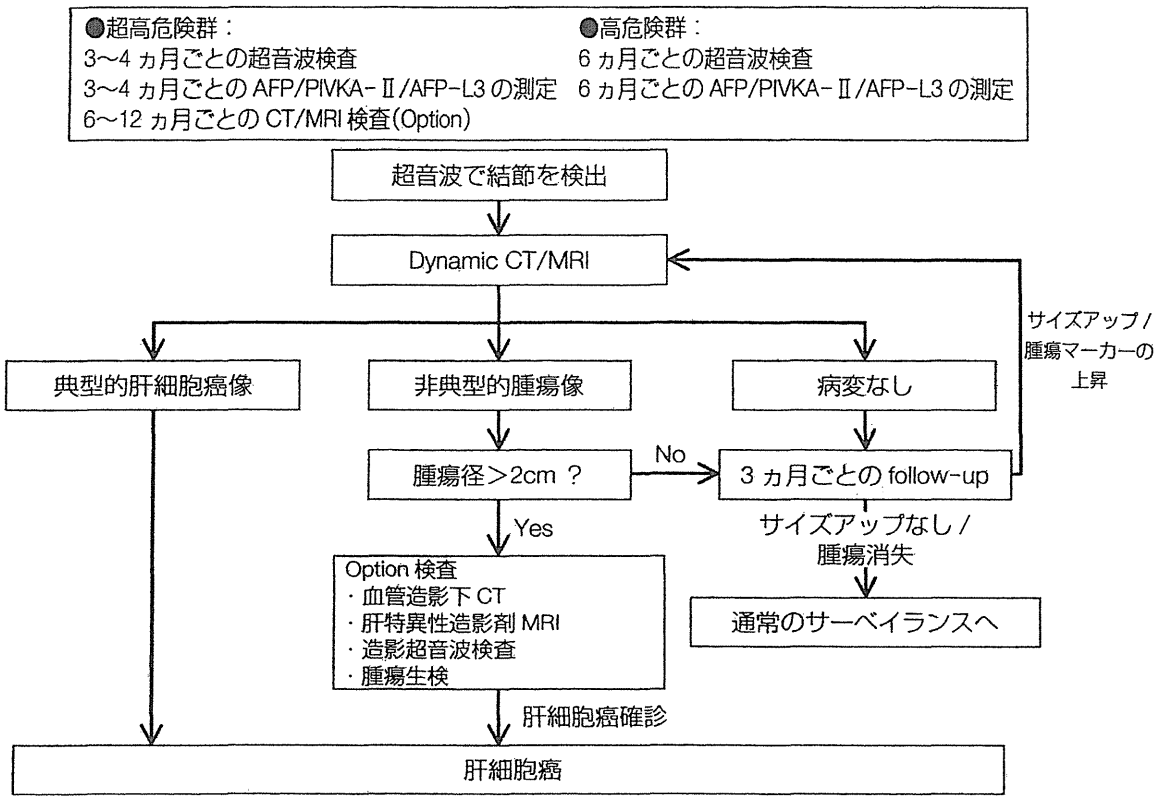


図 1. 肝細胞癌診断アルゴリズム
（日本肝臓学会編，「科学的根拠に基づく肝癌診療ガイドライン 2009 年版」より引用）

高率で可能になっている．治療の適応ある症例には積極的に抗ウイルス療法をすることが，最終的な肝細胞癌の予防につながると考えられる．肝細胞癌のサーベイランスに関しても原因，病態に応じたサーベイランス方法，間隔の設定により少なくとも肝細胞癌が小さい段階で発見することが可能である．問題は肝炎ウイルス症例を的確に発見し，適切な医療に結びつけることである．平成 14 (2002) 年より全国で肝炎ウイルス検診がおこなわれたが，適切な医療がされているかは疑問である．肝炎対策基本法も施行され，地域に応じた診療体制・連携の確立が急がれる．

文 献

1) 田中純子，片山恵子：肝癌発生の疫学．内科 **104**：614-620，2009
2) Donato F, Tagger A, Gelatti U *et al*：Alcohol and hepatocellular carcinoma：the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol* **155**：323-331, 2002
3) Komura T, Mizukoshi E, