硬変(primary biliary cirrhosis：PBC)に矛盾しない(compatible)組織像を示すものでAMAが陽性のもの，あるいは，③組織学的検索の機会はないが，AMAが陽性で，しかも臨床像および経過からPBCと考えられるものもPBCと診断される．

どのような治療がありますか

▶この病気を完全に治す薬はまだできていませんが，ウルソ®という薬に進行を抑える働きがあることがわかり，現在，世界中でこの病気に対して使われています．副作用としては下痢，腹痛などの胃腸障害をきたすことがありますが，多くの方ではほとんど認められず，長期にわたって服用することができます．

▶最近，高脂血症の治療に使われているベザフィブラートが，ウルソ®の効果がみられない人にも有効であることが明らかにされ，使用されています．

確立された根治的治療法はないため，対症的治療にとどまるが，病期・病態に応じた対策が必要である．初期から中期では免疫反応による炎症と胆汁うっ滞に対して，胆汁うっ滞が持続している時点では胆汁うっ滞に基づく症状と合併症に対して，肝硬変に至ると肝硬変に伴う門脈圧亢進症(食道静脈瘤)，肝癌，腹水，脳症などの合併症に対する治療が必要となる．

ウルソデオキシコール酸(UDCA，ウルソ®)はALP，γ-GTPなどの血液生化学検査値の改善をもたらすのみでなく，線維化の進行，死亡/移植までの期間も延長することが複数のランダム化比較試験で証明されている．そのため，現在第一選択薬とされ，病初期から投与される．1日量で通常600 mg，効果が少なければ900 mgまで増量できる．90%の症例では胆道系酵素の低下がみられるが，進行した症例では効果が期待できない．ウルソ®の効果がよくない場合は，高脂血症薬のひとつであるベザフィブラートが用いられる．作用機序はUDCAと異なるためUDCAとの併用が勧められるが，PBCに対しての保険適用はない．PBC-AIH(autoimmune hepatitis：自己免疫性肝炎)オーバーラップ症候群で肝炎の病態が強い場合や，自己免疫性胆管炎(autoimmune cholangitis：AIC)の初期には副腎皮質ホルモンが併用される．

症候性PBCでは胆汁うっ滞に基づく症状，特に瘙痒，高脂血症とビタミンDの吸収障害による骨粗鬆症に対する治療は重要である．門脈圧亢進症をきたしやすく，胃・食道静脈瘤は肝硬変に至る前に出現することがあるので，定期的な観察が必要である．進行例では肝癌の併発にも留意する．肝硬変に進展した場合は，腹水，肝性脳症などの合併症への対応が必要となる．病期が進むと，内科的治療に限界が生じ，肝移植の適応となる．脳死肝移植が少ないわが国では，すでに生体部分肝移植が定着しており，移植成績も欧米の脳死肝移植例と同様に良好である．

日常生活ではどのような注意が必要ですか

▶「肝硬変」という文字が病名に付けられていますが，症状がない，あるいは軽い時期に診断されることがほとんどですので，多くの方は肝硬変には至っていません．症状がない方は普通の方と同様に生活して構いません．

▶実際に肝硬変に至っている方，黄疸が出現している方は，その時期に応じた養生法がありますので，主治医に尋ねてください．

病名は「肝硬変」となっているが，現在は早期に診断することができるようになり，またUDCAが進展を遅らせる効果もあることから，現在診断されている大部分(70～80%)の患者は肝硬変には至っていない．無症候性PBCでは

日常生活に特別の制限はない．症候性PBCでは症候，今後起こりうる合併症，肝予備能の低下に応じた生活指導，食事指導が必要となる．

黄疸の増強に伴い，倦怠感，瘙痒感が増強し，骨粗鬆症が進み，痛みや骨折の危険性が増すので，予防・注意が必要である．さらに進行すると，黄疸の増強とともに腹水の貯留や脳症がみられるようになる．自覚症状としては現れないが，食道・胃静脈瘤が潜在性に進行し，突然の破裂をきたすこともある．また，肝癌も併発する可能性もある．定期的に診察を受け，それぞれの時期に応じた療養が大切である．

急変した場合どうしたらよいでしょうか

この病気は，進行していない限り肝硬変に至ってなければ急変することはまずありません．

急変する可能性は，唯一食道・胃静脈瘤の破裂です．そのため日頃，食道・胃静脈瘤の存在を消化管内視鏡検査で確認しておく必要があります．

ウイルスマーカー陰性の肝障害で突然の食道静脈瘤をきたした場合は，本疾患の可能性を考えて検索する．本疾患では，肝硬変に至らずとも門脈圧亢進症をきたしやすい．

妊娠や出産に影響はありますか？

無症候性PBCの患者は妊娠を避ける必要はない．症候性PBCでは，瘙痒感，黄疸の増強や食道静脈瘤の悪化・破裂の危険性が増す可能性がある．

一般的に肝硬変患者では妊娠が成立しにくいとされているが，病期の早いPBCの場合は一般人と変わらず妊娠が成立する．一方，症候性PBCでは，黄疸の増強や食道静脈瘤の悪化・破裂の危険性が増すことが報告されており，妊娠は控えたほうがよい．妊娠している可能性のある婦人または特に妊娠の最初の時期ではウルソ®，ベザフィブラートのいずれも投与は中止する．第3期では，胆汁うっ滞に対して必要であればウルソ®は投与可能である．妊娠によりPBCの胆汁うっ滞が増悪し，瘙痒感が増強する可能性がある．進行した，あるいはすでに肝硬変となっているPBC患者では，ほかの肝硬変患者同様のモニターを必要とし，妊娠第2期では食道・胃静脈瘤のスクリーニングのため上部消化管内視鏡検査の施行が望まれる．

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適切な栄養投与はすべての医療の基本である。しかし卒前の栄養学教育は乏しく、臨床で先輩の話を鵜呑みにするのも少々危うい。ならば正しい知識を「塾」で学ぼう。本書では、栄養管理のエキスパートが練習問題（症例）をもとに、Q&A方式で「目からウロコ」のクリニカルパールを伝授する。資格認定試験にも役立つ「栄養管理に必要な生化学の知識」も収録。栄養学が、そしてベッドサイドが、好きになる1冊！

Influence of *ITPA* Polymorphisms on Decreases of Hemoglobin During Treatment with Pegylated Interferon, Ribavirin, and Telaprevir

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Polymorphisms of the inosine triphosphatase (*ITPA*) gene influence anemia during pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy, but their effects during triple therapy with PEG-IFN, RBV, and telaprevir are not known. Triple therapy for 12 weeks, followed by PEG-IFN and RBV for 12 weeks, was given to 49 patients with RBV-sensitive (CC at rs1127354) and 12 with RBV-resistant (CA/AA) *ITPA* genotypes who had been infected with hepatitis C virus (HCV) of genotype 1. Decreases in hemoglobin levels were greater in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$), as well as at the end of treatment (−2.9 ± 1.1 vs. −2.0 ± 0.86, $P = 0.013$). Risk factors for hemoglobin <11.0 g/dL at week 4 were female gender, age >50 years, body mass index (BMI) <23, and CC at rs1127354 by multivariate analysis. RBV dose during the first 12 weeks was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$), but the total RBV dose was no different between them (49 ± 17% and 54 ± 18% of the target, $P = 0.531$). Sustained virological response (SVR) was achieved in 70% and 64% of them, respectively ($P = 0.724$). *Conclusion: ITPA* polymorphism influences hemoglobin levels during triple therapy, particularly during the first 12 weeks while telaprevir is given. With careful monitoring of anemia and prompt adjustment of RBV dose, SVR can be achieved comparably frequently between patients with CC and CA/AA genotypes. (HEPATOLOGY 2011;53:415-421)

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; HCV, hepatitis C virus; IFN, interferon; IL28B, *interleukin 28B;* ITPA, *inosine triphosphatase; PEG-IFN, pegylated interferon; RBV, ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virological response.*

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Worldwide, 123 million people are estimated to have been infected with hepatitis C virus (HCV),[1] and ≈30% of them develop fatal liver disease such as cirrhosis and hepatocellular carcinoma.[2,3] Currently, the standard of care therapy for patients infected with HCV is pegylated interferon (PEG-IFN) and ribavirin (RBV) for 48 weeks.[4-6] However, the combined treatment can induce a sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after treatment completion, in at most 50% of patients infected with HCV-1, the genotype most prevalent and least responsive to IFN-based therapies.

