE3染色では，MALTリンパ腫に特徴的なlymphoepithelial lesion（LEL）の

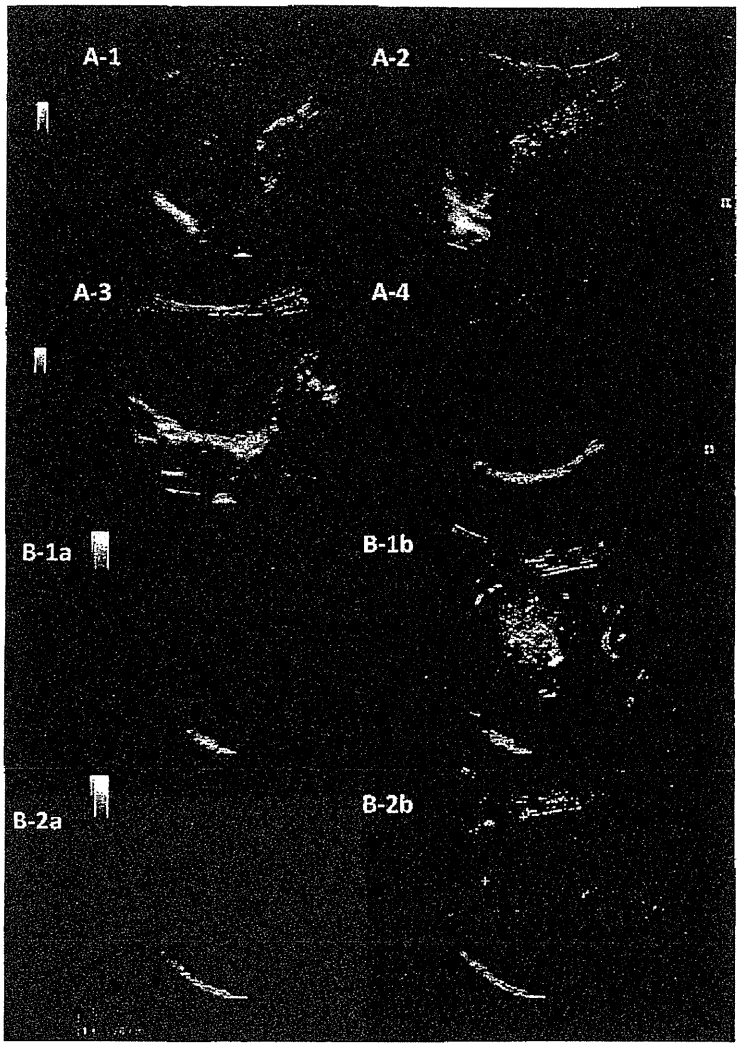

Fig. 1　Abdominal US
A-1, 2, 3 and 4. Abdominal US showed hypoechoic nodules with unclear boundary. B-1a, 1b, 2a and 2b. Contrast-enhanced US with Sonazoid showed the nodules enhanced in the vascular phase, and showed them with contrast defect in the postvascular phase.

所見（異型リンパ球がグリソン鞘より浸潤し胆管上皮が破壊されている）を認めた（Fig. 6）．CD20陽性，CD3陰性であり，B細胞性であることが示唆され，MALTリンパ腫であると確定診断に至った．

臨床経過：胃の生検病理にてH.Pylori陽性であり，除菌療法を施行した．また，骨髄生検では悪性所見を認めず，肝内多発病変で，PET-CTにて肺門縦隔リンパ節にも異常集積を認めたため，血液内科にてリツキシマブ併用化学療法（dose modified CHO-R）を5コース施行した．施行後のPET-CT，造影USでは，肝内病変の縮小および消失を認めた．また，血液検査では，初診時より約1年経過した時点で，肝機能はほぼ正常化し，Pltは$5.2\times10^4/mm^3$と横ばいであった．また，腫瘍マーカーはAFP 9.1 ng/ml，IL-2R 1097 U/mlと低下

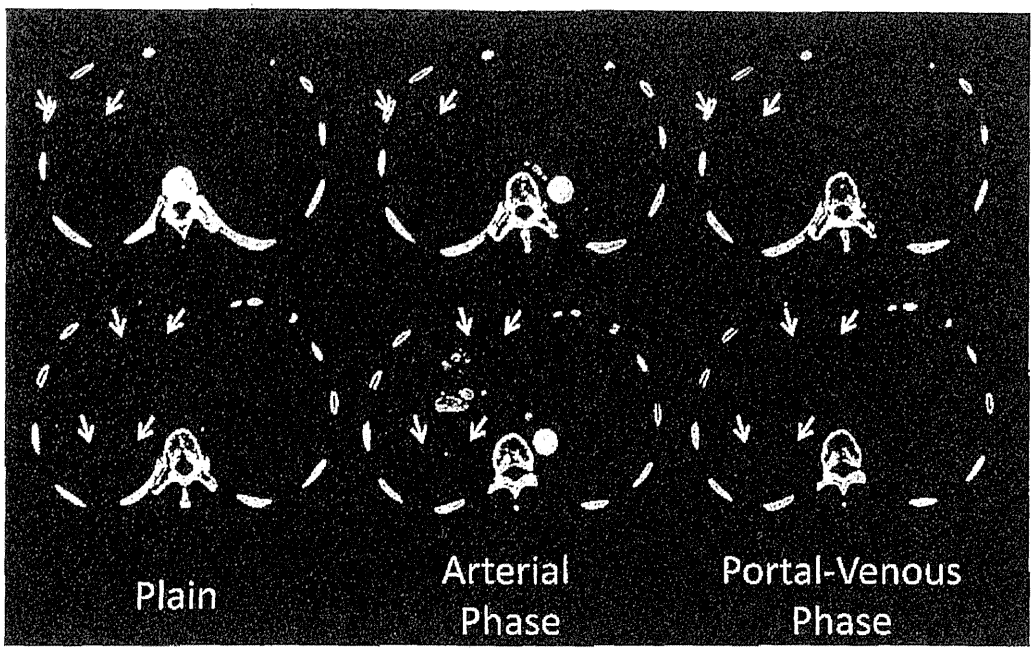

Fig. 2 Abdominal CT
Plain CT showed low density tumors with unclear boundary. By dynamic CT, arterial phase showed the tumors enhanced faintly, and portal-venous phase showed low density tumors compared with surrounding liver. (arrow)

