Table 1 Clinical characteristics of 207 patients with 321 nodules.

	Typical-HCC (n=199, 313 nodules)	Atypical-HCC (n=8, 8 nodules)	*p* value
Age (years)[a]	68.9±7.3	70.6±6.9	NS
Gender (male/female)	131/68	5/3	NS
Hepatitis viruses (B/C/B+C/non-B, non-C)	28/144/2/25	0/7/0/1	NS
Child-Pugh classification (A/B/C)	162/32/5	6/2/0	NS
Total Bilirubin (mg/dl)[a]	0.9±0.5	1.1±0.4	NS
Average tumor size (mm)[a]	28.8±15.2	15.5±3.5	0.0129

NS: not significant.
[a]Values are the mean±SD.

対象と方法

対象は2008年2月から2009年6月の間に当院にて血管造影下CTを行った374例のうち，同時期にソナゾイド®を用いた造影超音波もしくはEOB・プリモビスト®造影MRI（以下，EOB造影MRI）が施行された典型的肝細胞癌症例もしくは肝細胞癌疑い（非典型的肝腫瘍）症例の207例321結節である．なお，すべての画像検査は2カ月以内に施行され，肝内に結節が多発する場合は，同一肝内に3結節以内の症例を対象とし，非典型的肝腫瘍は腫瘍生検が行われた症例を対象とした．

患者・腫瘍背景はTable 1の通りであり，典型的肝細胞癌と非典型的肝腫瘍において腫瘍径において有意差が認められた．

使用装置および撮像方法は以下の通りである．

X線CT装置は東芝社製X vision Real（IVR-CT/Angio system）を使用し，CT during arterial portography（以下，CTAP）は造影剤70 ml/を2 ml/秒で注入し，造影開始30秒後より1相をスライス厚7 mmで撮像，CT during hepatic arteriography（以下，CTHA）は造影剤25 mlを1.2 ml/秒で注入し，造影開始10秒後と50秒後より各1相をスライス厚7 mmで撮像した．CTAPは周囲肝実質と比較して低吸収，等吸収，高吸収，CTHAは造影開始10秒後に周囲肝実質より血流の増加が認められるものを高吸収とし，等吸収そして低吸収と分類した．

超音波装置は東芝社製Aplio XGを使用し，ソナゾイド®は以前のわれわれの検討[5]から推奨量の半量である0.0075 ml/kgをボーラス注入し15秒から60秒までを血管相，10分後を後血管相として撮像した．それぞれの時相は，血管相では周囲肝実質より血流の増加が認められるものをhyper，同等をiso，低下をhypo，また後血管相はそれぞれ等エコー，低エコー，欠損に分類した．なお後血管相ではソナゾイド®の輝度は確認できるが周囲肝よりもエコーレベルが低いものを低エコーとし，ソナゾイド®の輝度が全く認められないものを欠損とした．

MRI装置はPHLIPS社製Achieva 1.5T Novaを使用した．EOB・プリモビスト®は0.1 ml/kgを1.5 ml/秒で注入し，後押し用の生理食塩水は35 mlを2 ml/秒で注入した．造影前T1強調像はFFE（fast field echo）のdual echo法にてin phaseおよびopposed phaseを，Dynamic-studyはTFE（turbo field echo）の3D収集にて動脈2相，門脈相，後期相の計4相を撮像し，造影後はTSE（turbo spin echo）法にてT2強調像，TFEの3D収集にて肝細胞造影相の順に撮像し，Dynamic-studyの撮影を開始するタイミングはBolusTrak法を用い，腹部大動脈の濃染を確認後とし，肝細胞造影相は15分後に撮像した．Dynamic-studyの動脈相で濃染が認められるものと，濃染が認められないものに分類し，肝細胞造影相では周囲肝実質と比較して低信号，等信号，高信号と分類した．検討項目は以下の2項目である．

①CTAPで低吸収かつCTHAで高吸収が認められた典型的肝細胞癌199例313結節における造影超音波所見とEOB造影MRI所見の対比を検討した．

②CTAPで低吸収ないし等吸収かつCTHAで高吸収が認められず，腫瘍生検が施行された非典型的肝腫瘍8例8結節のMRI T1強調像所見，EOB造影MRI肝細胞造影相所見，Bモード超音波所見，造影超音波所見，CTAPとCTHA所見および病理組織所見の対比を検討

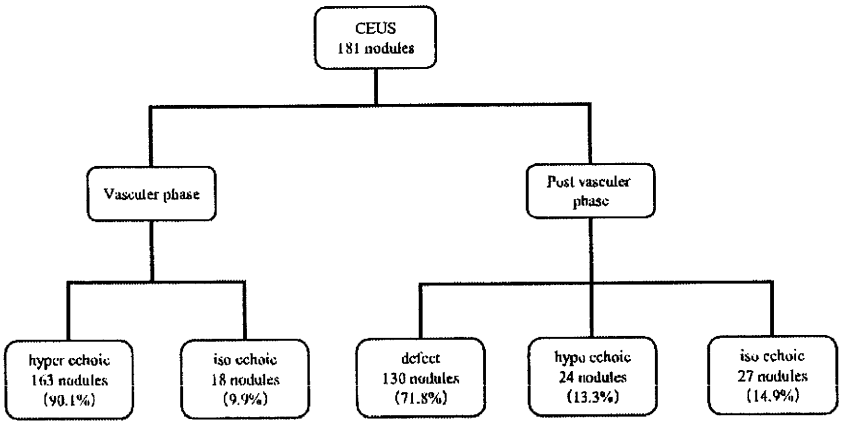

Fig. 1 Findings of contrast-enhanced ultrasound (CEUS) with perfluorobutane for patients with hepatocelluler carcinoma with typical imaging features on CTAP/CTHA. Detectability on the post-vascular phase, seen as a defect or hypo echogenic nodule, was 85.1%.

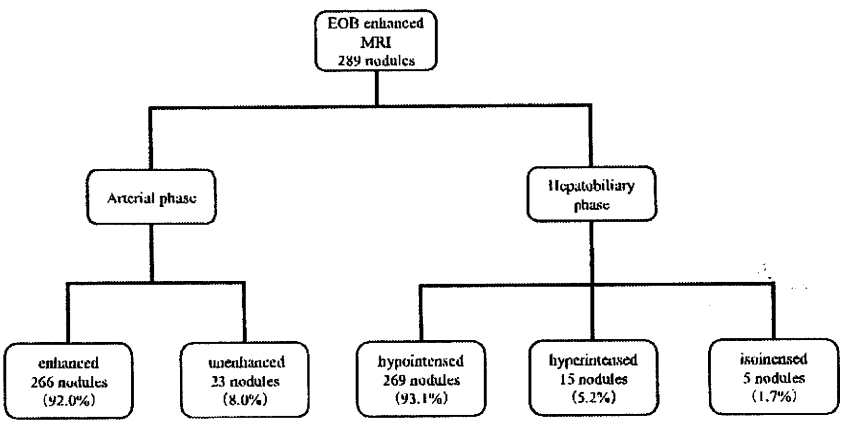

Fig. 2 Findings of enhanced MRI with Gadolinium-ethoxybenzyl-diethlenetriamine pentaacetic acid for patients with hepatocelluler carcinoma with typical imaging findings on CTAP/CTHA. Detectability on the hepatobiliary phase, seen as a hypo- or hyper-intensity nodule, was 98.3%.

した.

統計は t 検定，Fisher の直接確立検定もしくは X^2 検定を用い，$P<0.05$ を統計学的に有意差ありと判定した.

結　果

①検討項目1

造影超音波が施行されたのは136例181結節で，血管相でhyperは163結節(90.1%)で残り18結節(9.9%)はisoであった．後血管相では欠損が130結節(71.8%)，低エコーが24結節(13.3%)，等エコーが27結節(14.9%)であった（Fig. 1）．EOB造影MRIが施行されたのは160例289結節で，動脈相で濃染が認められたものは266結節（92.0%）で，残り23結節（8.0%）は濃染が認められなかった．肝細胞造影相では低信号が269結節（93.1%），高信号が15結節（5.2%），等信号が5結節（1.7%）であった（Fig. 2）．なお肝細胞造影相で等信号であった5結節はすべて造影超音波が施行されており，血管相では5結節ともhyperであり，さらに後血管相では4結節が欠損で1結節が低エコーであった.

なお腫瘍径は造影超音波施行結節が26.8±12.9 mm，EOB造影MRI施行結節が28.5±14.9 mmで，$p=0.2832$

Table 2 Findings on each imaging modality and by pathology for HCC lesions lacking the typical imaging findings on CTAP/CTHA.