Recently, Fellay et al.[7] reported that polymorphisms of the inosine triphosphatase (*ITPA*) gene in chromosome 20 (20p13) influence RBV-induced anemia in a genome-wide association study (GWAS). Single nucleotide polymorphism (SNP) at rs1127354 for proline-to-threonine substitution (P32T) in the second of eight

exons in the *ITPA* gene, as well as that at rs7270101 in the second intron, affects the expression of ITPA.[8-11] Patients infected with HCV-1 carrying the CC genotype at rs1127354 are more prone to develop anemia than those with CA/AA genotypes during the combination therapy, and the decrease in hemoglobin is greater in patients with the AA than AC/CC genotypes at rs7270101.[7] Their observations have been extended to many patients in a large-scale trial with pegIFN-α-2a on Caucasian and African Americans,[12] as well as in the Japanese receiving PEG-IFN-α-2b and RBV who were infected with HCV-1.[13]

For improving SVR in HCV-1 patients, protease inhibitors have been added to the standard treatment with PEG-IFN and RBV, and increased SVR by ≈20%.[14-16] However, such a gain in efficacy is not without trade-offs, represented by aggravation of anemia. Early decreases in hemoglobin levels during the triple therapy reach 4 g/dL, and they exceed ≈3.0 g/dL in the standard treatment.[14,15] Because there have been no reports focusing on the influence of *ITPA* genotypes on anemia developing in patients during triple therapy, hemoglobin levels were followed in 61 Japanese patients with HCV-1 who had received it. The results were correlated with polymorphisms at rs1127354 in the *ITPA* gene because the Japanese are monoallelic at rs7270101 and have the AA genotype exclusively.[11]

Patients and Methods

Study Cohort. This retrospective cohort study was performed in 61 patients with chronic hepatitis C who met the following inclusion and exclusion criteria. Inclusion criteria were: (1) diagnosed with chronic hepatitis C; (2) HCV-1 confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels ≥5.0 log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (4) Japanese aged from 20 to 65 years at the entry; and (5) body weight between ≥40 kg and ≤120 kg at the time of registration. Exclusion criteria were: (1) decompensated liver cirrhosis; (2) hepatitis B surface antigen in serum; (3) hepatocellular carcinoma or its history; (4) autoimmune hepatitis, alcoholic liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis C; (5) chronic renal disease or creatinine clearance ≤50 mL/min at the baseline; (6) hemoglobin ≤12 g/dL, neutrophil ≤1,500/mm^3 or platelet ≤100,000/mm^3 at baseline.

Of the 61 patients, 44 (72%) had received IFN-based treatment before. Relapse occurred in 29 (47%) and the remaining 15 (25%) did not respond (null-responders). All patients gave consent for analysis of SNPs in *ITPA* and interleukin 28 (*IL28B*) genes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Toranomon Hospital. Written informed consent was obtained from each patient.

Triple Treatment with PEG-IFN-α-2b, RBV, and Telaprevir. Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan), 750 mg, was administered 3 times a day at an 8-hour (q8) interval after each meal. Pegylated-IFN-α-2b (PEG-Intron, Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.32-1.71 μg/kg) once a week. RBV (Rebetol, Schering Plough) 200-600 mg was administered after breakfast and dinner. The RBV dose was adjusted by body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ≈≤80 kg; and 1,000 mg for ≥80 kg. The triple therapy with PEG-IFN-α-2b, RBV, and telaprevir was continued for 12 weeks, and then switched to PEG-IFN-α-2b and RBV for an additional 12 weeks. It was withdrawn when hemoglobin levels decreased <8.5 g/dL. After the therapy was completed or discontinued, patients were followed for 24 weeks for SVR.

The RBV dose was cut by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1,000 mg) when hemoglobin decreased <12 g/dL, and by another 200 mg when it was below <10 g/dL. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dL at baseline and those in whom it decreased by 1 g/dL to <13 g/dL within a week. PEG-IFN dose was reduced by one-half when the leukocyte count decreased <1,500/mm^3, neutrophil count <750/mm^3, or platelet count <80 × 10^3/mm^3; PEG-IFN was withdrawn when they decreased <1,000/mm^3, 500/mm^3, or 50 × 10^3/mm^3, respectively.

The triple therapy was withdrawn or stopped temporarily when hemoglobin decreased <8.5 g/dL. In patients in whom hemoglobin increased ≥8.5 g/dL within 2 weeks after the withdrawal, treatment was resumed with PEG-IFN and RBV 200 mg. A reduction of telaprevir (MP-424) dose was not permitted. It was discontinued when severe side effects appeared, whereas PEG-IFN and RBV were continued. Growth factors were not used for elevating hemoglobin levels.

Determination of ITPA Genotypes. *ITPA* (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described.[17,18]

Statistical Analyses. Continuous variables between groups were compared by the Mann-Whitney test (*U* test), and discontinuous variables by the chi-square test

Table 1. Baseline Characteristics of the 61 Patients Infected with HCV-1 Who Received Triple Therapy with Pegylated-Interferon, Ribavirin, and Telaprevir

		ITPA Genotypes at rs1127354	
	Total	CC	CA + AA
Demographic data			
Number	61	49	12
Sex (male/female)	34/27	28/21	6/6
Age (years)	56 (23-65)	55 (23-65)	58 (28-62)
Body weight (kg)	61.5 (41.0-92.9)	61.5 (41.0-92.9)	62.1 (44.4-81.1)
Body mass index (kg/m^2)	22.6 (17.6-32.4)	22.2 (17.6-32.4)	22.9 (17.8-26.5)
Genotypes of the *IL28B* gene			
rs8099917 (for 59 patients)			
(TT/TG + GG)	33/26	27/21	6/7
rs12979860 (for 57 patients)			
(CC/CT + TT)	30/27	36/22	4/5
Laboratory data			
Hemoglobin (g/dL)	14.4 (12.5-16.6)	14.4 (12.5-16.6)	14.2 (12.8-16.3)
Platelets (x 10^4/mm^3)	17.8 (9.1-33.8)	17.7 (9.1-33.8)	19.5 (13.1-31.6)
Albumin (g/dL)	3.9 (3.2-4.6)	3.9 (3.2-4.6)	3.9 (3.5-4.1)
Alanine aminotransferase (U/L)	39 (12-175)	41 (12-175)	28 (17-57)
Aspartate aminotransferase (U/L)	32 (15-137)	35 (15-137)	28 (20-35)
HCV RNA (log IU/mL)	6.7 (5.1-7.6)	6.8 (5.7-7.6)	6.6 (5.1-7.5)
HCV genotype 1a/1b	1/60	1/48	0/12
Previous IFN-based treatment			
Treatment naïve	17	12 (24%)	5 (42%)
Relapsed	29	23 (47%)	6 (50%)
Null response	15	14 (29%)	1 (8%)

Data are median values (range) or n.

and Fisher's exact test. Kaplan-Meier analysis and the log-rank test were applied to estimate and compare decreases of RBV dose between groups. Factors evaluated for influence on hemoglobin decrease by univariate analysis were: sex; age; body mass index (BMI); body weight; hemoglobin levels; initial PEG-IFN and RBV doses; amino acid substitutions in the HCV core protein; number of amino acid substitutions in the interferon sensitivity determining region; and *IL28B* polymorphisms (at rs8099917 and rs12979860). Factors associated with a decrease in hemoglobin levels ($P < 0.10$) were assessed by multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence interval (CI) was determined. All analyses were performed using SPSS software (SPSS II v. 11.0, Chicago, IL), and a P-value < 0.05 was considered significant.

Results

Triple Therapy in Patients with HCV-1 Infection. Baseline characteristics of the 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 in the *ITPA* gene are compared in Table 1. They all were infected with HCV-1. There were no significant differences between them, except that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were higher in patients with CC than CA/AA genotypes ($P = 0.041$ and $P = 0.008$, respectively). Overall, *IL28B* genotypes resistant to PEG-IFN and RBV, TT/TG at rs8099917, and CC/CT at rs12979860 were rather frequent, and possessed by 44% and 47%, respectively, of the patients. This was due to inclusion of 15 nonresponders to previous IFN-based therapies, corresponding to 25% of the 61 patients studied, most of whom (14/15 [93%]) possessed IFN-resistant genotypes (TT/TG and CC/CT). Six of them had low hemoglobin levels (<13 g/dL) at baseline and were started with an RBV dose decreased by 200 mg; they included five with CC and one with CA genotypes of the *ITPA* gene.