を認めており，現在も経過観察中である．

考　察

MALTリンパ腫は1983年にIsaacsonら[3]によって提唱された粘膜関連リンパ組織（MALT）に発生する低悪性度の悪性リンパ腫であり，胃に好発する．肝原発の悪性リンパ腫は稀で[2]，そのほとんどはdiffuse large B-cell lymphoma（DLBCL）であり，肝原発悪性リンパ腫におけるMALTリンパ腫の頻度は10%未満とされている[3)-6)]．また，MALTリンパ腫のDLBCLへの進展も報告されている．

これまでの報告例をみると，単発例が多く，画像所見が多様で，画像所見のみでの確定診断が困難であることから，HCCと術前診断され，切除後に病理所見で確定診断に至る例も少なくない．

この症例は肝内多発病変と，肺門縦隔リンパ節以外には明らかな病変は認めず，リンパ節病変と肝病変の両方が原発として発生したか，どちらかが他方からの転移かは不明である．また，低悪性度の腫瘍であるため転移の可能性は低く，肺門縦隔リンパ節の病変は肉芽腫性炎症の可能性もあり，本当にMALTリンパ腫かどうかも不明である．肝内に病変の主座があり，肝原発のMALTリンパ腫の可能性が高いと考えられる．

画像所見について，これまでの報告例の頻度の多い所見と本症例を比較し，Table 2にまとめた．

これまでの報告によると，US所見については，橋口ら[7]は，観察可能であった10例全例で低エコーの腫瘤性病変として描出され，腫瘍辺縁に高エコー域を認める症例もあり，辺縁は4例で明瞭，6例で不明瞭であったと報告しており，坂口ら[8]は，10例中7例が低エコー，1例が高エコーを呈し，2例では病変が検出されなかったと報告している．三輪ら[9]は，14例中12例が低エコーを示し，稀に周囲が高エコーとなり，境界は不明瞭な症例が多いと報告している．CT所見については，橋口ら[7]は，単純CT所見の記載された8例全例で低吸収域腫瘤として認められ，造影CTにて11例中5例が早期相で淡く造影され，そのうち4例は平衡相でwash outされたが，うち1例は造影効果の残存が認められ，残

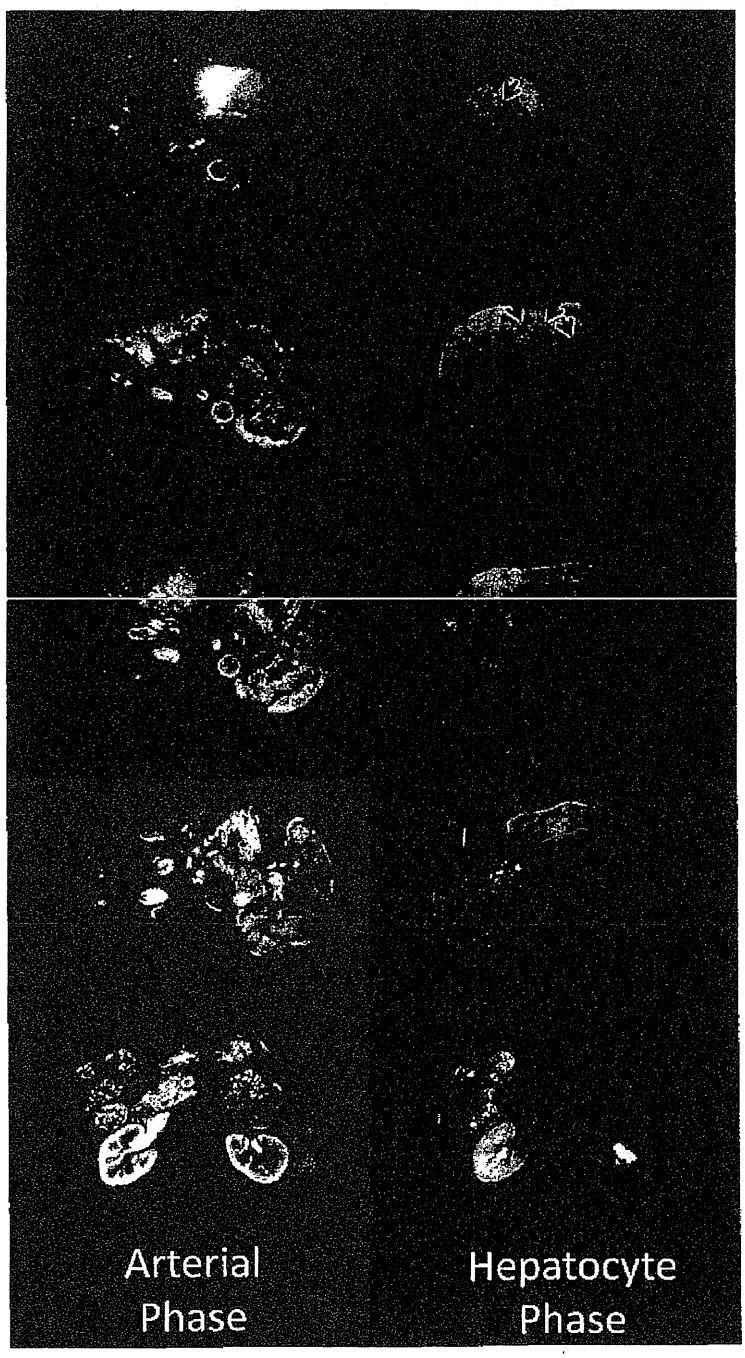

Fig. 3 Gd-EOB-MRI
Gd-EOB-MRI showed multiple tumors which were enhanced in arterial phase, and became the lesions with low intensity in hepatocyte phase. Portal vein was observed running through the S3 tumor. (arrow)