Case	Segment	Size (mm)	MRI T1WI	EOB MRI Hepatobiliary phase	B-mode US	CEUS Vascular phase	CEUS Post vascular phase	CTAP	CTHA	Pathologic features
Case 1	S5	17	hyper	hypo	hypo	hypo	iso	iso	low	w-d HCC
Case 2	S6	14	iso	hypo	bright loop	hyper	iso	iso	low	w-d HCC
Case 3	S7	10	iso	hypo	bright loop	hyper	hypo	low	iso	w-d HCC
Case 4	S3	18	hypo	hypo	hypo	hypo	iso	low	low	w-d HCC
Case 5	S5	12	iso	hypo	hypo	hypo	iso	iso	iso	w-d HCC
Case 6	S6	17	hyper	hypo	hypo	hypo	iso	iso	iso	w-d HCC
Case 7	S6	15	hyper	hypo	hyper	hypo	iso	iso	low	w-d HCC
Case 8	S7	21	hyper	hypo	hypo	hypo	iso	low	low	w-d HCC

w-d HCC: well-differentiated HCC

と有意差は認められなかった．

②検討項目2（Table 2）

8結節の平均腫瘍径は15.5±3.5 mmであった．腫瘍生検の結果は8結節すべてが高分化型肝細胞癌であった．MRI T1強調像は高信号が4結節，等信号が3結節，低信号が1結節であり，EOB造影MRIの肝細胞造影相は8結節すべてが低信号を呈した．Bモード超音波は高エコーが1結節，bright loopが2結節，低エコーが5結節であった．また造影超音波の血管相ではhyperが2結節，hypoが6結節であった．さらに後血管相では1結節の低エコーを除きすべて等エコーであった．CTAPでは3結節が低吸収で5結節が等吸収であり，CTHAでは5結節が低吸収，3結節が等吸収であった．症例としてCase 1（Fig. 3），Case 2（Fig. 4）を提示する．

考　察

肝細胞癌の診断は，ここ20数年間で各種画像診断と病理学的対比により多くのことが解明され，その病態は血流と密接に関連していることが証明された[6)~8)]．さらに肝細胞癌のうち悪性度が進行したものではKupffer細胞数が変動していることが報告された[9)10)]．このKupffer細胞数の多寡は超常磁性酸化鉄（super paramagnetic iron oxides：SPIO）MRI検査および今回の検討で用いたソナゾイド®により診断が可能となった[2)]．すなわち肝細胞癌の生物学的悪性度が血流診断のみならずKupffer細胞数という面からも可能となった．加えてソナゾイド®は血管相において肝腫瘍の血流の多寡も診断でき，リアル性に富み身体的負担が少ない面からみても肝細胞癌の画像診断において非常に有用な検査法といえる[11)]．さらに新しいMRI造影剤であるEOB・プリモビスト®は胆汁産生能を持つ正常の肝細胞内に特異的に取り込まれる造影剤で，肝細胞から胆汁中に排泄され，T1短縮効果を有する常磁性の陽性造影剤である．肝細胞内への取り込みそして毛細胆管への排泄の機序は完全には明らかになっていないがOATP1（organic anion transporter）やMRP2（multidrug resistance-associated protein 2）などの関与が考えられている[12)13)]．EOB造影MRIでは動脈相で肝腫瘍の血流の多寡が診断でき，さらに門脈相以降ではEOB・プリモビスト®が次第に肝細胞に取り込まれてゆくために，投与20分後の肝細胞造影相では高い肝/腫瘍コントラストが認められる．一般的に肝細胞癌の場合，肝細胞造影相にて低信号となるが[14)]，胆汁産生能を持ついわゆる“green hepatoma”や偽腺管構造が存在する場合は高信号となることが報告されている[15)16)]．このEOB・プリモビスト®登場により肝細胞癌の診断は血流およびKupffer細胞数に関する診断に加え，肝細胞内へのEOB・プリモビスト®の取り込み能に関しても診断が可能となり，まさしく肝細胞癌の画像診断は新たな局面を迎えたといえる．

肝細胞癌の血流動態を評価する画像検査としては造影CT，ガドリニウムを用いた造影MRI，血管造影などが挙げられるが，特に血管造影下CTは侵襲的ではあるが典型的肝細胞癌ではCTAPによる門脈血流欠損像に基づく存在診断とそれに続くCTHAによる門脈血流欠損部位の濃染による質的診断を可能とし，肝細胞癌に対する画像診断のゴールド・スタンダードとされてき

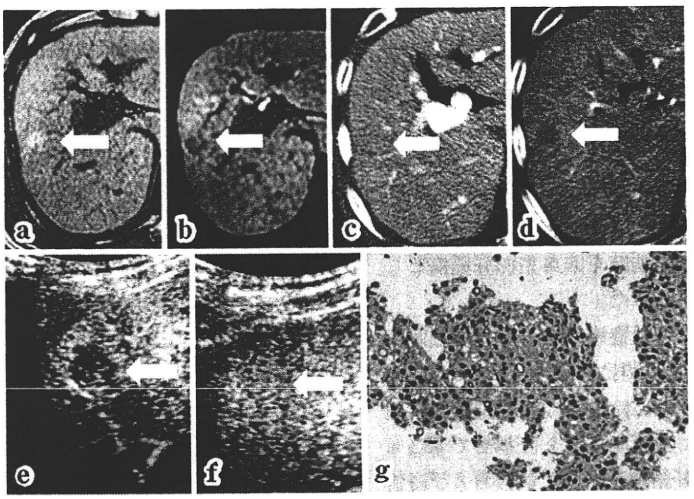

Fig. 3 Case 1, well-differentiated HCC measuring 17 mm in diameter.
(a) The nodule was visualized as a faintly hyperintense nodule on T1-weighted MRI (arrow).
(b) The nodule was identified as a hypointense nodule on the hepatobiliary phase of Gd-EOB-DTPA enhanced MRI (arrow).
(c) The nodule was depicted as an isodense nodule on CTAP (arrow).
(d) The nodule was seen as a low-density nodule on CTHA (arrow).
(e) The nodule was visualized as a hypo-echogenic nodule on the vascular phase of CEUS (arrow).
(f) The nodule was observed as an iso-echogenic nodule on the post-vascular phase of CEUS (arrow).
(g) Liver tissue fragments demonstrated a dense population of small cells with an increased N/C ratio and low nuclear roundness. This sample was diagnosed as a well-differentiated hepatocellular carcinoma.

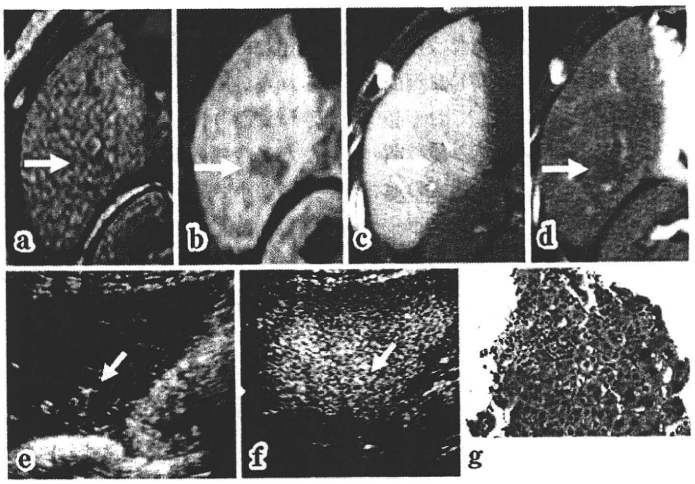

Fig. 4 Case 2, well-differentiated HCC measuring 14 mm in diameter.
(a) The nodule was visualized as an isointense nodule by T1-weighted MRI (arrow).
(b) The nodule was seen as a hypointense nodule on the hepatobiliary phase of Gd-EOB-DTPA enhanced MRI (arrow).
(c) The nodule was identified as an isodense nodule on CTAP (arrow).
(d) The nodule was depicted as a low-density nodule on CTHA (arrow).
(e) The nodule was visualized as a partially hyper-echogenic nodule on the vascular phase of CEUS (arrow).
(f) The nodule was seen as an iso-echogenic nodule on the post-vascular phase of CEUS (arrow).
(g) A tissue fragment was composed of areas of small and large cells. In small cells area, fibrotic tissue with inflammatory cells was seen in peripheral regions. This sample was diagnosed as a well-differentiated hepatocellular carcinoma.