Modification of RBV Dose During Triple Therapy. RBV dose was reduced by ≥200 mg in all 61 patients studied during triple therapy because hemoglobin had decreased <12.0 g/dL in them. During the first 12 weeks of therapy while telaprevir was given, the proportion of patients receiving the full RBV dose differed between those with CC and CA/AA genotypes (Fig. 1). RBV dose reduction was started earlier in the 49 patients with CC than the 12 with CA/AA genotypes (2.6 ± 1.3 vs. 4.8 ± 3.1 weeks after the start, respectively, $P = 0.010$). Thus, during the first 12 weeks with telaprevir the RBV dose was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the next 12

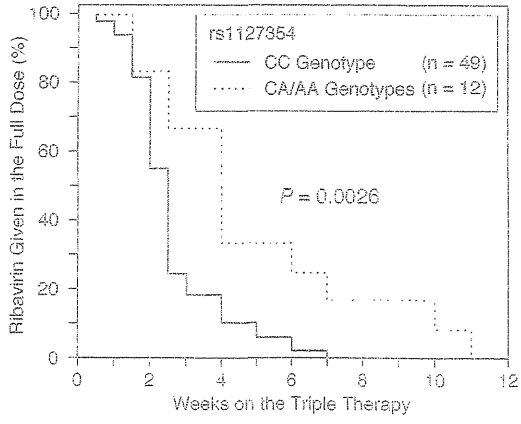

Fig. 1. Patients who received the full ribavirin dose during 12 weeks on triple therapy. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared.

weeks without telaprevir, in contrast, the RBV dose was somewhat larger in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). The total RBV dose during 24 weeks on therapy was comparable between the 49 patients with CC and the 12 with CA/AA genotypes (49 ± 17% vs. 54 ± 18%, $P = 0.531$). In patients with the CC genotype, the RBV dose was no different between those who achieved SVR and those who did not (50 ± 18% vs. 47 ± 13%, $P = 0.728$). The RBV dose did not differ either in patients with CA/AA genotypes with and without SVR (57 ± 17% vs. 48 ± 20%, $P = 0.368$).

The total dose of PEG-IFN was comparable among 49 patients with CC and 12 with CA/AA genotypes (87 ± 23% vs. 86 ± 20% of the target, $P = 0.488$). The total telaprevir dose was no different either between them (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$). Telaprevir was discontinued in 10 of the 49 (20%) patients with CC and 5 of the 12 (42%) with CA/AA genotypes ($P = 0.147$).

Decreases in Hemoglobin Levels During Triple Therapy. Figure 2 compares decreases in hemoglobin levels between 49 patients with CC and 12 with CA/AA genotypes of the *ITPA* gene. Data of six patients were omitted because the triple therapy was withdrawn 4-10 weeks after the start, including five with CC and one with CA genotype. Hemoglobin decreased more in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$). During week 8 through 12, hemoglobin reached the nadir of approximately −4 g/dL both in patients with CC and CA/AA genotypes. Thereafter, differences in hemoglobin decrease started to widen between patients with CC and CA/AA genotypes and were significant at week 20 (−3.0 ± 1.2 vs. −2.4 ± 0.88 g/dL, $P = 0.048$) and week 24 (−2.9 ± 1.1 vs. −2.0 ± 0.85 g/dL, $P = 0.013$).

SVR was achieved by 35 (71%) of the 49 patients with CC and 8 (67%) of the 12 with CA/AA genotypes ($P = 0.736$). Hemoglobin levels did not differ between them 24 weeks after the completion of triple therapy (−0.57 ± 1.1 vs. −0.17 ± 0.87 g/dL, $P = 0.271$). Of the 32 patients with TT genotype of the *IL28B* gene at rs8099917, 30 (94%) gained SVR, more frequently than 10 of the 26 (38%) with TG/GG genotypes ($P < 0.001$). Likewise, 29 of the 30 (97%) patients with CC genotype at rs12979860 achieved SVR, more frequently than 11 of the 27 (41%) with CT/TT genotypes ($P < 0.001$).

Factors Influencing Decreases in Hemoglobin Levels. Hemoglobin decreased <11 g/dL at week 4 during the triple therapy in 27 of the 61 (44%) patients. Factors for hemoglobin <11.0 g/dL were female gender, age >50 years, body weight <60 kg, BMI <23, and baseline hemoglobin <15 g/dL, as well as the CC genotype of the *ITPA* gene, in the univariate analysis (Table 2). Of them, female gender, age >50 years, BMI <23, and the CC genotype remained significant in the multivariate analysis. Hemoglobin levels lowered <8.5 g/dL during the triple therapy in 13 of the 61 (21%) patients. Factors for hemoglobin <8.5 g/dL were female gender, age >60 years, body weight <60 kg, BMI <23, and baseline hemoglobin <14 g/dL in the univariate analysis (Table 3). Of

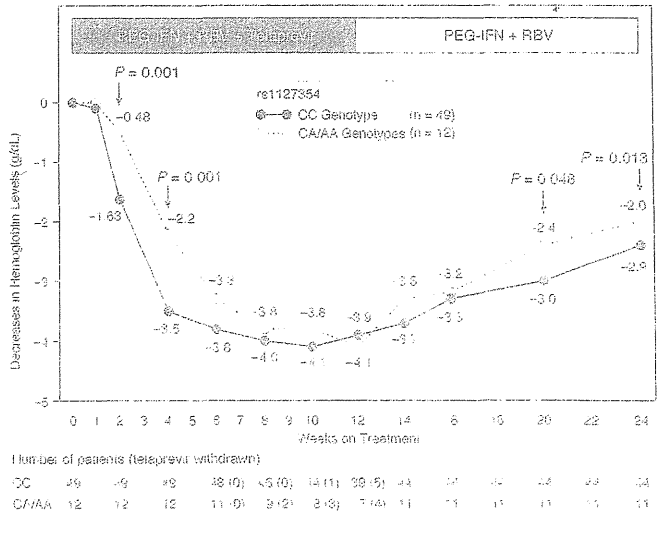

Fig. 2. Decreases in hemoglobin levels during triple therapy with telaprevir, PEG-IFN, and RBV. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared. Patients evaluated at each timepoint are indicated below, with the number of patients in whom telaprevir was withdrawn (PEG-IFN and RBV continued) in parentheses.

Table 2. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Low Hemoglobin Levels (< 11.0 g/dL) at Week 4 of Triple Therapy

	Univariate Analysis		Multivariate Analysis	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	14.3 (4.1-50.0)	< 0.001	29.41 (3.8-250.0)	0.001
Age (> 50 years)	4.3 (1.0-17.5)	0.030	7.3 (1.1-47.6)	0.039
Body weight (< 60 kg)	11.5 (3.4-38.2)	< 0.001		
Body mass index (< 23)	8.4 (2.6-27.1)	< 0.001	17.2 (2.6-112.0)	0.003
Hemoglobin (< 15g/dL)	14.2 (3.5-57.4)	< 0.001		
ITPA gene (CC genotype)		0.062	36.8 (2.5-550.2)	0.009

Abbreviations: OR, odds ratio; CI, confidence level.

them, only age and body weight remained significant in the multivariate analysis.

Discussion

Anemia is a substantial risk in the standard of care therapy with PEG-IFN and RBV.[4-6] Triphosphorylated RBV accumulates in erythrocytes of patients who receive RBV, increasingly with RBV dose and duration, and causes oxidative damage to erythrocyte membranes toward extravascular hemolysis by the reticuloendothelial system.[19,20] Inosine triphosphate accumulates also in erythrocytes of individuals who have mutations in the *ITPA* gene, and results in benign red-cell enzymopathy.[8] The expression of *ITPA* is genetically controlled and reduced in individuals who have point mutations in the *ITPA* gene.[8-11] As another achievement of GWAS in hepatology,[21] in the wake of polymorphisms of the *IL28B* gene that influence the response to PEG-IFN and RBV,[22-24] polymorphisms in the *ITPA* gene has been reported to influence anemia caused by RBV.[7] How inosine triphosphate protects erythrocytes from hemolysis caused by RBV needs to be sorted out by *in vivo* and *in vitro* experiments. Inosine triphosphate may prohibit the accumulation of RBV in erythrocytes, or rather, it might act directly toward prohibition of hemolysis.

In the present study, 61 patients infected with HCV-1 received triple therapy with PEG-IFN, RBV, and telaprevir in the first 12 weeks followed by PEG-IFN and RBV in the second 12 weeks. Then the RBV dose and hemoglobin were compared between patients with CC and CA/AA genotypes in the *ITPA* gene. Two polymorphisms in the *ITPA* gene, in close linkage disequilibrium with an r^2 value of 0.65,[7] have been recognized in Caucasians (rs1127354 and rs7270107); the respective CA/AA and AC/CC genotypes decrease the activity of inosine triphosphatase and protect against anemia induced by RBV.[7,12] Because the Japanese are monoallelic at rs7270107 and possess the AA genotype exclusively,[11,25] only polymorphisms at rs1127354 were examined.

Of the 61 patients, 49 possessed the RBV-sensitive CC genotype and the remaining 12 had RBV-resistant CA/AA genotypes. Hemoglobin levels decreased both in patients with CC and CA/AA genotypes. They lowered ≈4 g/dL during weeks 8-12 on the triple therapy with telaprevir, and increased thereafter (Fig. 2). Between the two groups of patients, differences in hemoglobin decrease were greatest at week 4 (1.3 g/dL), as in the standard treatment with PEG-IFN and RBV.[7,12,13]

When anemia and other side effects occurred, doses of RBV, PEG-IFN, and telaprevir were modified. Of the 61 patients studied, 27 (44%) were women and most of them were in old age. Beyond 50 years of age, women are less responsive than men to the standard treatment with PEG-IFN and RBV, probably because estrogens with an antifibrotic potential decrease after menopause.[26] Stringent precautions had to be taken, therefore, by reducing the RBV dose in the patients in whom hemoglobin levels decreased <12 g/dL, rather than the conventional threshold of <10 g/dL.

Reductions of RBV dose due to anemia in patients who receive PEG-IFN and RBV are influenced by *ITPA* polymorphisms.[12] Also, in patients who had received the triple therapy the RBV dose had to be reduced more in

Table 3. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Very Low Hemoglobin Levels (<8.5 g/dL) During Triple Therapy

	Univariate Analysis		Multivariate Analysis	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	6.1 (1.5-25.1)	0.007		
Age (>60 years)	6.8 (1.8-26.0)	0.004	10.1 (1.9-53.9)	0.007
Body weight (<60 kg)	23.8 (2.9-200.0)	<0.001	33.3 (3.4-333.3)	0.003
Body mass index (<23)	14.1 (1.7-125.0)	0.001		
Hemoglobin (<14 g/dL)	4.3 (1.2-15.6)	0.023		

Abbreviations: OR, odds ratio; CI, confidence level.

patients with CC than CA/AA genotypes during the first 12 weeks while they received telaprevir (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the second 12 weeks off telaprevir, the RBV dose was somewhat greater in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). Thus, the total RBV dose during 24 weeks of therapy was comparable between patients with CC and CA/AA genotypes (51 ± 15% and 57 ± 18%, $P = 0.724$). Likewise, the total dose of PEG-IFN (87 ± 23% vs. 86 ± 20% of the target, $P = 0.806$), as well as that of telaprevir (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$), was no different between patients with CC and CA/AA genotypes. SVR was achieved comparably frequently in them (71% vs. 67%, $P = 0.736$).

Decreases in hemoglobin levels during the first 12 week were similar between the current triple therapy cohort and previous patients receiving PEG-IFN and RBV.[12,13] The conservative hemoglobin levels chosen for RBV dose reduction may be a possible confounding factor on the impact of *ITPA* variants in anemia, which would have been greater should the RBV dose not be reduced in patients with RBV-sensitive CC genotypes.

ITPA polymorphisms at rs1127354 were associated with RBV-induced anemia in Japanese patients, without involvement of those at rs7270107 reported in Caucasian and African-American patients.[13] Thus, *ITPA* polymorphisms at rs1127354 would play a major role in protecting patients from RBV-induced anemia. CC/CA genotypes at rs1127354 occurs in 6% of the Caucasian population, much less often in the Oriental population, at 16%.[25,27] Although AC/CC genotypes at rs7270107 occurs in 13% of Caucasians, they do not exist in Orientals.[11,25] Obviously, different polymorphisms need to be examined in patients of distinct ethnicities when the influence on RBV-induced anemia is to be evaluated.

In confirmation of our previous report,[28] the triple therapy achieved SVR more frequently in patients with CC than CT/TT genotypes of *IL28* at rs12979860 (96% vs. 41%, $P < 0.001$). About two-thirds of studied patients accomplished SVR with the triple treatment, although one-fourth of them were nonresponders to previous IFN-based treatments; they are known to respond poorly to repeated treatments. This would lend further support to the efficacy of triple therapy being higher than treatment with pegylated IFN and RBV.

There are strong points in this study. First, *ITPA* polymorphisms influence RBV-induced anemia in the triple therapy. Second, polymorphisms at rs1127350, without involvement of those at rs7270107, protect against RBV-induced anemia. Third, the triple therapy can be applied with high efficacy by careful monitoring of hemoglobin and prompt modification of RBV dose. There are weak points in this study as well. First, it was a retrospective cohort study conducted in a small size of patients, especially those with CA/AA genotypes at rs1127350, and included null-responders to previous IFN-based therapies; the real impact of *ITPA* polymorphisms on RBV-induced anemia may have been obscured. Second, the study was conducted in Japanese patients, and the results may or may not be extended to patients of different ethnicities with distinct genetic backgrounds. Hopefully, the results presented herein will promote future studies in which the influence of the *ITPA* polymorphism on RBV-induced anemia will be pursued in larger scale and on patients of various ethnicities around the world.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Efficacy and Safety of Combination Therapy of Natural Human Interferon Beta and Ribavirin in Chronic Hepatitis C patients

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Abstract

Objective The aim of this study was to evaluate the efficacy and safety of combination therapy of natural human interferon-beta and ribavirin for patients for whom prior interferon therapy was discontinued due to depression induced by interferon-alpha.

Methods Inclusion criteria were as follows; 1) HCV-genotype 1b, 2) serum HCV RNA level of ≥100 KIU/mL, 3) stopping the prior interferon-alpha monotherapy or combination therapy of interferon-alpha and ribavirin due to the appearance of depression. A total of 14 were enrolled in this prospective cohort study. The treatment period of combination therapy was 48 weeks. Depression states, reflected by Beck depression inventories and Hamilton depression rating scale, were assessed during combination therapy. Nonparametric procedures were employed for the analysis of background features of the patients with sustained virological response (SVR) and without SVR. A p value of <0.05 was considered to indicate a significant difference.

Results Five of 14 patients (37.5%) had SVR by the intention to treat analysis. The SVR rate in patients who showed negative HCV RNA at 12 and 24 weeks after the initiation of combination therapy was 100% (4/4) and 83.3% (5/6), respectively. All of the patients continued the combination therapy owing to disappearance of severely adverse events contained the exacerbation of depression. Combination therapy did not yield a statistical difference in Beck depression inventories and Hamilton depression rating scale.

Conclusion The combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

Key words: chronic hepatitis C, depression, natural interferon-beta, ribavirin, HCV genotype 1b

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Introduction

The combination therapy of peginterferon-alpha and ribavirin has been widely recommended as a first choice for chronic hepatitis C patients with high virus-load (1-5). However, one big problem of the combination therapy is the treatment-related side effect (6, 7). In particular, physicians in charge tend to avoid the combination therapy of peginterferon-alpha and ribavirin for chronic hepatitis C patients with depression or interferon (IFN)-reduced depression.

IFN-beta-related side effects are mild and few compared to therapy of IFN-alpha (6-8). In particular, IFN-beta-induced mental disorders are mild compared to those induced by IFN-alpha (9). Moreover, IFN-beta could be given to elderly patients aged ≥70 years because of the mild side effects (10). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with genotype 1b and a high virus load (11, 12). The combination therapy of IFN-

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beta and ribavirin has the possibility to show the strong effect for hepatitis C virus (HCV) and mild side effects originating from the treatment (13-15). We have reported that the combination of IFN-beta plus ribavirin therapy is effective and safety for HCV patients with high virus load and depressive state (14). However, the previous study was retrospective and a prospective study is necessary to evaluate the efficacy and safety of combination therapy of IFN-beta and ribavirin for HCV patients with high virus load and depressive state.

Thus, in the present study, we performed a prospective study to examine the efficacy and safety of combination therapy of IFN-beta and ribavirin in HCV genotype 1b patients who had stopped the IFN therapy due to depression induced by IFN-alpha. At the same time, depression states, reflected by Beck depression inventories (BDI) and Hamilton depression rating scale (Ham-D), were assessed during combination therapy (16, 17).

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of $\geq$100 KIU/mL before treatment; 3) stopping of IFN-alpha therapy due to depression appearance during the prior IFN-alpha treatment; 4) Ham-D of <18; 5) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 6) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 7) white blood cell (WBC) >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/mL; follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy of IFN-beta and ribavirin as well as the potential adverse reactions to each patient and informed consent was obtained from each patient. This study was approved by the Human Ethics Review Committee of Toranomon Hospital.

From December 2007 to May 2008, 14 HCV patients were enrolled in this prospective cohort study at the study hospital. A sustained virological response (SVR) was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (18).

Laboratory investigation

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80℃ until analysis. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (19). Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (18). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (20). The core protein of HCV-1b was determined by the previous report (21). Next, the genetic variations near the IL28B gene (rs8099917), reported as the pretreatment predictors of treatment efficacy and clinical outcome, were investigated (22-26). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Clinical evaluation and biochemical and hematological tests were performed at 1, 2, and 4 weeks in the first month after the initiation of combination therapy. After that, these evaluations were done at monthly intervals. The patients were followed by both physicians of hepatology and psychiatry.

Combination therapy of IFN-beta and ribavirin

Treatment was provided for 48 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) by six times a week for 4 weeks, followed by three times a week for 44 weeks. The total dose was 936MU. Ribavirin (Rebetol, MSD KK., Tokyo, Japan) was given at the dose prescribed based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for $\leq$60 kg, 800 mg for >60 kg and $\leq$80 kg, and 1,000 mg for >80 kg).

Evaluation of the psychic state

The psychiatrist in charge evaluated the scores of BDI and Ham-D prospectively. BDI shows the subjective symptom of the depressive patients and Ham-D shows the objective evaluation by the psychiatrist. Scores on the BDI were divided the following; severe, 29-63; moderate, 20-28; mild, 14-19; and minimal, 0-13. Scores on the Ham-D were divided the following; very severe, >23; severe, 19-22; moderate, 14-18; mild, 8-13; and normal $\leq$7 (27).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher's exact test. The following variables were evaluated as prognostic factors: sex, age, BDI score, Ham-D score, a HCV RNA level, IL28B (genetic variation in rs8099917), variation of HCV-core, biochemical factors (AST, ALT, gamma glutamyltransferase, total cholesterol), white blood cell (WBC), hemoglobin, platelet count, HCV RNA 4, 12, 24 week after the initiation of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Table 1. **The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR ***

	Total	SVR (n=5)	Non-SVR (n=9)	p value†
Age (years old)	62.1 ± 4.3	62.4 ± 4.2	61.9 ± 4.6	0.797
Sex (male/female)	6/8	2/3	4/5	0.898
Previous IFN therapy (combination/monotherapy)	8/6	3/2	5/4	0.898
Duration of previous IFN therapy (week)	11.9 ± 7.8	11.6 ± 10.2	12.0 ± 7.1	0.699
HCV-RNA (KIU/mL)	2588± 1455	2228± 1807	2788± 1296	0.759
Core aa70 (Wild/Mutant)	6/8	3/2	3/6	0.438
BDI score	11.9 ± 10.3	12.2 ± 14.2	11.7 ± 8.4	0.518
Ham-D score	3.5 ± 4.1	3.6 ± 5.5	3.4 ± 3.5	0.606
IL28B (genetic variation in rs8099917, genotype TT/TGorGG)	7/7	5/0	2/7	0.042
AST (IU/L)	50 ± 24	46 ± 37	52 ± 17	0.112
ALT (IU/L)	68 ± 33	60 ± 35	72 ± 32	0.518
GGT (IU/L)	55 ± 59	25 ± 5	72 ± 69	0.813
Total cholesterol (mg/dL)	175 ± 30	166 ± 35	179 ± 28	0.298
White blood cell(10^3/mm^3)	4.39 ± 1.24	4.16 ± 1.02	4.52 ± 1.39	0.898
Hemoglobin (g/dL)	14.1 ± 1.1	14.2 ± 1.5	14.0 ± 0.9	0.898
Platelet(10^4/mm^3)	15.8 ± 4.8	19.9 ± 2.4	13.5 ± 4.1	0.019
HCV RNA (+/-) 4W	11/3	2/3	9/0	0.083
HCV RNA (+/-) 12W	10/4	1/4	9/0	0.012
HCV RNA (+/-) 24W	8/6	0/5	8/1	0.004

Data are number of patients (percentage) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDI, Beck depression inventories; GGT, gamma-glutamyltransferase; Ham-D, Hamilton depression rating; HCV, hepatitis C virus;
*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 44 weeks.
†Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher' exact test.

Result

Clinical characteristics of the patients

A total of 14 patients treated with IFN-beta +ribavirin were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age =62.1 years, male/female =6/8, and HCV-RNA =2,588±1,455 KIU/mL. Patients were classified into two groups according to the difference of response: SVR (n=5), Non-SVR (n=9).

Efficacy of treatment

Five of 14 patients (37.5%) had SVR by the intention to treat analysis. Table 1 shows the differences in the clinical background between patients with SVR and those without SVR. The negativity rate of HCV RNA 12 weeks after the initiation of combination therapy was 80% (4/5) in SVR group and 0%(0/9) in Non-SVR group (p=0.012). The negativity rate of HCV RNA 24 weeks after the initiation of combination therapy was 100% (5/5) in SVR group and 11.1% (1/9) in Non-SVR group (p=0.004). Next, the platelet count in SVR group was significantly higher than that in Non-SVR group.

On the IL28B (genetic variation in rs8099917), all seven patients with TG or GG at IL28B showed non-SVR. On the other hand, five of the seven patients with TT at IL28B showed SVR. The TT at IL28B that is associated with SVR was statistically significant in the present study (p=0.042).

Safety and tolerance of combination therapy

Of the 14 patients treated with IFN-beta + ribavirin included in this study, four patients necessitated a reduced dose of ribavirin due to the appearance of hemoglobin level <10 g/dL and two patients needed a reduced dose of IFN-beta due to WBC count of <2,000/mm^3. Three patients had dipstick proteinuria of +1 at 4 week after the initiation of combination therapy. This proteinuria continued during combination therapy. However, no patient discontinued combination therapy because of treatment related adverse events related to exacerbation of depression. Fig. 1 shows the changes of BDI scores in 14 patients treated with IFN-beta + ribavirin. BDI scores during combination therapy were lower than that at the initiation time of treatment. Fig. 2 shows the changes of Ham-D scores in 14 patients. There was no statistically significant difference in changes of Ham-D scores during combination therapy compared to that at the initiation time of treatment.

Regarding the prescription of antidepressant and anti-anxiety drugs, antidepressants, such as sulpiride, and amitriptyline hydrochloride, were given to three patients at the

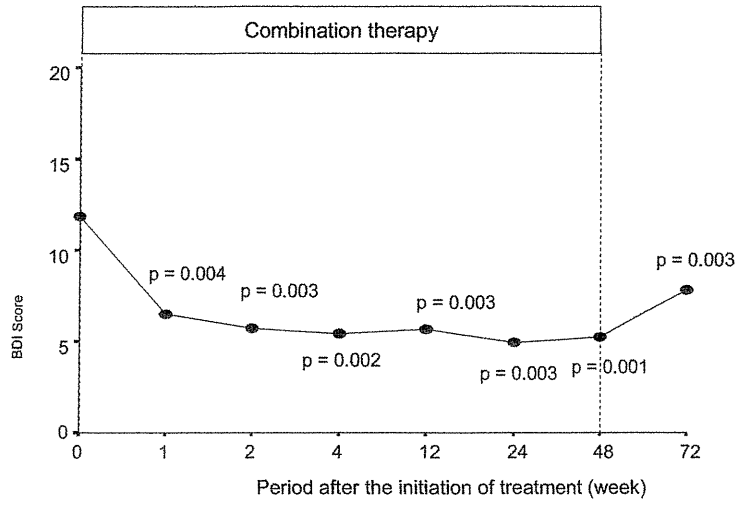

Figure 1. **The change of BDI score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the BDI-2 score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

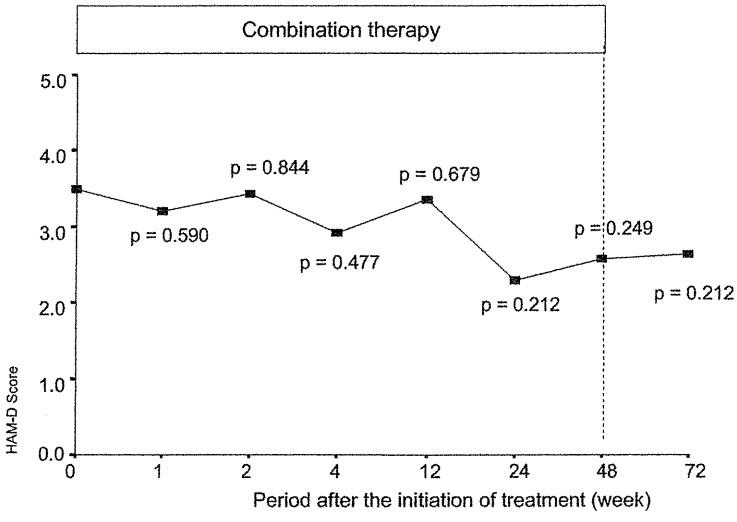

Figure 2. **The change of Ham-D score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the HAM-D score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

start of IFN therapy and to four patients during IFN therapy. Anti-anxiety drugs, such as etizolam, alprazolam, were given to four patients at the start of IFN therapy and to five patients during IFN therapy.

The changes of WBC, hemoglobin, and platelet count after the initiation of combination therapy are shown in Fig. 3. WBC and hemoglobin levels were decreased during combination therapy. On the other hand, the platelet count decrease was statistically significant at 1, 2, and 4 weeks after the initiation of combination therapy compared to that at the initiation time of treatment. After that, the platelet count recovered to the base line at 12, 24, and 48 weeks after the initiation of combination therapy.