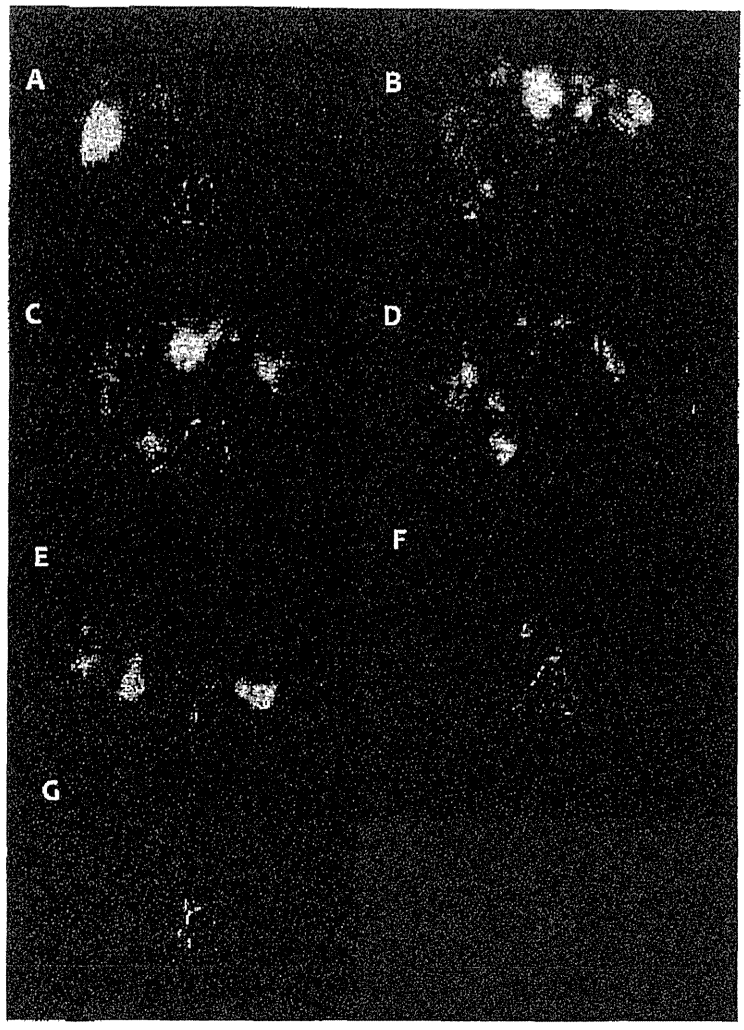

Fig. 4　PET-CT
PET-CT showed multiple abnormal accumulation of fluorodeoxyglucose in the liver (A, B, C, D and E), hilar lymph node (F) and mediastinal lymph node (G).

る6例中5例では造影効果は認められず，1例では腫瘍辺縁から造影される転移性肝腫瘍様の造影パターンであったと報告している．坂口ら[8]は，単純CTでは14例中，11例で低吸収域，3例では病変が検出されず，造影CTでは13例中10例は造影効果を認めず，3例で不均一に造影されたと報告している．三輪ら[9]は，単純CTで腫瘍は肝実質より低吸収域もしくは等吸収域であり，造影CTでは19例中12例で腫瘍が造影されたと報告しており，HCCや胆管癌などに比べ，腫瘍内部は均一な画像を示すことが多いとも報告している．Gd-EOB-MRI所見については，橋口ら[7]は，Gd-EOB-MRIの施行された2例では，動脈相で淡く造影され，平衡相で肝実質よりも低信号を呈したと報告している．また，T1強調像，T2強調像所見について，橋口ら[7]，坂口ら[8]は，MRIが施行された例は全例で，T1強調像で肝実質よりも低信号，T2強調画像で高信号であったと報告してお

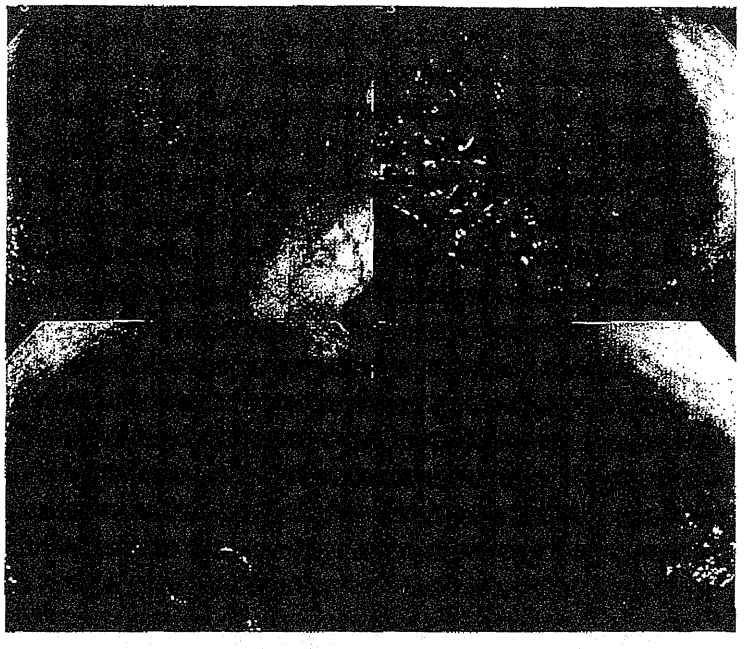

Fig. 5 Esophagogastroduodenoscopy
Esophagogastroduodenoscopy showed esophageal varices and chronic gastritis, but no tumors. Biopsy of stomach showed the existence of Helicobacter pylori.

り，三輪ら[9]も，T1強調像で肝実質よりも低信号，T2強調像で高信号であった症例が多かったと報告している．PET所見については，橋口ら[7]，三輪ら[9]は，PETの施行された3例では全例で異常集積を認めたと報告している．また，腫瘍内部を門脈や肝静脈などの構造が貫いて走行する所見が肝原発MALTリンパ腫などの肝悪性リンパ腫には特徴的とされるが，三輪ら[9]は，27例中4例で腫瘍の内部に通常の脈管が残る画像が示されていたと報告している．今回の症例でも，過去の報告例と同様な肝原発MALTリンパ腫を疑わせる所見は認められるものの，上記のように画像所見の多彩さから，画像所見のみで確定診断に至ることは難しいと考えられる．

三輪ら[9]の報告では27例中，9例が肝腫瘍生検によりMALTリンパ腫と診断されているが，それ以外の多くの症例では外科切除後の病理診断で初めて確定診断がなされていた．画像上HCCと矛盾しない所見である場合や，病変が小さい場合などには，腫瘍生検による術前診断は難しい場合もあるが，HCCとして非典型的と考える場合には，治療方針を決定するためにも肝腫瘍生検による組織診断が重要と考える．

MALTリンパ腫の発症には，持続する慢性炎症の関与があるとされている．胃MALTリンパ腫でのH.Pyloriによる慢性胃炎との関連が有名である．肝原発MALTリンパ腫でも，基礎疾患として，HCVウイルス感染，HBVウイルス感染による慢性肝炎や肝硬変，原発性胆汁性肝硬変，肝回虫感染などの何らかの慢性肝障害を有する症例が約4割と報告されている[10]．また，HBs抗原陽性例が約2割，HCV抗体陽性例が約3割との報告もある[8]．HCVを伴った肝B細胞性リンパ腫に対しインターフェロン治療を行い，HCVの消失とともにリンパ腫が寛解したとの報告もある[11]．肝原発MALTリンパ腫に，胃のH.Pylori感染の合併や胃癌の合併が報告されており[9]，H.Pylori感染は胆道感染とも関連するという報告もある[12]．しかし肝原発MALTリンパ腫と胃のH.Pylori感染との関連について，一定の見解は得られていない．

肝原発MALTリンパ腫の治療については，これまで

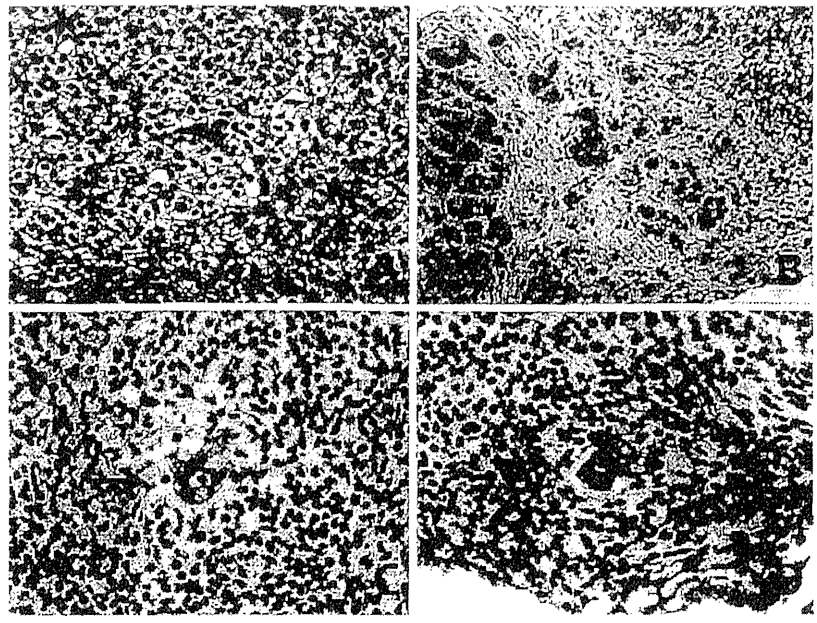

Fig. 6 *Histology of biopsy specimen of liver tumor*
A) Proliferation of centrocyte-like cells are observed and suggested the diagnosis of malignant lymphoma (hematoxylin-eosin stain, ×40). B) Atypical lymphocytes are invading and destroying hepatocytes (immune stain for hepatocyte, ×20). C) Lymphoepithelial lesion which is a characteristic finding of MALT lymphoma is observed (immune stain for cytokeratin AE1/AE3, ×40). D) Atypical lymphocytes are negative for CD3 suggesting the diagnosis of B-cell lymphoma (immune stain for CD3, ×40).

Table 2 Comparison of image findings between previously reported cases and present case

	Common findings of previously reported cases	Present case
US	Hypoechoic nodule with unclear boundary[7)~9)]	Hypoechoic nodule with unclear boundary
CT	・Plain CT; low density[7)~9)] ・Dynamic CT; enhanced/not enhanced[7)~9)]	・Plain CT; low density ・Dynamic CT; enhanced faintly in arterial phase, and low density in portal-venous phase
MRI	・Gd-EOB-MRI; enhanced in arterial phase, and low intensity in hepatocyte phase.[7)] ・T1WI; low intensity[7)~9)] ・T2WI; high intensity[7)~9)]	・Gd-EOB-MRI; enhanced in arterial phase, and low intensity in hepatocyte phase. ・T1WI; faintly low intensity or iso intensity ・T2WI; faintly high intensity
PET	Abnormal accumulation[7)9)]	Abnormal accumulation

の報告によると，外科的切除単独，化学療法単独，術後化学療法や術後放射線療法の併用療法など，様々な治療がなされているが，低悪性度である組織学的特徴により，比較的予後は良好である．しかし，治療方針については一定の見解が得られていないのが現状である．Thieblemontら[13]は，MALTリンパ腫のうち，初回治療後の胃病変の再発率が11%であるのに対し，胃外病変の再発率は44%と高率であったと報告している．また，肝原発MALTリンパ腫切除約7年後に肺に再発した例も2例報告されており[14)15)]，外科切除のみでは再

発の観点から不十分である可能性がある．また，Page ら[16]は早期からの多剤併用化学療法が有用であると提唱している．また，H.Pylori 除菌が有効である可能性もある．本症例では肝内多発病変に加えリンパ節病変も認め，また，MALT リンパ腫からの高悪性度の DLBCL への移行の可能性も否定できないため，H.Pylori 除菌後に化学療法を施行し，現在肝内病変の縮小を認めている．近年，画像診断の進歩により切除前に肝腫瘍生検により診断される症例が増えてきており，今後も症例を蓄積し，治療方針を確立していく必要がある．

結　語

C型肝硬変を背景として肝内多発病変を呈し，治療前に肝腫瘍生検にて組織診断を行い，化学療法導入が可能になった多発肝 MALT リンパ腫1例を経験したため，文献的考察とともに報告した．

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本論文内容に関連する著者の利益相反：なし

A case of multiple hepatic MALT lymphoma with cirrhosis related to hepatitis C virus

Yuka Takagawa, Naoto Kawabe, Senju Hashimoto, Masao Harata, Michihito Murao, Yoshifumi Nitta, Takuji Nakano, Hiroaki Shimazaki, Yuko Mizuno, Toshiki Kan, Kazunori Nakaoka, Masashi Oki, Kentaro Yoshioka*

We report a case of a 70-year-old woman with multiple hepatic tumors. She was a type C cirrhosis patient. Abdominal US showed hypoechoic nodules with unclear boundary. Contrast-enhanced US with Sonazoid showed the same sites enhanced in the vascular phase, and showed contrast defect in the postvascular phase. Plain CT showed low density tumors with unclear boundary. In dynamic CT, arterial phase showed the tumors enhanced faintly, and portal-venous phase showed low density tumors compared with surrounding liver. Gd-EOB-MRI showed multiple tumors which were enhanced in arterial phase, and showed low intensity in hepatocyte phase. Portal vein was observed in the S3 tumor. PET-CT showed multiple abnormal accumulation in the liver, mediastinal lymph node, and hilar lymph node. We underwent a liver tumor biopsy because the tumors were atypical as HCC, and showed an elevation of IL-2R. Biopsy histology showed the finding of MALT lymphoma. Thus we underwent chemotherapy. This case is a valuable case because multiple lesions of hepatic MALT lymphoma with type C cirrhosis is rare, and chemotherapy has become possible after diagnosis by tumor biopsy.