た．一方，早期肝細胞癌を含む非典型的肝腫瘍ではCTAPで低ないし等吸収，CTHAでも低ないし等吸収を呈する場合が多く典型的肝細胞癌像とは対照的である[17]．今回の検討でも血管造影下CTをゴールド・スタンダードとし，CTAPで低吸収かつCTHAで高吸収が認められた場合を典型的肝細胞癌とした．さらにCTAPで低吸収ないし等吸収かつCTHAで高吸収が認められない結節，すなわち非多血性の肝結節を非典型的肝腫瘍としてそれぞれを検討の対象とした．

今回の検討では典型的肝細胞癌に対しては造影超音波，EOB造影MRIとも動脈相にて90％以上に多血性の所見が認められた．さらに検出能に関しては造影超音波後血管相で欠損および低エコーを合わせて85.1％であり，EOB造影MRI肝細胞造影相では低および高信号を合わせて98.3％となり，特にEOB造影MRIは血管造影下CTとほぼ同等の検出能が認められ，肝細胞癌検出に関しては非常に有用な低侵襲的検査法と考えられた．ただし今回の検討では造影超音波施行結節とEOB造影MRI施行結節の腫瘍径に有意差は認められないものの，対象結節数が異なっていることに検討の限界があると考えられる．また造影超音波後血管相の評価を造影剤注入10分後としており，さらに遅い時間で評価した場合には検出能が増加する可能性も考えられた．加えて，EOB造影MRI肝細胞造影相は肝細胞内へのEOB・プリモビスト®の取り込み能を，造影超音波後血管相ではKupffer細胞数の多寡をみており，同じ典型的肝細胞癌でも検査対象が異なることを確認しておく必要がある．特にEOB・プリモビスト®の取り込み能に関してはNaritaら[18]の報告の通り肝細胞癌の分化度の進行や胆汁産生能よりもOATP1 B3などのトランスポーターの活性の関与が大きいとされており，血管造影下CTにて典型的肝細胞癌と診断された肝結節でもEOB造影MRI肝細胞造影相で低信号とならない結節が存在したことが考えられた．

続いてCTHAで等～乏血性と診断された非典型的肝腫瘍の場合，腫瘍生検で全例が高分化型肝細胞癌であり，MRI T1強調像で50％が高信号で，さらにEOB造影MRIの肝細胞造影相は全例が低信号を呈し検出可能であった．CTAPで62.5％が等吸収で37.5％が低吸収すなわち門脈血流低下の所見が認められた．Bモード超音波では62.5％が低エコーで，37.5％に高エコーもしくはbright loopの所見が認められ，脂肪成分の存在が考えられた．造影超音波血管相では多血性の所見が25％，乏血性の所見が75％認められたが，後血管相では8結節中1結節（12.5％）のみが検出されただけであり，高分化型肝細胞癌の検出能に関してはEOB造影MRI肝細胞造影相が非常に有能な検査法であることが示唆された．なおCase 3のようにCTHAで非多血性と診断され，多血性の所見が造影超音波血管相で認められた症例が存在したのは，おそらく両者の時間・空間分解能の差によるものと考えられた．さらにCase 2は造影超音波血管相で不均一な濃染が認められ，高分化型肝細胞癌の脱分化の所見がとらえられていた可能性が考えられた．

組織学的に早期の高分化型肝細胞癌と診断されるものの中には，血行動態に変化のないものや，Kupffer細胞機能が維持されるものがある[19]．そのため結節内血流からみればCTAPで周囲肝に比べ等～やや低吸収（門脈血流同等～やや低下）あるいは一部低吸収（門脈血流一部欠損），CTHAで周囲肝と比較して低～等吸収（動脈血流低下～同等）あるいは一部高吸収（動脈血流一部増加）として認められ，またKupffer細胞機能からみればソナゾイド®造影後血管相において等エコーとして認められる[20]．これらの結節は超音波検査や単純CT・MRI，造影平衡相でのわずかな吸収値・信号値の変化から発見されるが，画像診断上は肝細胞癌の診断には至らないことも多いとされている．EOB・プリモビスト®はこれらの検査とは全く異なった生物学的特性を画像化しているため，血行動態やKupffer機能に変化がみられない結節でも検出できる可能性を持つことが報告されており[4]，今回の検討も同様の結果が認められた．ただし今回の検討では非典型例では典型例に比べて腫瘍径が小さく，各画像検査所見に影響を及ぼした可能性もある．

一般的にCTHAで非多血性肝細胞結節と診断された場合，鑑別には高分化型肝細胞癌をはじめ前癌・境界病変などが含まれる[17]が，さらに腫瘍生検や他の画像所見を総合してその診断をすすめることが重要であると考えられた．今回の検討では腫瘍生検が施行された非典型的肝腫瘍は全例がEOB造影MRI肝細胞造影相で検出され，かつ高分化型肝細胞癌であった．そのためEOB造影MRI肝細胞造影相で検出された肝結節は，動脈相で多血性の所見が認められていなくても造影超音波所見，患者の状態や肝予備能などを考慮して治療方針を決定していくことが重要であると考えられた．また経過観察する場合には，将来的に結節に多血性の変化が生じる可能性があるので，厳重なfollow upが必要と考えられた．

結　語

EOB造影MRIは肝細胞癌および非典型的肝腫瘍の検出に非常に有用な検査方法と考えられた．特にEOB造影MRI肝細胞造影相で検出された非典型的肝腫瘍は高分化肝細胞癌の可能性が高く，造影超音波所見や肝予備能などを総合的に判断して治療方針を決定していくことが重要であると考えられた．

文　献

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Diagnosis of Hepatocellular Carcinoma by Contrast-Enhanced Ultrasound with Perfluorobutane and Enhanced Magnetic Resonance Imaging with Gd-EOB-DTPA

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Aim：We evaluated the usefulness for the diagnosis of hepatocellular carcinoma (HCC) of a contrast-enhanced ultrasound (CEUS) method using a Kupffer cell-specific contrast agent, perfluorobutane, as well as enhanced magnetic resonance imaging (MRI) using a hepatocyte-specific contrast agent, gadolinium-ethoxybenzyl-diethlenetriamine pentaacetic acid (Gd-EOB-DTPA).

Materials and methods：We analyzed the results of enhanced MRI with Gd-EOB-DTPA and/or CEUS with perfluorobutane in the examination of 338 HCC nodules in 217 patients that were also studied by computed tomography (CT) during arterial portography (CTAP) and during hepatic arteriography (CTHA). A diagnosis of HCC was confirmed for 313 nodules in 199 patients by the presence of typical findings for HCC on CTAP and CTHA. For eight additional nodules in eight patients without the typical imaging findings on CTAP/CTHA, HCC was confirmed by the pathologic findings of the biopsied specimen.

Result：Three hundred fourteen patients underwent enhanced MRI with Gd-EOB-DTPA, while 206 patients underwent CEUS with perfluorobutane. For liver nodules possessing the typical imaging findings of HCC, 284 of 289 nodules (98.3%) were detected as hypo- or hyperintense nodule on the hepatobiliary phase of enhanced MRI with Gd-EOB-DTPA, and 154 of 181 nodules (85.1%) were detected as a defect or low-echogenic nodule on the post-vascular phase of CEUS with perfluorobutane. For HCC nodules lacking the typical imaging findings, all eight nodules were detected on the hepatobiliary phase of enhanced MRI with Gd-EOB-DTPA as hypointense nodules. In contrast, only one of the eight nodules (12.5%) could be detected on the post-vascular phase of CEUS with perfluorobutane. All eight nodules were confirmed to be well-differentiated HCC by histopathological examination.

Conclusions：Enhanced MRI with Gd-EOB-DTPA is an excellent imaging modality to detect HCC and is superior to CEUS with perfluorobutane. This imaging modality can detect well-differentiated HCC, even those lesions lacking the typical imaging findings. This method may not detect a subset of HCC with typical imaging findings, however, making the combination of different imaging modalities is important in diagnosis.

Key words: hepatocellular carcinoma　perfluorobutane　Gd-EOB-DTPA
contrast-enhanced ultrasound　magnetic resonance imaging

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肝癌診療のアルゴリズム 2010

肝癌の診断
(2) 肝癌スクリーニングにおける腫瘍マーカー

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Key words: 肝細胞癌, AFP, AFP-L3 分画, PIVKA-Ⅱ

要 旨

肝細胞癌の代表的な腫瘍マーカーには, AFP, AFP-L3 分画および PIVKA-Ⅱの3種類がある. AFP は特異度が低いが肝機能検査とリンクして評価すれば感度の高いマーカーとなり, AFP-L3 分画は陽性率が低いがきわめて特異性の高いマーカーである. また PIVKA-Ⅱは3者のなかでもっとも陽性率が高く, 特異度も高いマーカーである. 腫瘍マーカーは組み合わせによる評価が推奨され, とくに AFP-L3 分画と PIVKA-Ⅱの組み合わせが良好な結果であった. 悪性度評価では各マーカーの陽性率は進行度とともに上昇し, さらに AFP-L3 分画および PIVKA-Ⅱと切除標本の病理学的検討でもそれぞれのマーカーは生物学的悪性度の評価に適していることが判明した.

はじめに

現在, 肝細胞癌の代表的な腫瘍マーカーには AFP(α-fetoprotein)[1], AFP-L3 分画(レンズ豆結合性 AFP)[1,2] および PIVKA-Ⅱ(protein induced by vitamin K absence or antagonist-Ⅱ)[3] の3種類がある. 肝細胞癌の腫瘍マーカーに求められるのは, 1 存在診断(早期診断, 進展度診断(Stage 分類)], 2 質的診断(鑑別診断, 悪性度診断), 3 治療効果判定・再発診断の3点であるが, これらをすべて満たす腫瘍マーカーは存在しない. しかし, 3種類の腫瘍マーカーを組み合わせることによりその診断性は向上する.

本稿では, 肝細胞癌のスクリーニングにおける3種類の腫瘍マーカーについてその臨床的意義について概説する.

Ⅰ. AFP

- AFP は特異度が低くカットオフ値をどこに設定しているかにもよるが, 肝機能検査とリンクして評価すれば感度の高い腫瘍マーカーとなりうる.