Discussion

In the present study, we have described the efficacy and safety of combination therapy of IFN-beta and ribavirin for patients for whom IFN therapy was discontinued due to depression induced by IFN-alpha. The patients with HCV genotype 1b and HCV-load of $\geq$100 KIU/mL were enrolled. We could evaluate the relationship between IL-28 or HCV core mutation and SVR in the combination therapy of IFN-beta and ribavirin for genotype 1b and high virus load. The present study was limited to exclude the subjects with Ham-D score of more than 18. Patients with Ham-D score of more than 18 were defined as severe depression state. It is possible that high score of Ham-D enhance the dropout

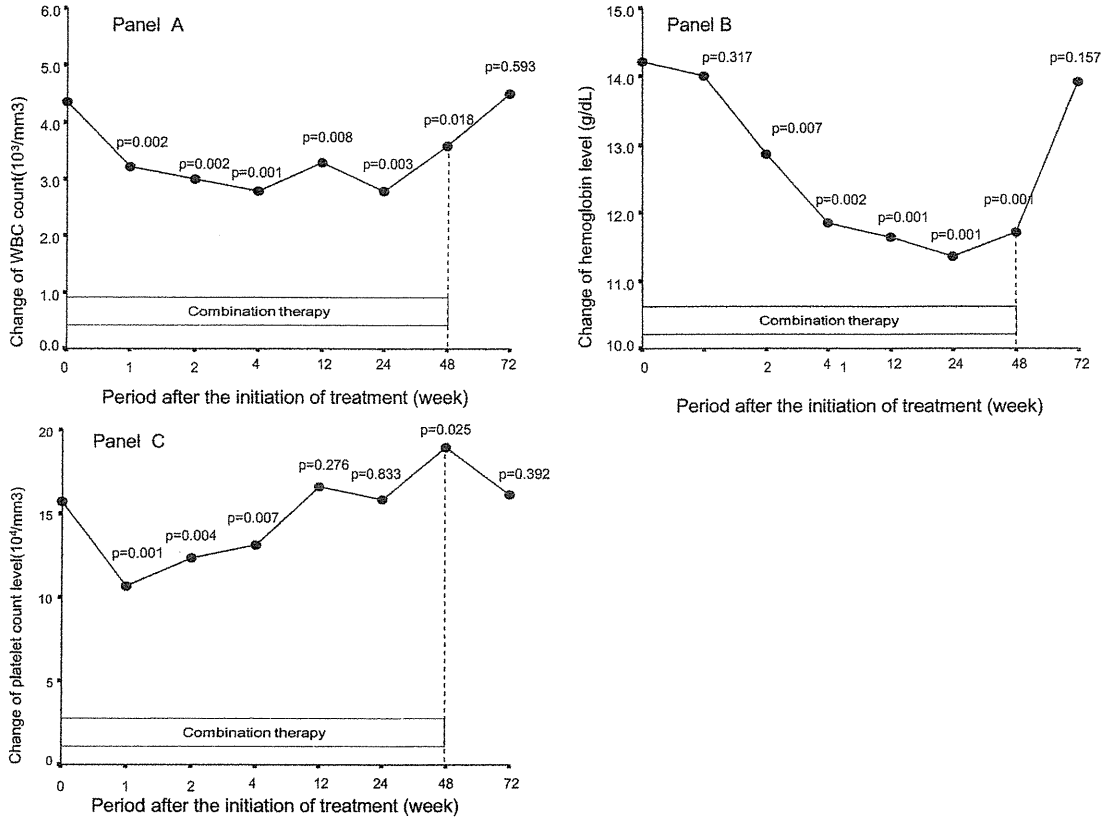

Figure 3. The change of complete blood cell count after the initiation of combination therapy. Panel A; The change of white blood cell count. Panel B; The change of hemoglobin level. Panel C; The change of platelet count.

due to combination therapy and aggravation of depressive state. Thus, we excluded the patients with Ham-D score of more than 18 in the present study. Moreover, the number of 14 patients enrolled was a small size. Another limitation is that the present study was not a randomized controlled study. Several findings from the present study have direct implications for combination therapy of IFN-beta and ribavirin for chronic hepatitis C in the future. First, the drop-out rate due to depressive state in combination therapy of IFN-beta and ribavirin was low. This result was similar to that in the previous study (14). The result by this prospective study confirmed that combination therapy of IFN-beta and ribavirin reduced the aggravation of depressive state compared with combination therapy of peginterferon-alpha and ribavirin.

Second, 5 out of 14 patients treated with combination therapy of IFN-beta and ribavirin had SVR. The SVR rate in the present study was almost the same to that in the previous study.

Third, SVR had a tendency to occur in patients with negativity of HCV RNA at 12 and/or 24 weeks after the initiation of combination therapy. All of the patients with positive HCV RNA at 24 weeks after the initiation of combination therapy showed non-SVR. This result agreed with our previous report (14). Thus, positive HCV RNA at 24 weeks after the initiation of combination therapy of IFN-beta and ribavirin suggests that the possibility of SVR is low. Next, patients with a high platelet count tended to show SVR. In general, a high platelet count suggests slight fibrosis of liver. Thus, the result raises the possibility that slight hepatic fibrosis enhance the efficacy of combination therapy.

Finally, SVR in combination therapy of IFN-beta + ribavirin was associated with IL-28B in the present study. None of the seven patients with genotype TG or GG at the genetic variation in rs8099917 near the IL28B gene had SVR. The results suggested that only patients with genotype TT might have the possibility of getting SVR. On substitution of core amino acid (aa) 70, two of eight patients with mutant type of core aa 70 showed SVR. The result shows that patients with mutant type of core aa 70 have the possibility of getting SVR. Several authors have reported that virus clearance in combination therapy of peginterferon-alpha and ribavirin is associated with HCV mutations in the core region and IL-28B (21-26). The present study confirmed that IL-28B was related with SVR for HCV patients with genotype 1b and high virus load.

IFN-beta is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to those of IFN-alpha. IFN-beta-induced mental disorders are mild compare to those induced by IFN-alpha. Out of 7,250 HCV patients treated with IFN in our hospital, 960 (13.2%) were

given IFN-beta. The mechanism of the better tolerability of IFN-beta and ribavirin is unclear. However, the following mechanism might be considered: 1) IFN-beta is not recombinant IFN but produced from human white blood cell. Thus, IFN-beta has a tendency not to produce some immune complex relating to IFN-related side effects. 2) IFN-beta might have different intracellular mechanisms compared to IFN-alpha. Although the receptor of IFN alpha and beta are common, intracellular mechanisms could differ. Our results described above suggest that combination therapy of IFN-beta and ribavirin is one possible method for patients who have HCV-genotype 1, high virus load and depressive state of Ham-D scale of <18. In conclusion, the combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

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Original Article

Highly sensitive AFP-L3% assay is useful for predicting recurrence of hepatocellular carcinoma after curative treatment pre- and postoperatively

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Aim: The micro-total analysis system (μTAS), a fully automated immunoassay system using microchip capillary electrophoresis, is highly sensitive and able to quickly assay the AFP-L3%. The clinical usefulness of this system was studied.

Methods: We retrospectively enrolled 250 patients who underwent curative treatment for primary hepatocellular carcinoma (HCC) (93 patients underwent hepatic resection and 157, radiofrequency ablation [RFA]).

Results: The sensitivity for μTAS AFP-L3% was 40.3% at the cutoff value of 5% in a range of AFP less than 20 ng/mL where the conventional method was unable to determine AFP-L3%. The sensitivity for AFP-L3% remained high even at stage I and at tumor size less than 2 cm (42.5% and 46.0%, respectively). Recurrence rate of patients with AFP-L3% greater than 5% was significantly higher than that of patients with less than 5% ($P = 0.001$). Furthermore, in resected patients, the postoperative AFP-L3% remained elevated with value greater than 5% was related to HCC recurrence ($P = 0.001$). Multivariate analysis revealed that multiple tumors ($P = 0.004$), preoperative AFP-L3% greater than 5% ($P = 0.003$), albumin less than 3.5 g/dL ($P = 0.008$), and RFA ($P = 0.003$) were significant prognostic factors of recurrence.

Conclusions: The μTAS was found to be a highly sensitive assay for AFP-L3% in patients with curative treatment of HCC. A cutoff value of 5% was useful for predicting recurrence after the curative treatment and detecting small tumors and early stage HCC. Additionally, postoperative AFP-L3% was found to be a prognostic factor of HCC recurrence.

Key words: hepatocellular carcinoma, highly sensitive AFP-L3%, micro-total analysis system

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common malignancy and the third leading cause of cancer-related death in the world.[1] Assays of three tumor markers, α-fetoprotein (AFP), Lens culinaris agglutinin-reactive fraction of α-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP), are helpful for HCC surveillance and diagnosis in parallel with imaging.[2–5] Among such markers, AFP is the most frequently assayed in the world, and adopted in the guidelines of the European Association for the Study of the Liver (EASL)[6] and The Asian Pacific Association for the Study of the Liver (APASL)[7] and also in the surveillance guidelines in Japan,[8] while the markers are not yet recommended for HCC surveillance by the American Association for the Study of Liver Disease (AASLD).[9] AFP level has been reported to be related to both disease stage and histological progression of HCC.[10,11] However, AFP level is often elevated even in patients with benign liver disease, and the low specificity of AFP has thus been a cause of concern for use as a HCC marker.[12–14] Aoyagi *et al.*[15] and Taketa *et al.*,[16] who focused on HCC-specific glycoform, found that the carbohydrate chain of AFP derived from HCC is fucosylated, leading to the discovery of AFP-L3 fraction highly specific for HCC. The rate of AFP-L3 in

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total AFP (AFP-L3%) has been reported to be useful for HCC diagnosis in many studies,[17–20] but is not sufficiently sensitive because it has been conventionally determined by lectin affinity electrophoresis and antibody affinity blotting method,[21] or liquid-phase binding assay on an auto-analyzer (LiBASys),[22] with a clinical sensitivity of about 20% among patients with curable small HCC.[17–19] Recently, a micro-total analysis system (μTAS) based on lectin-affinity electrophoresis using microfluidics technology has been put into clinical use to quickly determine the AFP-L3% with high sensitivity.[23] The μTAS is a system enabling simultaneous determination of AFP, AFP-L3%, and DCP, and is expected to be useful in assistance of detecting HCC.[24,25]

In the present study, AFP-L3% was assayed using this system in HCC patients who underwent curative resection or radiofrequency ablation (RFA) of HCC at our hospital, to investigate the clinical sensitivity and the relationship of the AFP-L3% with prognosis of HCC recurrence.