Key words: hepatic MALT lymphoma　type C cirrhosis　liver tumor biopsy　PET-CT　H.Pylori

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Effects of branched-chain amino acids and zinc-enriched nutrients on prognosticators in HCV-infected patients: A multicenter randomized controlled trial

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Abstract. Branched-chain amino acids (BCAAs) and trace element deficiencies are associated with poor prognosis in hepatitis C virus (HCV)-infected patients. The aim of this study was to investigate the effects of BCAA and zinc-enriched supplementation on prognostic factors in HCV-infected patients. Fifty-three HCV-infected patients were enrolled in this multicenter randomized controlled trial. The patients were assigned to either the placebo (n=27) or supplement group (n=26; 6,400 mg/day BCAAs and 10 mg/day zinc) and were followed up for 60 days. Primary outcomes were prognostic factors for chronic liver disease, including the serum BCAA-to-tyrosine ratio (BTR), zinc levels and α-fetoprotein (AFP) levels. There were no significant differences in any of the prognostic factors between the placebo and supplement groups at baseline. In the supplement group, the BTR and zinc levels were significantly increased compared with the placebo group (BTR: 5.14±1.59 vs. 4.23±1.14, P=0.0290; zinc: 76±11 vs. 68±11 μg/dl, P=0.0497). No significant differences were observed in AFP levels between the groups in the whole analysis. However, a stratification analysis showed a significant reduction in ΔAFP levels in the supplement group, with elevated AFP levels compared with the other groups (-2.72±3.45 ng/ml, P=0.0079). It was demonstrated that BCAA and zinc-enriched supplementation increased the BTR and zinc levels in the HCV-infected patients. Furthermore, the supplementation reduced the serum AFP levels in patients who had elevated serum AFP levels at baseline. Thus, BCAA and zinc-enriched supplementation may prolong the survival of HCV-infected patients by improving amino acid imbalance and zinc deficiency, and by partly downregulating AFP.

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Abbreviations: BCAAs, branched-chain amino acids; BTR, BCAA-to-tyrosine ratio; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment for insulin resistance; AFP, α-fetoprotein, BMI, body mass index

Key words: chronic hepatitis C, prognosticator, tumor marker, nutritional therapy, valine, leucine, isoleucine

Introduction

The liver plays a central role in nutritional metabolism and, therefore, metabolic disorders, including amino acid imbalances and trace element deficiencies, occur frequently in patients with chronic liver disease (1). Branched-chain amino acids (BCAAs) are essential amino acids that play a crucial role in albumin synthesis and ammonia detoxification in patients with cirrhosis (2). In the clinical setting, reductions in serum BCAA levels can be assessed by measurement of the serum BCAA-to-tyrosine ratio (BTR) (1). Low serum BTR, along with low serum albumin levels, have recently been reported to be a predictive factor in the development of hepatocellular carcinoma (HCC), and confer a poor prognosis in patients with cirrhosis (3,4).

Zinc is a trace element that activates >300 metalloenzymes, including DNA polymerase (5). In addition, zinc stabilizes zinc finger proteins, which bind to DNA and modulate the transcription of target genes, including tumor

suppressor genes (6). In patients with chronic liver disease, serum zinc levels decrease as the severity of liver disease increases (7), and low serum zinc levels are known to be associated with the development of HCC and poor prognosis in hepatitis C virus (HCV)-infected patients (8,9). Thus, serum zinc levels are a significant metabolic prognosticator in HCV-infected patients.

BCAAs and zinc are pharmacological nutrients that exert diverse biological effects (1). A decreased risk of hepatocarcinogenesis and mortality has been reported in patients with chronic liver disease treated with BCAA (4,10,11) or zinc (8,9) supplementation. Therefore, a supplement containing both BCAAs and zinc may have the potential to improve prognostic factors in patients with chronic liver disease. Aminofeel® is a commercially available BCAA and zinc-enriched supplement. We previously examined the usefulness of the supplement and demonstrated that it improves insulin resistance, taste sensitivity and adherence to interferon therapy in HCV-infected patients (12-16). However, it remains unclear whether BCAA and zinc-enriched supplementation improves prognostic factors, including the platelet count, serum albumin levels, homeostasis model assessment for insulin resistance (HOMA-IR) value, serum BTR and zinc levels in HCV-infected patients. In addition, to the best of our knowledge, the impact of the supplement on serum α-fetoprotein (AFP) levels has never been investigated. Therefore, we performed a prospective multicenter randomized controlled trial to investigate the effects of a BCAA and zinc-enriched supplement on prognostic factors in HCV-infected patients.

Subjects and methods

Ethical considerations. This study was designed in 2009 by the steering committee of Research on Hepatitis, The Ministry of Health, Labour and Welfare of Japan (principal investigator, Michio Sata, MD), to evaluate the usefulness of BCAA and zinc supplementation in patients with chronic HCV infection. The study protocol was approved by the Ethical Committee of Human Experimentation in Kurume University School of Medicine (approval no. 09152, UMIN000012815), and is in accordance with the Helsinki Declaration of 1975, as revised in 1983. Written informed consent for participation in the study was obtained from each subject.

Subjects. HCV-infected patients aged 65 years or older who had serum albumin levels ≥3.5 and <4.0 g/dl were recruited for the study. The exclusion criteria were as follows: i) currently undergoing interferon therapy; ii) positivity for hepatitis B surface antigen or serum hepatitis B virus DNA; iii) presence of autoimmune hepatitis, alcoholic liver disease (ethanol consumption >50 g/day), primary biliary cirrhosis, primary sclerosing cholangitis, hemochromatosis or Wilson's disease; iv) presence of cardiac or renal disease or severe psychiatric disease; v) presence of HCC or within a year of treatment for HCC; vi) presence of esophageal or gastric varices at risk of rupture; vii) presence of diabetes mellitus with anti-diabetic medication; viii) history of consumption of the BCAA and zinc-enriched supplement; ix) having taken a BCAA-related medication or a BCAA-containing supplement within the preceding 90 days; and x) currently taking a trace element-related medication or a trace element-containing supplementation.

Study design and participants. We performed a prospective multicenter randomized controlled trial in an outpatient setting in six medical institutions in Japan. From 2010 to 2012, 54 HCV-infected patients who fulfilled the inclusion criteria were enrolled in the study. One patient withdrew consent for participation before randomization. The stratified randomization method was used to achieve balance among groups in terms of subjects' baseline characteristics. Randomization was performed centrally, and both patients and investigators were blinded to the patients' group assignment. The patients were allocated at a 1:1 ratio to the placebo (n=27) or supplement group (n=26). Two patients in the placebo group were withdrawn from the study due to withdrawal of consent (n=1) and anemia (n=1). Two patients in the supplement group were withdrawn due to general fatigue (n=1) and the appearance of a rash (n=1). Thus, four patients were excluded from the statistical analysis on day 60 owing to lack of data. Finally, 92.6% (25/27) of the patients in the placebo group (age, 74±5 years; female/male, 17/8) and 92.3% (24/26) of patients in the supplement group (age, 74±5 years, female/male, 15/9) completed the 60-day treatment period, and the efficacy and safety of the treatment were assessed (Fig. 1).