AFP は分子量約7万で4%の糖を含む糖蛋白であり, 胎生期には生理的に体内に存在する. 組織学的に診断された慢性肝炎 71 例, 肝硬変 90 例, 異型結節 13 例, 早期肝細胞癌(高分化型肝細胞癌)14 例, 進行肝細胞癌(最大径 3 cm 以下, 中・低分化型肝細胞癌)82 例を用いたわれわれの検討では(図1), カットオフ値を 20 ng/ml とすると, 慢性肝炎, 肝硬変, 異型結節, 早期肝細胞癌および進行肝細胞癌の陽性率はそれ

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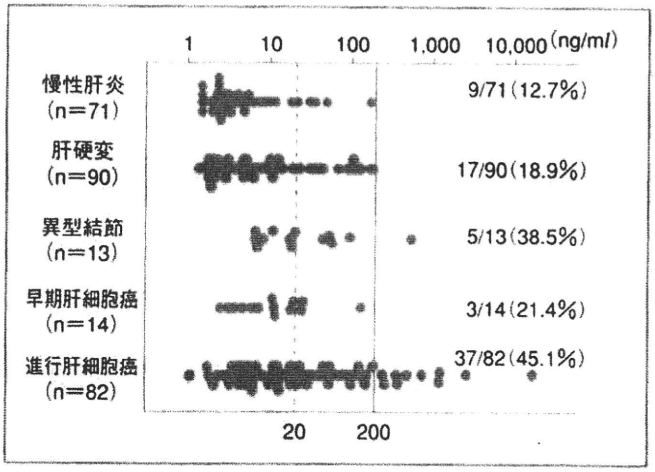

図1　慢性肝疾患におけるAFP

ぞれ 9/71 (12.7%), 17/90 (18.9%), 5/13 (38.5%), 3/14(21.4%)および37/82(45.1%)で,感度41.7%,特異度82.2%であった. 一方, カットオフ値を200 ng/ml とすると, 慢性肝炎, 肝硬変, 異型結節, 早期肝細胞癌および進行肝細胞癌の陽性率はそれぞれ0/71(0.0%), 0/90(0.0%), 1/13(7.7%)および0/14(0.0%), 10/82(12.2%)で, 感度10.4%, 特異度99.4%であった. カットオフ値を上げることによって特異度は増したが感度が著しく低下した.

このようにAFPは慢性肝疾患など種々の状態で増加するため, 軽度上昇の場合には, 肝細胞癌との鑑別にはほかの血清学的検査の動態および画像診断などを参考とする必要があり, かつ経過を追うことが重要である[1].

Ⅱ. AFP-L3分画

この項のポイント

- AFP-L3分画は小肝細胞癌での陽性率は低いがきわめて特異性の高いマーカーである.
- 最近, 高感度AFP-L3分画の測定が可能となり, とくにAFPが低値例での有用性が期待される.

AFP-L3分画はAFPの特異性を向上させることを目的として, AFPの複合型糖鎖の癌性変化の一つをとらえたものである. カットオフ値は10%を採用することが多く[4], 慢性肝炎, 肝硬変, 異型結節, 早期肝細胞癌および進行肝細胞癌の陽性率はそれぞれ0/71(0.0%), 1/90(1.1%), 0/13(0.0%), 0/14(0.0%)および18/82(22.0%)で, 感度18.8%, 特異度99.4%であった(**図2**). このように特異度は高いものの感度は低いため小さな肝細胞癌の発見は単独では限界があると考えられる. しかし, 小さくても上昇例では進行肝細胞癌と診断できる[5]. なお, AFP-L3分画は肝不全時に上昇することがあり, 解釈には注意が必要である[1].

最近, 高感度AFP-L3分画の測定が可能となった. 従来法ではAFP-L3分画の測定が可能なAFPは10 ng/ml 以上であったが, 高感度法では2 ng/ml 以上となった. 高感度AFP-L3分画の測定はとくにAFPが低値例で威力を発揮すると考えられ, AFPが20 ng/ml 未満でChild-Pugh AもしくはBの肝細胞癌270例の検討ではカットオフ値を5%とした場合, 感度41.5%, 特異度85.1%, 陽性的中率65.5%, 陰性的中率68.1%であった. さらにStage別の感度ではStage Ⅰ(n=89):34.8%, Stage Ⅱ(n=127):42.5%であった.

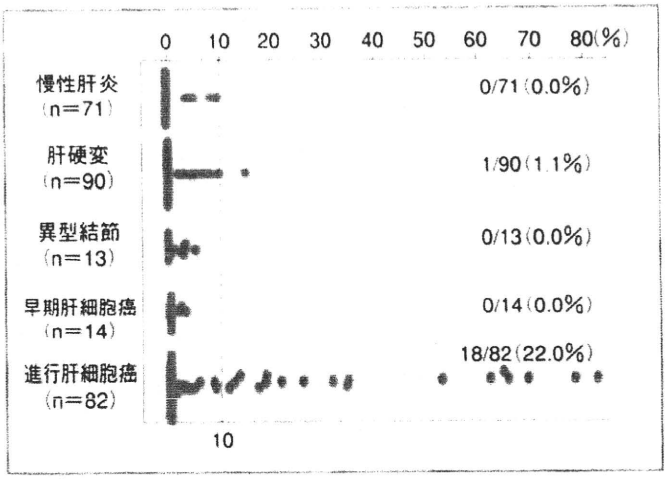

図2　慢性肝疾患における AFP-L3 分画

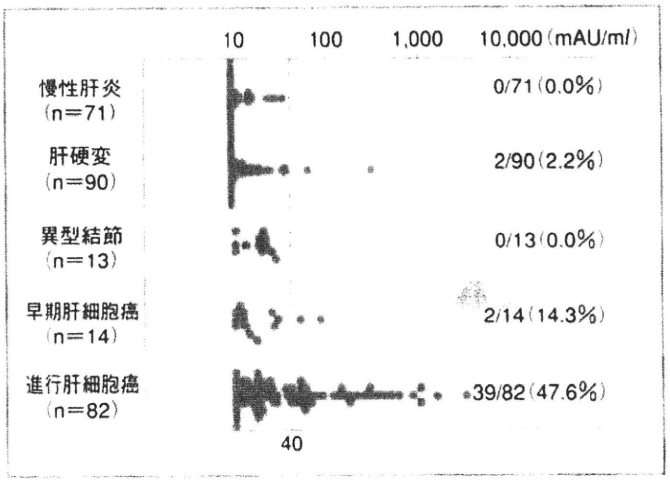

図3　慢性肝疾患における PIVKA-Ⅱ

Ⅲ. PIVKA-Ⅱ

- PIVKA-Ⅱは三つの腫瘍マーカーのなかでもっとも小肝細胞癌で陽性率が高く，特異度も高い．ただし，ワルファリンやセフェム系抗生物質を投与されたときに上昇することがあり，その解釈には注意を要する

PIVKA-Ⅱは des-γ-carboxy prothrombin (DCP)とも呼ばれ，凝固活性のない異常プロトロンビンである．カットオフ値は 40 mAU/ml で，慢性肝炎，肝硬変，異型結節，早期肝細胞癌および進行肝細胞癌の陽性率はそれぞれ 0/71(0.0%)，2/90(2.2%)，0/13(0.0%)，2/14(14.3%)および 39/82(47.6%)で，感度 42.7%，特異度 98.9%であった(**図3**)．単独の陽性率は三つの腫瘍マーカーのうちでもっとも高く，特異性も優れていた．しかし，早期肝細胞癌での陽性率は高いとはいえない．なお，PIVKA-Ⅱは黄疸が長期続いてビタミン K 欠乏をきたしたとき(閉塞性黄疸，肝内胆汁うっ滞など)やビ

タミンKサイクルを阻害するワルファリンや広域スペクトラムの抗生物質(セフェム系)を投与されたときに上昇することがあり，その解釈には注意を要する．

Ⅳ．腫瘍マーカーの組み合わせによる診断の有用性

この項のポイント

- 腫瘍マーカーは組み合わせによる評価が推奨される．

前述のように，単独での腫瘍マーカー測定での肝細胞癌診断には限界がある．各々の腫瘍マーカーの相関は弱いかもしくは認められないため，組み合わせての測定が勧められる[6]．3 cm以下の肝細胞癌での組み合わせ診断の結果を**表1**に示す．AFP-L3分画とPIVKA-Ⅱの組み合わせ測定が感度46.9%，特異度98.3%，陽性的中率93.8%，陰性的中率77.0%ともっとも良好であった．

なお前述の高感度AFP-L3分画ではAFPが20 ng/ml未満でChild-Pugh AもしくはBの肝細胞癌270例の検討ではカットオフ値を5%とした場合，PIVKA-Ⅱと組み合わせることにより感度63.7%，特異度77.3%，陽性的中率65.6%，陰性的中率75.7%であった．さらにStage別の感度ではStage Ⅰ(n=89)：44.9%，Stage Ⅱ(n=127)：71.7%であった．