METHODS

Patients

BETWEEN 2003 AND 2007, a total of 724 patients were diagnosed with primary HCC at the Department of Hepatology, Toranomon Hospital. Of these, 250 patients who underwent curative resection ($n = 93$) or RFA ($n = 157$) for HCC were included in the present study. The demographic characteristics of patients are shown in Table 1. Serum samples were obtained immediately before treatment and 30 to 120 days (median 83 days) after surgical resection, and stored at −80°C.

The present study was retrospective in design and approved by the Toranomon Hospital Clinical Committee, with written consent obtained from patients or patients' legally acceptable representatives.

Diagnosis of HCC

Hepatocellular carcinoma was diagnosed by image modalities in most cases. If a hepatic nodular lesion was found on screening by ultrasonography (US), the patient underwent dynamic computed tomography (CT) and/or dynamic magnetic resonance imaging (MRI). Furthermore, when a liver nodule exhibited hyper-attenuation in the arterial phase of dynamic study and washout in the portal or delayed phase, or exhibited typical hyper vascular staining on digital subtraction angiography, the nodule was diagnosed as HCC according to the AASLD guidelines.[9] When the nodule did not

Table 1 Demographics of study population

Characteristics		All patients ($n = 250$)	Patients with resection ($n = 93$)	Patients with RFA ($n = 157$)	*P*-value
Age (years)	Range (Median)	35–84 (64)	35–80 (62)	38–87 (67)	0.004
Gender	Male/Female (%)	179(72)/71(28)	72(77)/21(23)	107(68)/50(32)	NS
Infection of hepatitis virus	HCV/HBV/Others (%)	169(68)/52(21)/29(11)	46(49)/32(34)/15(16)	123(78)/20(13)/14(9)	<0.001
Tumor size (mm)	Range (Median)	8–83 (20)	10–83 (25)	8–40 (17)	<0.001
Tumor number	Single/multiple (%)	193(77)/57(23)	71(76)/22(24)	122(78)/35(22)	NS
Albumin (g/dL)	Range (Median)	2.4–4.7 (3.6)	2.4–4.7 (3.7)	2.6–4.4 (3.6)	0.006
Bilirubin (mg/dL)	Range (Median)	0.3–4.1 (0.9)	0.3–3.1 (0.8)	0.3–4.1 (1.0)	0.001
AST (IU/L)	Range (Median)	15–446 (48)	15–446 (40)	16–258 (54)	0.001
PLT ($x10^4/mm^3$)	Range (Median)	2.7–31.6 (12.0)	3.8–31.6 (14.5)	2.7–24.6 (10.7)	<0.001
PT (%)	Range (Median)	39–125 (91)	67–124 (94)	39–125 (89)	0.026
Preoperative AFP (ng/mL)	Range (Median)	1.1–20 893 (12)	1.3–20 893 (11.8)	1.1–2388 (12.0)	NS
Preoperative DCP (mAU/mL)	Range (Median)	1–1774 (18)	7–1774 (23)	1–1253 (16)	<0.001

AFP, α-fetoprotein; AST, aspartate aminotransferase; DCP, des-gamma-carboxy prothrombin; NS, Not significance; PLT, platelet count; PT, prothrombin time; RFA, radiofrequency ablation.

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appear with the above-noted typical imaging features, a fine needle aspiration biopsy was carried out, followed by histological examination and diagnosis. Tumor stage on imaging findings was assessed on the basis of the Tumor Node Metastasis (TNM) classification of the Liver Cancer Study Group of Japan.[26]

Measurements of AFP, AFP-L3%, and DCP

α-fetoprotein, AFP-L3%, and DCP were assayed using a microchip capillary electrophoresis and liquid-phase binding assay on the μTASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The minimal detection limit of the μTAS was 0.3 ng/mL for AFP, and AFP-L3% was measurable when its concentration was above 0.3 ng/mL.

Follow-up protocol

Physicians examined patients every 4 weeks after curative treatment, and liver function and tumor markers were also measured once every month. After completion of HCC eradication, recurrence was surveyed with contrast-enhanced three-phase CT every 3 months.

Statistical analysis

We determined sensitivity and recurrence rate of HCC at diagnosis with AFP at the cutoff value set to 20 ng/mL. AFP-L3% cutoff values was set to 3%, 5%, 7%, and 10%.

Differences in the patient characteristics and laboratory data between the resection and RFA groups were examined with the χ^2 test and Mann–Whitney's *U*-test. Differences in the positive rates of AFP and AFP-L3% were evaluated by the Cochran–Armitage trend test. Recurrence rates were analyzed using the Kaplan–Meier method, and differences in the curves were tested using the log-rank test. Independent risk factors associated with recurrence were studied using the Cox proportional hazards model. Probabilities of less than 0.05 were considered significant. The Cochran–Armitage trend test was performed using the JMP statistical software version 9 (SAS Institute, Cary, NC, USA). Other data analysis was performed using SPSS statistical software version 10 (SPSS Inc., Chicago, IL, USA).

RESULTS

Sensitivity for AFP and AFP-L3%

OVERALL, THE SENSITIVITY for AFP was 38.0% when the cutoff value was set to 20 ng/mL. The sensitivity for AFP-L3% was 66.4%, 47.2%, 31.6%, and 18.8% at a cutoff value of 3%, 5%, 7%, and 10%, respectively (Table 2A).

Table 2 Sensitivity (A) All patients ($n = 250$) (B) Patients with AFP < 20 ng/mL ($n = 154$), and (C) Patients with AFP ≥ 20 ng/mL ($n = 96$)

	Analyte	Cutoff value	Sensitivity (%)
	AFP	20 ng/mL	38.0
(A)	AFP-L3%	3%	66.4
		5%	47.2
		7%	31.6
		10%	18.8
(B)	AFP-L3%	3%	54.5
		5%	40.3
		7%	24.0
		10%	12.3
(C)	AFP-L3%	3%	85.4
		5%	58.3
		7%	43.8
		10%	29.2

We compared the sensitivities in the groups of 154 patients with AFP less than 20 ng/mL (Table 2B) and 96 patients greater than 20 ng/mL (Table 2C). The sensitivity for AFP-L3% was 54.5%, 40.3%, 24.0%, and 12.3% in the patient group with low AFP and 85.4%, 58.3%, 43.8%, and 29.2% in the patient group with high AFP, with the cutoff value at 3%, 5%, 7%, and 10%, respectively. The sensitivity for AFP-L3% was higher in the high AFP patient group at respective cutoff values, but relatively high even in the low AFP patient group.

Sensitivity for AFP-L3% by tumor stage and size

Table 3A shows the sensitivity for AFP and AFP-L3% by tumor stage and Table 3B shows the sensitivity by maximal tumor size. The sensitivity for AFP-L3% increased with tumor progression at the cutoff values of 7% and 10% ($P = 0.021$ and 0.011, respectively, by the Cochran–Armitage trend test); however, the sensitivities were 65.0% and 42.5% and remained at a high level even for patients with stage-I tumors when the cutoff values were 3% and 5%, respectively.

When analyzed by tumor size, no significant difference observed at all the cutoff values. The sensitivity was 68.0% and 46.0% in patients with tumor size less than 2 cm and remained high at AFP-L3% of cutoff 3% and 5% regardless of tumor size, respectively.

Relationship of AFP and AFP-L3% with HCC recurrence

Hepatocellular carcinoma recurred in 151 (60.4%) patients during a median follow-up period of 4.2 years

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Table 3 Sensitivity by tumor stage and size (A) by tumor stage and (B) by tumor size

(A)

Analyte	Cutoff value	Stage I ($n = 120$)	Stage II ($n = 103$)	Stege III ($n = 27$)	*P*-value
AFP	20 ng/mL	38.3%	37.9%	40.7%	NS
AFP-L3%	3%	65.0%	67.0%	70.4%	NS
	5%	42.5%	50.5%	55.6%	NS
	7%	25.0%	35.9%	44.4%	0.021
	10%	12.5%	23.3%	29.6%	0.011

(B)

Analyte	Cutoff value	≤2 cm ($n = 150$)	2–3 cm ($n = 66$)	3–5 cm ($n = 25$)	>5 cm ($n = 9$)	*P*-value
AFP	20 ng/mL	42.7%	33.3%	36.0%	11.1%	0.057
AFP-L3%	3%	68.0%	71.2%	48.0%	55.6%	NS
	5%	46.0%	54.5%	36.0%	44.4%	NS
	7%	28.0%	42.4%	24.0%	33.3%	NS
	10%	15.3%	27.3%	16.0%	22.2%	NS

AFP, α-fetoprotein; NS, not significant.