Intervention and assessment protocols. In the supplement group, the patients were given two sachets of the BCAA and zinc-enriched supplement containing 6,400 mg/day BCAAs and 10 mg/day zinc (Aminofeel, Seikatsu Bunkasya Co., Inc., Chiba, Japan), once after breakfast and again at bedtime (Fig. 2). In the placebo group, the patients were administered a sachet of placebo after breakfast and another at bedtime. Although the BCAAs, trace elements and vitamins were replaced with corn starch in the placebo (Table I), the appearance and taste of the placebo were similar to those of the supplement.

On days 0 and 60, we evaluated body mass index (BMI), subjective symptoms (fatigue, sleeplessness, muscle cramps, loss of appetite and taste disorders) using a visual analog scale (Fig. 2). A visual analog scale is a horizontal line, 100 mm in length, anchored by word descriptors for subjective symptoms at each end. The patients marked on the line the point that they felt represented their current subjective symptom. The visual analog scale score was determined by measuring the distance (in mm) from the left-hand end of the line to the point that the patient had marked, on which 0 mm indicates an absence of symptoms and 100 mm indicates the worst symptom.

The following blood biochemical parameters were measured in all the patients on days 0 and 60: White blood cell count, hemoglobin levels, platelet count, total protein levels, albumin levels, BTR, prothrombin time (international normalized ratio), ammonia levels, zinc levels, fasting blood glucose levels, hemoglobin A1c levels, fasting immune reactive insulin levels, HOMA-IR, total cholesterol levels, aspartate aminotransferase levels, alanine aminotransferase levels, γ-glutamyl transpeptidase levels, alkaline phosphatase levels, blood urea nitrogen levels, creatinine levels, AFP levels and HCV RNA levels. All the biochemical parameters were measured by standard clinical methods using venous blood samples

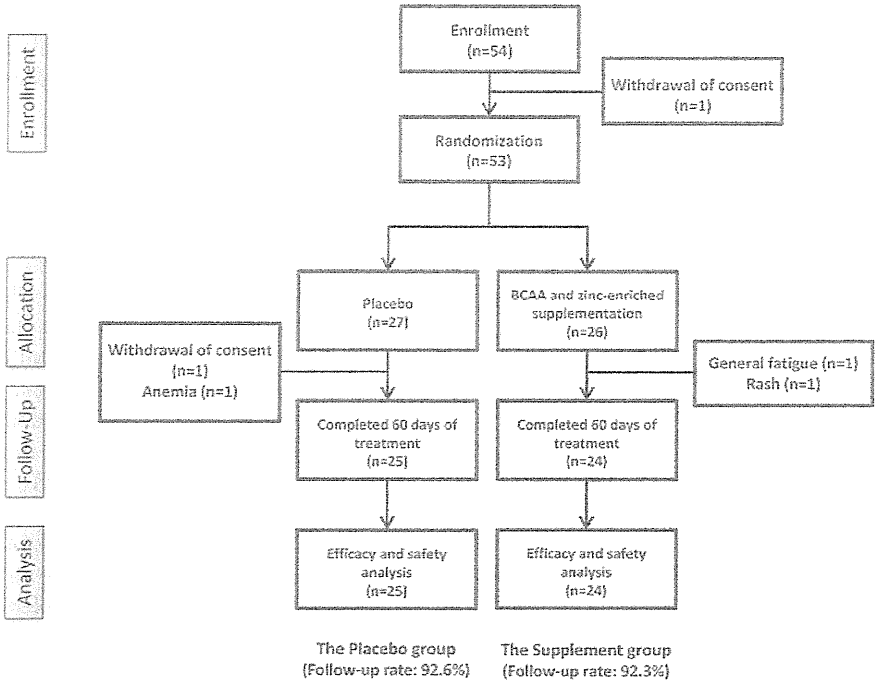

Figure 1. Flow diagram of the study. A total of 54 HCV-infected patients were enrolled. During the course of the study, one patient withdrew consent before randomization and four patients dropped out during the trial. In total, 49 patients completed 60 days of treatment/placebo, and the efficacy and safety of the treatment was assessed. The follow-up rates were 92.6% (25/27) in the placebo group and 92.3% (24/26) in the supplement group.

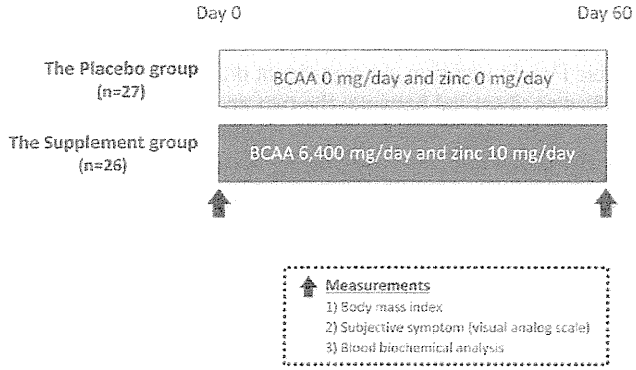

Figure 2. Intervention and assessment protocols used in the study.

taken the morning after a 12-h overnight fast, as previously described (17).

Outcomes. The primary outcomes were the following known prognostic factors for patients with chronic liver disease: Platelet count, serum albumin levels, serum AFP levels, HOMA-IR value, serum BTR and serum zinc levels. The secondary outcomes were BMI, subjective symptoms and biochemical examinations, including liver and renal functions, glucose metabolism and HCV RNA levels.

Stratification analysis according to serum AFP levels. As the serum AFP levels had a wide distribution, we performed a stratification analysis to assess the effects of the BCAA and zinc-enriched supplement on changes in serum AFP levels. The patients in both the placebo and supplement groups were further classified into two groups based on their serum AFP levels at baseline as follows: One group with AFP levels within the reference range (≤8.7 ng/ml) and another group with elevated serum AFP levels (>8.7 ng/ml). Changes in serum AFP levels were expressed as ΔAFP (day 60 AFP level vs. day 0 AFP level).

Safety monitoring. Safety was assessed in terms of vital signs, physical examination results, laboratory test results and clinical adverse events. When 19 patients (n=9 in the placebo group; n=10 in the supplement group) had completed the study, the data monitoring committee evaluated the safety and disadvantages of the groups and confirmed that there were no severe adverse events or disadvantages observed in either the placebo or supplement group. This evaluation was conducted to produce an interim report at the meeting of Research on Hepatitis (Ministry of Health, Labour and Welfare of Japan; principal investigator, Michio Sata).