Ⅴ．悪性度評価

この項のポイント

- AFPでのStageⅠとⅡを除き，各腫瘍マーカーの陽性率は進行とともに上昇した．

図4にAFP，AFP-L3分画，PIVKA-Ⅱの同時測定を行った肝細胞癌712例の進行度別の各腫瘍マーカーの陽性率を示した．AFPでのStageⅠとⅡを除き各腫瘍マーカーの陽性率は進行とともに上昇した．

腫瘍マーカーは肝細胞癌の生物学的悪性度の評価にも適している．肝切除例に対する病理学的検討では浸潤性発育，被膜浸潤，隔壁形成，門脈侵襲，肝静脈侵襲を有する例で有意にAFP-L3分画陽性の癌が多く[7]**(表2)**，また被膜浸潤，隔壁形成，門脈侵襲，肝静脈侵襲，肝内転移を有する例で有意にPIVKA-Ⅱ高値の癌が多かった**(表3)**．

表1　各腫瘍マーカーの組み合わせによる陽性率(n=270)

	AFP のみ	AFP-L3 のみ	PIVKA-II のみ	AFP+ AFP-L3	AFP+ PIVKA-II	AFP-L3+ PIVKA-II
Overall accuracy*	67.8%	70.7%	78.9%	68.1%	74.8%	80.0%
感度**	41.7%	18.8%	42.7%	42.7%	63.5%	46.9%
特異度*1	82.2%	99.4%	98.9%	82.2%	81.0%	98.3%
PPV*4	46.3%	74.7%	95.3%	56.9%	64.9%	93.8%
NPV*5	71.9%	69.5%	75.8%	72.2%	80.1%	77.0%

TP：true-positive, TN：true-negative, FP：false-positive, FN：false-negative

*：Overall accuracy：TP+TN/TP+FP+TN+FN, **：Sensitivity：TP/TP+FN

*1：Spesificity：TN/FP+TN, *4：Positive predictive value：TP/TP+FP

*5：Negative predictive value：TN/FN+TN

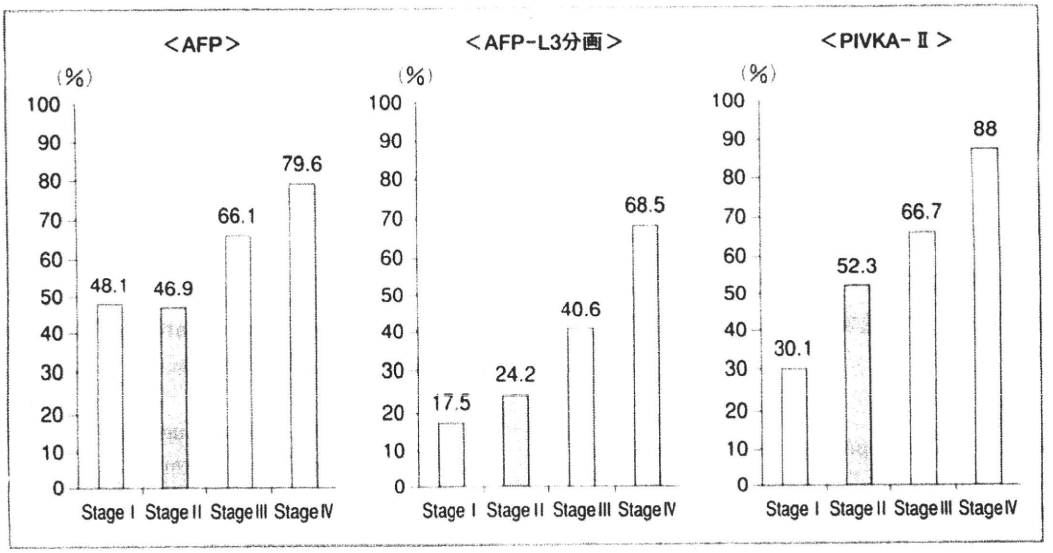

図4　肝細胞癌の進行度と陽性率(n=712)

表2　AFP-L3分画と組織学的所見(n=111)

	AFP-L3分画陽性(≧10%) (n=33)	AFP-L3分画陰性(<10%) (n=78)	*p* value
発育様式(膨張性/浸潤性)	27/6	76/2	0.0122
被膜形成(+/−)	16/17	43/35	NS*
被膜浸潤(+/−)	6/27	4/74	0.0295
隔壁形成(+/−)	18/15	25/53	0.0444
門脈侵襲(+/−)	6/27	3/75	0.0317
肝静脈侵襲(+/−)	4/29	1/77	0.0438
胆管侵襲(+/−)	1/32	0/78	NS*
肝内転移(+/−)	4/29	7/71	NS*
分化度(高/中・低)	6/27	23/55	NS*

*：NS：not significant

表3　PIVKA-Ⅱと組織学的所見(n=134)

	PIVKA-Ⅱ陽性(≧40 mAU/m*l*) (n=71)	PIVKA-Ⅱ陰性(<40 mAU/m*l*) (n=63)	*p* value
発育様式(膨張性/浸潤性)	64/7	59/4	NS*
被膜形成(+/−)	44/27	37/26	NS*
被膜浸潤(+/−)	15/56	5/58	0.0403
隔壁形成(+/−)	37/34	20/43	0.0228
門脈侵襲(+/−)	19/52	3/60	0.0008
肝静脈侵襲(+/−)	8/63	1/62	0.0358
胆管侵襲(+/−)	3/68	0/63	NS*
肝内転移(+/−)	14/57	3/60	0.0099
分化度(高/中・低)	12/59	15/48	NS*

*：NS：not significant

おわりに

肝細胞癌スクリーニングにおけるAFP, AFP-L3分画およびPIVKA-Ⅱにつき概説した．3種類のマーカーを単独の独立したマーカーとして用いず，複数測定しその組み合わせによって肝細胞癌の存在を考慮することが重要である．

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Summary

Clinical Utility of Tumor Markers for Screening of Hepatocellular Carcinoma

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Three tumor markers, alpha-fetoprotein (AFP), *lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP) are currently used for the diagnosis of hepatocellular carcinoma (HCC) in Japanese clinical practice. Despite its low specificity, AFP is highly sensitive for HCC when evaluated along with liver function test values. In contrast, AFP-L3 has a low degree of sensitivity but has a very high level of specificity for HCC. DCP has both high sensitivity and specificity for HCC. Recently, the combination of some of these tumor markers has been recommended for more accurate diagnosis of HCC. We found that the combination of AFP-L3 and DCP provided accurate diagnosis of HCC. Positive rates increased in association with the increase in HCC stage for all three tumor markers. The elevation of AFP-L3 or DCP strongly reflected several pathologic features of advanced HCC.

Key words: hepatocellular carcinoma, alpha-fetoprotein, *lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein, des-gamma-carboxy prothrombin

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Outcome in Partial Early Virologic Responders to Combination Therapy With Peginterferon and Ribavirin in Patients Infected With Hepatitis C Virus Genotype 1b

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The course and outcome in patients infected with hepatitis C virus (HCV) genotype 1b with partial early virologic response during combination therapy with peginterferon and ribavirin, in whom serum HCV RNA is detectable but has decreased by more than 2 $\log_{10}$ 12 weeks after the start of the therapy, has not been elucidated sufficiently. The outcome in this group of patients was investigated. Serum HCV RNA levels was measured every 4 weeks in 149 patients with HCV genotype 1b infection who underwent combination therapy for 48 weeks. In patients with partial early virologic response, the time point when serum HCV RNA became undetectable as well as the final virologic response to treatment was determined. Sixty-three patients (42.3%) had partial early virologic response. The time when serum HCV RNA became undetectable ranged from 16 to 48 weeks after the start of therapy. Serum HCV RNA remained detectable in 17 patients. The rates of sustained virologic response decreased with the delay of the time when serum HCV RNA became undetectable; sustained virologic responder was not found in patients in whom HCV RNA was still detectable at 24 weeks after the start of treatment. The degree of decrease in serum HCV RNA levels at 12 weeks corresponded to the rate of sustained virologic response in partial early virologic responders. The outcome of partial early virologic responders varied greatly, and close monitoring of serum HCV RNA is required for predicting the outcome of treatment in these patients. ***J. Med. Virol. 83:101–107, 2011.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis C; peginterferon and ribavirin; response-guided therapy; partial early virologic response; real-time PCR

INTRODUCTION

The current standard antiviral therapy for patients with chronic hepatitis C is combination therapy with peginterferon (PEG-IFN) and ribavirin [Ghany et al., 2009]. Although the current treatment regimen has increased markedly the rate of sustained virologic response, indicating eradication of hepatitis C virus (HCV), only approximately 50% of patients infected with HCV genotype 1 achieved sustained virologic response. The response of HCV during combination therapy, that is, the changes in serum HCV RNA levels after the start of therapy, has been shown to be important for predicting the treatment outcome [Zeuzem et al., 2001; Buti et al., 2002; Berg et al., 2003; Lee and Ferenci, 2008], and this has led to increased emphasis on "response-guided therapy" [Lee and Ferenci, 2008; Marcellin and Rizzetto, 2008]. An early virologic response, defined as either undetectable serum HCV RNA or HCV RNA levels decreased by >2.0 $\log_{10}$ from the pretreatment level 12 weeks after the start of therapy, is the most important predictor of sustained virologic response in patients infected with HCV genotype 1 [Fried et al., 2002; Davis et al., 2003]. Therefore, an early virologic response is a pivotal decision criterion for treatment guidelines [Ghany et al., 2009].