(0.2 to 7.8 years) after curative treatment. The cumulative recurrence rate was 21.5% at year 1, 53.5% at year 3, and 65.6% at year 5 after treatment. In these patients, the recurrence rate was analyzed by preoperative AFP and AFP-L3% (Fig. 1).

There was no significant difference in recurrence rate between the patient groups with AFP greater than and less than 20 ng/mL (Fig. 1a). On the other hand, the 1- and 3-year recurrence rates were 29.4% and 65.5% in patients with AFP-L3% greater than 5% and 14.5% and 42.7% in patients with AFP-L3% less than 5%, respectively, and significantly different between the two patient groups ($P = 0.001$) (Fig. 1b). When the cutoff value for AFP-L3% was set to 7% and 10%, recurrence rate tended to be high in the patient group with AFP-L3% greater than the cutoff value, though not to a significant difference (data not shown).

Relationship of pre- and postoperative AFP and AFP-L3% with recurrence rate in patients undergoing resection

To exclude the improper matching of other potential risk factors for recurrence between the resected and the RFA patients, the relationships of pre- and postoperative AFP and AFP-L3% with the recurrence rate of HCC were analyzed for 93 resected patients. Figures 2 and 3 show the recurrence rates with preoperative and postoperative, respectively.

On analysis by preoperative AFP, the 1- and 3-year recurrence rates were 17.9% and 51.7% in patients with AFP less than 20 ng/mL and 11.1% and 36.9% in patients with AFP greater than 20 ng/mL, respectively, showing that the recurrence was high in the patient group with lower AFP, but this is not statistically significant ($P = 0.121$) (Fig. 2a). In contrast, by preoperative AFP-L3% using a cutoff value of 5%, the 1- and 3-year recurrence rates were 10.0% and 33.6% in patients with AFP-L3% less than 5% and 21.4 and 59.5% in patients with AFP-L3% greater than 5%, with a significantly high recurrence rate in patients with AFP-L3% higher than 5% ($P = 0.013$) (Fig. 2b). In addition, using the cutoff values of 7% and 10%, there was no significant difference between groups (data not shown).

Similar analyses were performed using the serum samples obtained from 91 of 93 patients after resection. Preoperative level of AFP greater than 20 ng/mL decreased to the level of less than 20 ng/ml in 29 of 37 patients (78.4%). On the other hand, preoperative AFP levels below 20 ng/mL turned positive in only one of 54 (1.9%) patients after curative treatment. Similarly, preoperative level of AFP-L3% greater than 5% decreased to a level less than 5% only in 16 of 42 (38.1%) patients. Moreover, preoperative level of AFP-L3% less than 5% increased to a postoperative level of 5% or higher after treatment in seven of 49 patients (14.3%). Thereby AFP-L3% turning negative after treatment was rare.

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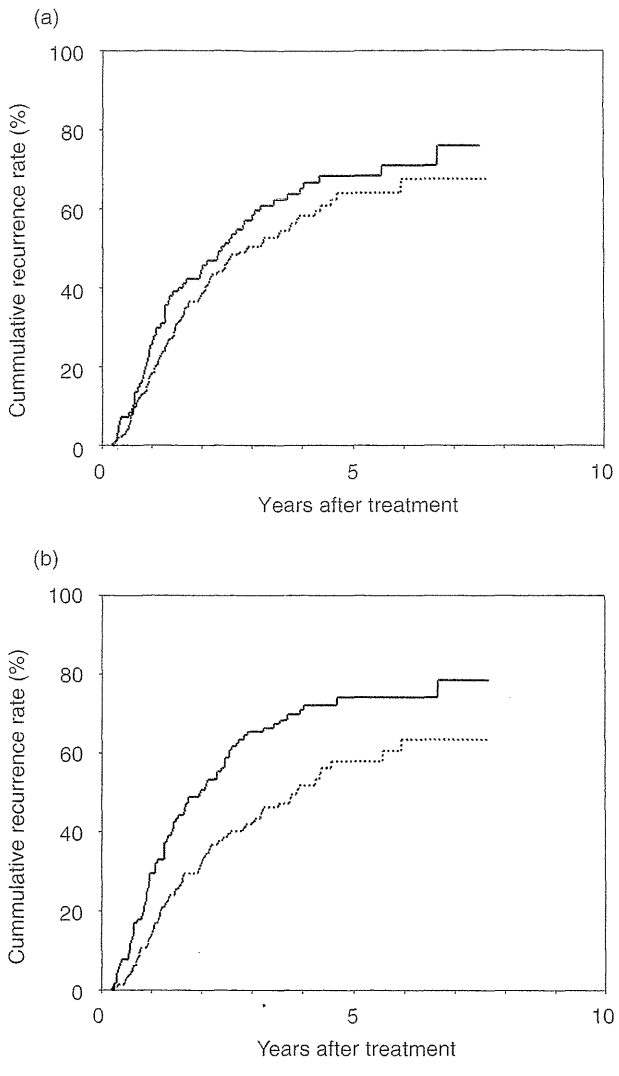

Figure 1 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for α-fetoprotein (AFP) and AFP-L3% in all patients. (a) Recurrence rate for AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for AFP-L3%: solid line, recurrence rate in patients with AFP-L3 ≥ 5%; broken line, recurrence rate in patients with AFP < 5%.

Comparing recurrence rates by postoperative AFP and AFP-L3%, the 1- and 3-year recurrence rates were 14.6% and 46.7% in patients with total AFP less than 20 ng/mL and 25.0% and 37.5% in patients with AFP greater than 20 ng/mL, with no significant difference between the two groups (Fig. 3a). In contrast, the 1- and 3-year recurrence rates were 14.7% and 43.5% in patients with AFP-L3% less than 5% and 29.3 and 64.4% in patients with AFP-L3% greater than 5%, with a significant difference between the two groups ($P = 0.001$) (Fig. 3b). With a cutoff value of 7% for AFP-L3%, no significant difference was observed between the two groups (data not shown). Only two patients had the postoperative AFP-L3% value greater than 10%. They developed HCC recurrence within 1 year and were suspected to have persistent HCC.

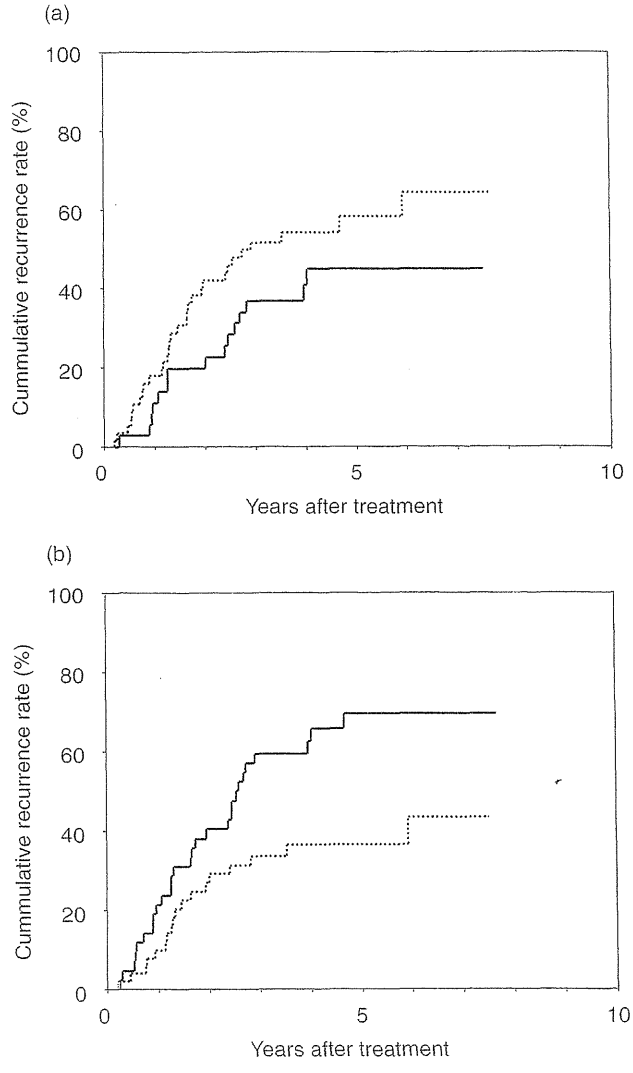

Figure 2 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for preoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for preoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for preoperative AFP-L3%: solid line, recurrence rate in patients with AFP-L3 ≥ 5%; broken line, recurrence rate in patients with AFP-L3 < 5%.

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