Considerations relating to the sample size and treatment period. Based on the BTR data in our previous pilot study (12), the sample size was calculated using Power and Sample Size Calculation v 3.0, as previously described (18). Briefly, we planned to study a continuous response variable in independent controls and experimental subjects with one control per experimental subject. In our previous pilot study, the response within each subject group was normally distributed with a standard deviation of 0.9 (12). If the true difference between the experimental and control means is 1, we would need to study a total of 36 subjects (18 experimental subjects and 18 control subjects) to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with a probability (power) of 0.9. The type I error probability associated with testing this null hypothesis is 0.05.

Table I. Contents of one sachet of placebo and one sachet of the branch-chain amino acid and zinc-enriched supplement.

	Placebo	Supplement
Valine (mg)	0.0	800.0
Leucine (mg)	0.0	1,600.0
Isoleucine (mg)	0.0	800.0
Zinc (mg)	0.0	5.0
Calcium (mg)	0.0	21.1
Magnesium (mg)	0.0	12.6
Copper (mg)	0.0	0.2
Selenium (μg)	0.0	49.6
Chromium (μg)	0.0	14.4
Vitamin A (μg)	0.0	315.0
Vitamin D (μg)	0.0	3.0
Vitamin E (mg)	0.0	6.4
Vitamin K (μg)	0.0	29.6
Vitamin C (mg)	0.0	40.0
Vitamin B1 (mg)	0.0	2.4
Vitamin B2 (mg)	0.0	2.6
Niacin (mg)	0.0	12.0
Vitamin B6 (mg)	0.0	2.4
Vitamin B12 (μg)	0.0	10.0
Folic acid (μg)	0.0	0.2
Pantothenic acid (mg)	0.0	6.8
Corn starch (mg)	3,487.0	0.0

The treatment period was also based on BTR data from our previous pilot study (12). In the previous study, we examined the effects of the BCAA and zinc-enriched supplement on serum BTR 30, 60 and 90 days after treatment. Considering that the most significant efficacy was observed 60 days after treatment, a 60-day treatment period was used in this study (12).

Statistical analysis. All the data are expressed as the number or mean ± standard deviation. Differences between the placebo and supplement groups were analyzed using the χ^2 test or Mann-Whitney U test. Statistical comparisons between multiple groups were performed using the Kruskal-Wallis test. $P<0.05$ was considered to indicate a statistically significant difference. All analyses were performed using JMP 10.0.2 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics. At baseline, there were no significant differences in age, gender and BMI between the placebo and supplement groups (Table II). No significant differences were observed in any subjective symptoms, including fatigue, sleeplessness, muscle cramps, loss of appetite and taste disorders between the two groups (Table II).

There were no significant differences in any of the prognostic factors, including platelet count, serum albumin and AFP levels, HOMA-IR value, serum BTR and serum zinc levels between the two groups (Table II). Moreover, no significant difference was observed in any of the biochemical examinations, including tests for liver and renal function, glucose metabolism and HCV RNA levels, between the groups at baseline (Table II).

Effects of BCAA and zinc-enriched supplementation on primary outcomes. On day 60, we evaluated the effects of the BCAA and zinc-enriched supplementation on the prognostic factors in the HCV-infected patients. There were no significant differences in platelet count, serum albumin and AFP levels, and HOMA-IR value between the placebo and supplement groups (Fig. 3A-D). Conversely, a significant increase in serum BTR was observed in the supplement group compared with the placebo group (Fig. 3E). In addition, a significant increase was observed in serum zinc levels in the supplement group compared with the placebo group (Fig. 3F).

Effects of the BCAA and zinc-enriched supplementation on secondary outcomes. On day 60, we also evaluated the effects of the BCAA and zinc-enriched supplement on BMI, subjective symptoms and biochemical parameters in the HCV-infected patients. There was no significant difference in BMI between the placebo and supplement groups (Table III). There was also no significant difference in fatigue, sleeplessness, muscle cramps, loss of appetite or taste disorders between the groups (Table III).

There was no significant difference in liver function test results, renal function test results, glucose metabolism or blood ammonia levels between the placebo and supplement groups (Table IV). In addition, no significant difference in HCV RNA levels was observed between the two groups (Table IV).

Stratification analysis according to serum AFP levels at baseline. As the serum AFP levels were widely distributed, a stratification analysis was performed according to the serum AFP levels at baseline to assess the effects of BCAA and zinc-enriched supplementation on changes in the serum AFP levels. A significant reduction in the ΔAFP levels was observed in the supplement group with an elevation in the AFP levels compared with the other groups (Fig. 4).

Safety. The incidence rates of adverse events during the study were 3.7% (1/27) and 7.7% (2/26) in the placebo and supplement groups, respectively. Two subjects discontinued the placebo due to withdrawal of consent (n=1) and anemia (Grade 2; n=1). Two subjects in the supplement group discontinued the treatment due to general fatigue (Grade 2; n=1) and rash (Grade 3; n=1). There was no significant deterioration in any biochemical parameters including liver and renal function. No Grade 4 or higher adverse events occurred during the study period.

Discussion

This was a multicenter randomized controlled trial designed to examine the effects of a BCAA and zinc-enriched supplement on prognostic factors in HCV-infected patients. No changes were observed in the platelet counts, serum albumin levels or HOMA-IR values; however, a significant increase was noted in the serum BTR and zinc levels in the patients administered

Table II. Patient characteristics at baseline.