Early virologic response has been subdivided into two categories, that is, complete early virologic response and partial early virologic response [Davis et al., 2003; Marcellin et al., 2007; Ghany et al., 2009]. A complete early virologic response is defined as undetectable HCV RNA in the serum 12 weeks after the start of

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combination therapy. A partial early virologic response is defined as a decrease in serum HCV RNA levels by more than 2 $\log_{10}$ from the pretreatment levels, without becoming undetectable. Patients who achieve a complete early virologic response have a high rate of sustained virologic response. In contrast, the outcome of patients with a partial early virologic response has not been documented fully. The present study examined the outcome for patients infected with HCV genotype 1 who had a partial early virologic response 12 weeks after the start of combination therapy.

PATIENTS AND METHODS

Between September, 2005 and June, 2007, a total of 228 patients with chronic hepatitis C underwent antiviral combination therapy with PEG-IFN and ribavirin for HCV infection at the Ogaki Municipal Hospital, Japan according to the Japanese treatment guidelines for chronic HCV infection [Izumi et al., 2010]. Among these patients, 152 were infected with HCV genotype 1b and had pretreatment HCV RNA level >5.0 $\log_{10}$ IU/ml by a quantitative Amplicor assay (Amplicor HCV monitor test, version 2.0, Roche Molecular Systems, Pleasanton, CA: detection limit, 615 IU/ml). Patients with HCV genotype 1a were not included because this genotype is not found in the general Japanese population. Of the 152 patients, 149 agreed to have their stored serum samples examined in this study. Table I shows the baseline characteristics of the 149 study patients. They comprised 72 (48.3%) men and 77 (51.7%) women with a mean age of 58.0 ± 9.2 years. Although 38 patients (25.5%) had a history of previous antiviral monotherapy with conventional IFN or combination therapy with conventional IFN and ribavirin, none of the patients had been treated with PEG-IFN and ribavirin previously. All patients had pretreatment HCV RNA levels >5.0 $\log_{10}$ IU/ml, because the administration of ribavirin in addition to PEG-IFN is not allowed by the Japanese National Medical Insurance in patients with pretreatment HCV RNA levels ≤5.0 $\log_{10}$ IU/ml. The average pretreatment HCV RNA level was 6.21 ± 0.55 $\log_{10}$ IU/ml. Among 136 patients who underwent a pretreatment liver biopsy, the grade of liver fibrosis according to the METAVIR score [The French METAVIR Cooperative Study Group, 1994] was F0 in 8 patients (5.9%), F1 in 79 patients (58.1%), F2 in 33 patients (24.3%), and F3 in 16 patients (11.7%), respectively. None of the patients were coinfected with hepatitis B virus or the human immunodeficiency virus. None of the patients had a history of alcohol abuse or intravenous drug use. For combination therapy with PEG-IFN and ribavirin, all patients received PEG-IFN alpha-2b (Pegintron; Schering-Plough, Tokyo, Japan) weekly and ribavirin (Rebetol; Schering-Plough) daily according to the manufacturer's recommendations. The doses of PEG-IFN and ribavirin were adjusted by patient body weight. Briefly, patients weighing ≤45 kg were given 60 μg of PEG-IFN alpha-2b once a week, those weighing >45 and ≤60 kg were given 80 μg, those weighing >60 and ≤75 kg were given 100 μg, those weighing >75 and ≤90 kg were given 120 μg, and those weighing >90 kg were given 150 μg. Patients weighing ≤60 kg were given 600 mg of ribavirin per day, those weighing >60 and ≤80 kg were given 800 mg of ribavirin per day, and those weighing >80 kg were given 1,000 mg of ribavirin per day. All patients were scheduled to undergo the standard 48-week treatment regimen. The extension of treatment duration from 48 to 72 weeks in patients in whom serum HCV RNA became undetectable between 12 and 24 weeks after the start of the therapy, and discontinuation of treatment at 24 weeks in patients in whom HCV RNA remained detectable at 24 weeks was not considered in the present study, because the benefits of these modifications of the treatment regimen were not established in Japan at

TABLE I. Baseline Characteristics of All Study Patients

	All patients (n = 149)
Age (years)	58.0 ± 9.2
Sex female/male)	77 (51.7)/72 (48.3)
History of interferon therapy (naive/retreatment)	111 (74.5)/38 (25.5)
Body weight (kg)	58.9 ± 10.1
Alanine aminotransferase (IU/L)	62.0 ± 27.8
Aspartate aminotransferase (IU/L)	52.3 ± 40.2
Gamma-glutamyl transpeptidase (IU)	52.3 ± 57.9
Alkaline phosphatase (IU/L)	258.4 ± 84.6
Albumin (g/dl)	4.16 + 0.35
Total bilirubin (mg/dl)	0.69 ± 0.29
White blood cell count (/μl)	5,138 ± 1,321
Hemoglobin (g/dl)	14.2 ± 1.4
Platelet count ($\times 10^3$/μl)	16.8 ± 5.2
Liver histology activity (A0/AI/A2/A3)[a]	3 (2.2)/76 (55.9)/45 (33.1)/12 (8.8)
Liver histology fibrosis (F0/FI/F2/F3)[a]	8 (5.9)/79 (58.1)/33 (24.3)/16 (11.7)
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	6.21 ± 0.55
Reduction of the peginterferon dose	41 (27.5)
Reduction of the ribavirin dose	64 (43.0)

HCV, hepatitis C virus.
Percentages are shown in parentheses.
[a]Liver biopsy was not performed in 13 patients.

the time in the present study was conducted. Dose modification or discontinuation of PEG-IFN or ribavirin was based on the manufacturer's recommendations. A sustained virologic response was defined as undetectable serum HCV RNA at 24 weeks after the end of therapy. Relapse was defined as detectable serum HCV RNA during the period between the end of treatment and 24 weeks thereafter, despite the undetectable serum HCV RNA at the end of treatment. No response was defined as detectable serum HCV RNA throughout the treatment period and thereafter (null or partial response) [Ghany et al., 2009].

After a patient provided informed consent, serum samples were obtained during the regular visit to the hospital just prior to starting treatment, and every 4 weeks during treatment and during the 24-week follow-up period. Serum samples were stored at −80°C until analysis. HCV RNA levels were measured in the serum samples using a real-time PCR-based quantitation method for HCV (HCV Cobas AmpliPrep/Cobas TaqMan System; Roche Molecular Systems; lower limit of quantitation, 1.7 $\log_{10}$ IU/ml; lower limit of detection, 1.0 $\log_{10}$ IU/ml) [Colucci et al., 2007; Pittaluga et al., 2008]. Patients were divided into three groups based on the HCV RNA levels in the serum sample that was obtained 12 weeks after the start of the combination therapy. Patients with undetectable serum HCV RNA at 12 weeks were considered to have a complete early virologic response. Patients with detectable serum HCV RNA at 12 weeks that had decreased by more than 2 $\log_{10}$ compared to the pretreatment levels were considered to have a partial early virologic response. The remaining patients whose serum HCV RNA at 12 weeks had not decreased by more than 2 $\log_{10}$ in compared to the pretreatment levels were considered to have a non-early virologic response. The clinical course and eventual outcome of partial early virologic responders were analyzed.

Statistical Analyses

Quantitative values are reported as the means ± SD. Between-group differences were analyzed by a chi-squared test. The differences in quantitative values between two groups were analyzed by a Mann–Whitney *U*-test. The times from the start of the therapy to the point when the serum HCV RNA became undetectable were compared by a log-rank test. Univariate and multivariate analyses using a logistic regression model were performed to identify factors that predict a sustained virologic response, including age, sex, body weight, serum alanine aminotransferase activity, serum aspartate aminotransferase activity, serum gamma-glutamyl transpeptidase levels, serum alkaline phosphatase values, serum albumin levels, total serum bilirubin values, white blood cell counts, hemoglobin, platelet counts, hepatitis activity grade (A0 and A1 vs. A2 and A3), liver fibrosis grade (F0 and F1 vs. F2 and F3), pretreatment HCV RNA levels, reduction in PEG-IFN dose, reduction in ribavirin dose, complete early virologic response, and partial early virologic response. All *P*-values were two-tailed, and $P < 0.05$ was considered as statistically significant.

The study protocol was approved by the institutional review board and complied with the Helsinki Declaration. All patients provided written informed consent to use their clinical data and serum samples before initiating this study.

RESULTS

Although the doses of PEG-IFN were reduced in 42 patients (27.5%) and the ribavirin doses were reduced in 74 patients (49.7%) during therapy, all patients adhered more than 80% to both PEG-IFN and ribavirin for the entire treatment period, and all patients more than 90% adhered to both drugs for the first 12 weeks of therapy (until an early virologic response was determined). No patients discontinued therapy. Based on the serum HCV RNA levels at 12 weeks after the start of the combination therapy, 63 of 149 patients (42.3%) were categorized as having a partial early virologic response. Of the remaining 86 patients, 63 (42.3%) and 23 (15.4%) were considered to have a complete early virologic response and non-early virologic response, respectively. The baseline characteristics of the patients with a complete, partial, and those without early virologic response are summarized in Table II. There were no significant differences between the baseline characteristics of the partial early virologic responders and the complete early virologic responders or patients without early virologic response, including clinical background, baseline laboratory data, liver histology, pretreatment HCV RNA levels, and the rate of patients with dose reductions of PEG-IFN or ribavirin during the treatment period.

As regards to the final outcome, 67 of 149 patients (45%) achieved a sustained virologic response, 41 patients (27.5%) relapsed, and the remaining 41 patients (27.5%) did not respond to treatment. Table III summarizes the outcomes of the combination therapy for the complete and partial early virologic responders, and patients without early virologic response. The rate of a sustained virologic response was significantly higher in complete early virologic responders than in partial early virologic responders ($P = 0.0139$). All but one patient who did not achieve early virologic response showed no response. In univariate and multivariate analyses for factors that predict a sustained virologic response (Table IV), patient age, serum alkaline phosphatase levels, serum albumin levels, platelet counts, fibrosis grade of the liver, and complete and partial early virologic responses were significantly associated with a sustained virologic response by a univariate analysis. Multivariate analysis showed that only a complete early virologic response was associated with a sustained virologic response.

The rate that the serum HCV RNA became undetectable after 12 weeks of treatment in partial early virologic responders is shown in Figure 1. The serum HCV RNA in partial early virologic responders became

TABLE II. Baseline Characteristics of Patients With Complete and Partial Early Virologic Response, and Patients Without Early Virologic Response

	Complete early virologic response (n = 63)	Partial early virologic response (n = 63)	Lack of early virologic response (n = 23)
Age (years)	57.2 ± 10.0	58.4 ± 8.9	59.9 ± 7.3
Sex (female/male)	30 (47.6)/33 (52.4)	36 (52.1)/27 (42.9)	11 (47.8)/12 (57.2)
History of interferon therapy (naive/retreatment)	48 (76.2)/15 (23.8)	45 (71.4)/18 (28.6)	18 (78.3)/5 (21.7)
Body weight (kg)	59.0 ± 10.1	59.3 ± 10.8	57.4 ± 8.6
Alanine aminotransferase (IU/L)	68.3 ± 66.4	55.8 ± 41.6	60.1 ± 28.8
Aspartate aminotransferase (IU/L)	55.2 ± 44.9	47.7 ± 32.7	55.9 ± 26.6
Gamma-glutamyl transpeptidase (1U)	51.1 ± 64.5	46.6 ± 51.5	70.2 ± 53.9
Alkaline phosphatase (1U/L)	246.3 ± 73.3	258.9 ± 79.3	290.4 ± 117.1
Albumin (g/dl)	4.20 ± 0.34	4.17 ± 0.31	4.02 ± 0.48
Total bilirubin (mg/dl)	0.64 ± 0.28	0.70 ± 0.28	0.79 ± 0.34
White blood cell count (/μl)	5,111 ± 1,162	5,293 ± 1,359	4,795 ± 1,576
Hemoglobin (g/dl)	14.0 ± 1.3	14.3 ± 1.4	14.3 ± 1.6
Platelet count ($\times 10^3$/μl)	173 ± 51	170 ± 51	148 ± 50
Liver histology activity (A0–1/A2–3)[a]	33 (58.9)/23 (41.1)	36 (61.0)/23 (39.0)	10 (47.6)/11 (52.4)
Liver histology fibrosis (F0–1/F2–3)[a]	39 (69.6)/17 (30.4)	38 (64.4)/21 (35.6)	10 (47.6)/11 (52.4)
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	6.25 ± 0.58	6.21 ± 0.55	6.15 ± 0.55
Reduction of the peg interferon dose	17 (27.0)	18 (28.6)	6 (26.1)
Reduction of the ribavirin dose	27 (42.9)	26(41.3)	11 (47.8)

HCV, hepatitis C virus.
Percentages are shown in parentheses.
[a]Liver biopsy was not performed in 7 complete, 4 partial, and 2 non-early virologic responders.

TABLE III. Comparison of the Treatment Outcome Between Complete and Partial Early Virologic Responses, and Lack of Early Virologic Response

	Sustained virologic response (n = 67)	Relapse (n = 41)	No response (n = 41)
Complete early virologic response (n = 63)	50 (79.4)	11 (17.4)	2 (3.2)
Partial early virologic response (n = 63)	17 (27.0)	29 (46.0)	17 (27.0)
Lack of early virologic response (n = 23)	0	1 (4.3)	22 (95.6)

TABLE IV. Univariate and Multivariate Analyses for Factors Associated With Non-Response to Combination Therapy With Peginterferon and Ribavirin

	Univariate analysis	Multivariate analysis	Odds ratio (95% CI
Age (years)	0.0218	0.5389	
Sex (female/male)	0.1503	—	
Body weight (kg)	0.1720	—	
Alanine aminotransferase (IU/L)	0.9982	—	
Aspartate aminotransferase (IU/L)	0.2671	—	
Gamma-glutamyl transpeptidase (IU)	0.1985	—	
Alkaline phosphatase (IU/L)	0.0112	0.7110	
Albumin (g/dl)	0.0057	0.7464	
Total bilirubin (mg/dl)	0.5332	—	
While blood cell count (/μl)	0.1074	—	
Hemoglobin (g/dl)	0.2923	—	
Platelet count ($\times 10^3$/μl)	0.0065	0.6926	
Liver histology activity (A0–1/A2–3)[a]	0.2716	—	
Liver histology fibrosis (F0–1/F2–3)[a]	0.0450	0.5981	
Pretreatment HCV RNA concentration ($\times 10^3$ IU/ml)	0.4979	—	
Reduction of the peginterferon dose	0.5966	—	
Reduction of the ribavirin dose	0.1131	—	
Complete early virologic response	<0.0001	<0.0001	23.265 (6.606–106.038)
Partial early virologic response[b]	0.0133	0.1087	

HCV, hepatitis C virus.
[a]Liver biopsy was not performed in 13 patient.
[b]In comparison between partial early virologic responders and non-early virologic responders.

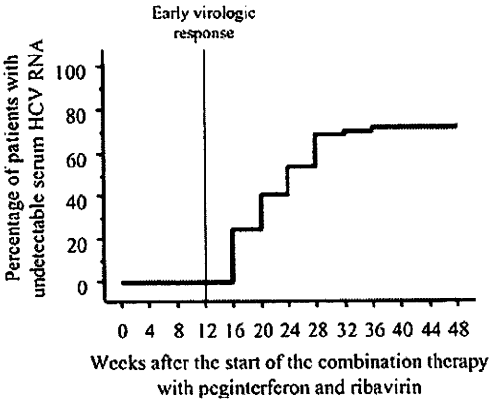

Fig. 1. Percentage of partial early virologic responders with undetectable serum HCV RNA based on the time after the start of the combination therapy with peginterferon and ribavirin.

undetectable in 16 of 63 patients (25.4%) at 16 weeks, in 10 patients (15.9%) at 20 weeks, in 8 patients (12.7%) at 24 weeks, in 9 patients (14.3%) at 28 weeks, and in 1 patient (1.6%) each at 32, 36, and 48 weeks after initiating combination therapy. In the remaining 17 patients (27%), the HCV RNA remained detectable throughout the treatment period. For the final outcome in 63 patients with a partial early virologic response, 17 patients (27%) achieved a sustained virologic response, 29 patients (46%) relapsed, and the remaining 17 patients (27%) did not respond to treatment. In contrast, 50 patients (79.4%) achieved a sustained virologic response and 13 patients (20.6%) relapsed among 63 patients with a complete early virologic response, and no patients achieved a sustained virologic response, one patient (4.3%) relapsed, and the remaining 22 patients (95.7%) did not respond among 23 patients without an early virologic response. Figure 2 shows the rate of sustained virologic responses in partial early virologic responders based on the time point when the serum HCV RNA became undetectable. Sustained virologic responses were achieved in 10 of 16 patients (62.5%) in whom serum HCV RNA became undetectable at 16 weeks after the start of the therapy, in 5 of 10 patients

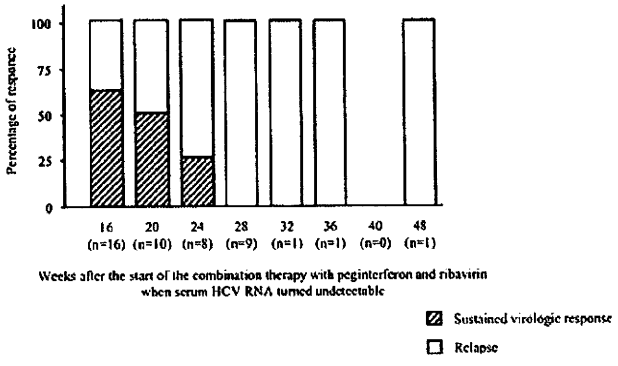

Fig. 2. Percentage of partial early virologic responders with a sustained virologic response based on the time period when the serum HCV RNA became undetectable after the start of combination therapy.