	Placebo group	Supplement group	P-value
No. of patients	25	24	-
Age (years)	74±5	74±5	0.7480
Gender (female/male)	17/8	15/9	0.7688
Body mass index (kg/m^2)	22.9±3.7	22.6±3.3	0.7820
Subjective symptoms evaluated by visual analog scale (mm)			
Fatigue	20±21	21±22	0.9679
Sleeplessness	19±24	19±25	0.9272
Muscle cramp	14±25	20±29	0.7054
Loss of appetite	11±18	20±24	0.2278
Taste disorder	5±14	8±16	0.1828
Biochemical prognosticators			
Platelet count ($10^4/\mu$l)	13.6±5.8	14.2±4.4	0.4776
Albumin (g/dl)	3.79±0.12	3.79±0.20	0.6448
α-fetoprotein (ng/ml)	15.6±32.1	8.0±8.6	0.3372
HOMA-IR	2.99±3.81	2.89±2.38	0.7491
BCAA-to-tyrosine ratio	4.43±1.06	4.93±1.33	0.3731
Zinc (μg/dl)	68±9	68±7	0.8281
Other biochemical parameters			
White blood cell count (/μl)	4182±1092	4504±1132	0.3787
Total lymphocyte count (/μl)	1449±658	1515±826	0.7501
Hemoglobin (g/dl)	12.8±1.4	13.1±1.4	0.4007
Aspartate aminotransferase (U/l)	51±18	47±16	0.5283
Alanine aminotransferase (U/l)	47±23	37±20	0.0799
γ-glutamyl transpeptidase (U/l)	33±15	36±34	0.3650
Alkaline phosphatase (U/l)	340±132	318±113	0.6170
Total protein (g/dl)	7.55±0.63	7.44±0.62	0.5552
Prothrombin time (international normalized ratio)	1.05±0.12	1.02±0.06	0.9366
Fasting blood glucose (mg/dl)	102±12	109±23	0.3123
Hemoglobin Alc (%)	5.2±0.4	5.5±0.6	0.0932
Fasting immune reactive insulin (μU/ml)	11.0±10.8	10.1±6.4	0.8204
Total cholesterol (mg/dl)	160±23	158±28	0.6671
Ammonia (μg/dl)	32±13	30±16	0.5598
Blood urea nitrogen (mg/dl)	16.2±4.8	16.9±4.0	0.3916
Creatinine (mg/dl)	0.65±0.29	0.73±0.25	0.0872
HCV RNA (log copy/ml)	6.1±1.0	6.2±0.8	0.9544

The data are expressed as the number or mean ± SD. Differences between the placebo and supplement groups were analyzed using the χ^2 test or Mann-Whitney U test. $P<0.05$ was considered to indicate a statistically significant difference. HOMA-IR, homeostasis model assessment for insulin resistance; BCAAs, branched-chain amino acids; HCV, hepatitis C virus.

the supplement for 60 days. Furthermore, the stratification analysis revealed a significant reduction in the ΔAFP levels in the supplement group with an elevation in the AFP levels compared with the other groups.

No significant increase was observed in the serum albumin levels in this study. However, to the best of our knowledge, we demonstrated for the first time that 6,400 mg/day BCAAs administered for 60 days was sufficient to increase the serum BTR in patients in the early stages of HCV-related chronic liver disease. The dose of BCAAs used in this study may have been insufficient to increase serum albumin levels over the period of 60 days. However, a lower serum BTR can predict decreases in serum albumin levels (19), suggesting that long-term administration of the supplement may maintain constant serum albumin levels. In addition, low serum BTRs have been recently reported to be a predictive factor in the development of HCC, intrahepatic distant recurrence of HCC and poor prognosis in patients with cirrhosis (2,3). Moreover, BCAA supplementation is known to increase serum BTR, leading to the suppression of hepatocarcinogenesis and

Table III. Effects of the branch-chain amino acid and zinc-enriched supplement on body mass index and subjective symptoms.

	Placebo group	Supplement group	P-value
No. of patients	25	24	
Body mass index (kg/m^2)	23.2±3.6	22.8±3.2	0.7505
Subjective symptoms evaluated by a visual analog scale, mm			
Fatigue	18±17	18±25	0.5486
Sleeplessness	25±26	18±24	0.3095
Muscle cramp	16±23	23±33	0.5357
Loss of appetite	15±19	16±19	0.6210
Taste disorder	10±21	4±5	0.4153

The data are expressed as the number or mean ± SD. Differences between the placebo and supplement groups were analyzed using the Mann-Whitney U test. P<0.05 was considered to indicate a statistically significant difference.

Table IV. Effects of the branch-chain amino acid and zinc-enriched supplement on biochemical examinations.

	Placebo group	Supplement group	P-value
No. of patients	25	24	
White blood cell count (/μl)	4142±1010	4475±965	0.2711
Total lymphocyte count (/μl)	1494±650	1478±708	0.9915
Hemoglobin (g/dl)	13.1±1.4	13.0±2.0	0.8100
Aspartate aminotransferase (U/l)	52±17	54±21	0.9521
Alanine aminotransferase (U/l)	48±21	45±28	0.4064
γ-glutamyl transpeptidase (U/l)	34±16	37±40	0.2417
Alkaline phosphatase (U/l)	355±132	308±109	0.2846
Total protein (g/dl)	7.77±0.71	7.57±0.57	0.4412
Prothrombin time (international normalized ratio)	1.02±0.09	1.00±0.05	0.8915
Fasting blood glucose (mg/dl)	105±14	109±25	0.8886
Hemoglobin A1c (%)	5.2±0.4	5.6±0.6	0.0402
Fasting immune reactive insulin (μU/ml)	14.0±10.9	9.5±4.6	0.2155
Total cholesterol (mg/dl)	165±26	163±29	0.8258
Ammonia (μg/dl)	35±13	29±15	0.1906
Blood urea nitrogen (mg/dl)	14.9±4.5	16.9±4.5	0.0927
Creatinine (mg/dl)	0.64±0.27	0.72±0.25	0.0871
HCV RNA (log copy/ml)	5.9±1.5	6.1±0.9	0.7837

The data are expressed as the number or mean ± SD. Differences between the placebo and supplement groups were analyzed using the Mann-Whitney *U* test. P<0.05 was considered to indicate a statistically significant difference.

improvement in survival in patients with cirrhosis (4,10,11). Taken together, it would be worthwhile to test the effect of the long-term administration of the supplement on hepatocarcinogenesis and prognosis in HCV-infected patients.

We also demonstrated that the administration of 10 mg/day zinc was sufficient to increase the serum zinc levels in patients in the early stages of HCV-related chronic liver disease. In patients with cirrhosis, zinc upregulates hepatic ornithine transcarbamylase activity, a key enzyme in the urea cycle, and enhances hepatic ammonia detoxification (20). A recent meta-analysis by Chavez-Tapia *et al* (21) demonstrated that oral zinc supplementation improved performance in the number connection test, a test for hepatic encephalopathy. Thus, the supplement tested here may be beneficial for patients with hyperammonemia. In addition, a decrease in serum zinc levels is known to predict the development of HCC and poor prognosis in HCV-infected patients (8,9). Moreover, decreased risks of hepatocarcinogenesis and mortality have been observed in HCV-infected patients with increased serum zinc levels owing to zinc supplementation (8,9). The data reported here indicate that the zinc-enriched supplement has the potential to suppress the onset of HCC and improve prognosis in HCV-infected patients.

When all of the data were analyzed, the serum AFP levels were not significantly decreased in the patients administered