(50%) with undetectable HCV RNA at 20 weeks, and in 2 of 8 patients (25%) with undetectable HCV RNA at 24 weeks. The remaining 12 patients whose serum HCV RNA became undetectable later than 24 weeks after the start of the therapy did not achieve a sustained virologic response.

When partial early virologic responders were stratified according to the decrease in HCV RNA levels at 12 weeks after starting therapy compared to the pretreatment levels, this decrease was $>4 \log_{10}$ in 44 patients (69.8%), between 3 and $4 \log_{10}$ in 10 patients (15.9%), and between 2 and $3 \log_{10}$ in 9 patients (14.3%). The percentages of patients with undetectable serum HCV RNA at each week after starting treatment are shown for each group in Figure 3. The serum HCV RNA in patients with more than a $4 \log_{10}$ decrease at 12 weeks became undetectable significantly earlier than either in those with a decrease of $3-4 \log_{10}$ ($P = 0.0020$) or in those with a decrease of $\leq 3 \log_{10}$ ($P < 0.0001$). The final outcome of therapy is summarized in Table V based on the decrease in HCV RNA levels at 12 weeks after the start of the therapy. The rates of sustained virologic responses were 36.4% in patients with a $>4 \log_{10}$ decrease, 10.0% in those with a $3-4 \log_{10}$ decrease, and 0% in those with a $\leq 3 \log_{10}$ decrease.

DISCUSSION

The serum HCV RNA at 12 weeks after initiating combination therapy has been reported to predict the final outcome of treatment in patients infected with HCV genotype 1. Among the three classifications based on serum HCV RNA at 12 weeks, a complete early virologic response is a strong predictor of a sustained virologic response, and a non-early virologic response is a strong predictor of the absence of a sustained virologic response. However, the outcome in patients with a partial early virologic response, which is an intermediate response between complete and non-early virologic responses, has not been studied. Therefore, it is unclear whether a partial early virologic response is a favorable response.

The present study investigated the course of patients with a partial early virologic response at 12 weeks after starting combination therapy. The serum HCV RNA levels were measured using a real-time PCR-based assay that is more sensitive than other assays reportedly. The high sensitivity of this assay is important because determining the response during treatment with a less sensitive assay may reduce the ability to predict the final treatment outcome [Berg et al., 2009; Matsuura et al., 2009]. A large portion of patients in the present study had a mild METAVIR score (<F2), which is not typically a candidate for antiviral therapy in Western countries. The decision to treat these patients is determined on a case-by-case basis according to the Japanese guidelines. Because the age of Japanese patients with HCV infection is generally higher than that of patients in Western countries and because the risk of developing hepatocellular carcinoma in Japanese

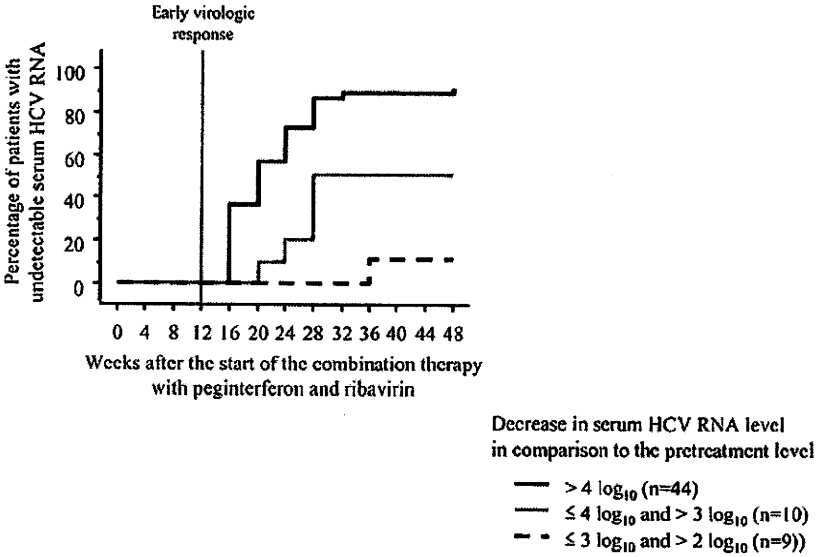

Fig. 3. Percentages of partial early virologic responders whose serum HCV RNA became undetectable based on the time period after the start of the combination therapy. Patients are stratified by the degree of decrease in the serum HCV RNA at 12 weeks compared to the pretreatment levels.

patients with mild liver fibrosis is reported to increase with age [Miki et al., 2008], antiviral therapy is considered for many patients with HCV with mild fibrosis.

The rate of a sustained virologic response for partial early virologic responders was between those of the complete early virologic responders and those without early virologic response. The sustained virologic response rate of partial early virologic responders in the present study was consistent with previous studies in patients who underwent the standard 48-week treatment regimen [Berg et al., 2006; Ferenci et al., 2006; Sanchez-Tapias et al., 2006]. The rate of a sustained virologic response was far lower than that of complete early virologic responders, indicating that a partial early virologic response is an unfavorable response, unless the treatment duration is potentially extended up to 72 weeks.

The changes in serum HCV RNA levels in patients with a partial early virologic response were largely heterogenous. The time interval between the start of therapy and when the serum HCV RNA became undetectable ranged between 16 and 48 weeks, and the serum HCV RNA did not become undetectable in 17 patients during the treatment period. None of the patients whose serum HCV RNA remained positive until 24 weeks after starting treatment achieved a sustained virologic response. This finding is consistent with previous reports, and discontinuing therapy should be considered for these patients [Ghany et al., 2009]. The rate of a sustained virologic response decreased as the time interval until the serum HCV RNA became undetectable increased, even in patients who achieved undetectable HCV RNA before 24 weeks. Although the current guidelines of the American Association for the Study of Liver Diseases recommend measuring serum HCV RNA at 24 weeks after the start of the therapy for patients who attained a partial early virologic response at 12 weeks, more frequent measurements of serum HCV RNA will improve the accuracy for predicting the final outcome.

The degree of decrease in HCV RNA levels at 12 weeks after starting therapy correlated with the time at which the serum HCV RNA became undetectable and the final outcome in partial early virologic responders. A greater decrease in HCV RNA levels at 12 weeks resulted in an earlier disappearance of HCV RNA from the serum and a higher rate of sustained virologic responses. In addition, no patient with a decrease of less than 3 $\log_{10}$ at 12 weeks achieved a sustained virologic response as a final outcome. The lack of a decrease in HCV RNA levels of more than 3 $\log_{10}$ at 12 weeks after initiating therapy indicates that therapy should be discontinued potentially. This potential discontinuation of therapy should be considered for patients without early virologic

TABLE V. Treatment Outcome in Patients With Partial Early Virologic Response According to the Decrease in Serum HCV RNA at 12 Weeks After the Start of Combination Therapy in Comparison to the Pretreatment Level

	Sustained virologic response (n = 17)	Relapse (n = 29)	No response (n = 17)
>4 $\log_{10}$ (n = 44)	16 (36.4)	24 (54.5)	4 (9.1)
≤4 $\log_{10}$ and >3 $\log_{10}$ (n = 10)	1 (10.0)	4 (40.0)	5 (50.0)
≤3 $\log_{10}$ and >2 $\log_{10}$ (n = 9)	0	1 (11.1)	8 (88.9)

response and even patients with a partial early virologic response, unless extending the treatment duration is considered.

There are several limitations of the present study. This study included patients who had received IFN-based antiviral therapy previously (25.5%), although they did not have a history of combination therapy with PEG-IFN and ribavirin. However, this study did not find any differences in the rate of retreated patients who achieved a partial, complete early virologic response, or those who did not achieve early virologic response. In addition, all study patients had undergone a 48-week treatment regimen and no patients had extended their treatment duration to 72 weeks or discontinued therapy at 24 weeks. Therefore, the outcomes of the partial early virologic responders in the present study were solely obtained from patients who underwent the standard 48-week treatment regimen of the combination therapy. As indicated in the results, extending or discontinuing treatment in partial early virologic responders based on when the serum HCV RNA becomes undetectable and the decrease in HCV RNA levels at 12 weeks compared to the pretreatment levels may improve the treatment outcome.

In conclusion, a partial early virologic response results in heterogeneous final outcomes. Although Marcellin et al. [2007] noted that patients with early virologic responses are highly heterogeneous and suggested that this patient group should be further subclassified into complete and partial early virologic responders, partial early virologic responders are still a heterogeneous subgroup. It is preferable to evaluate the serum HCV RNA repeatedly in this patient subpopulation in order to make a more accurate prediction of the final response. A detailed evaluation of the decrease in HCV RNA levels at 12 weeks after starting therapy compared to the pretreatment levels will allow more accurate prediction of the final response.

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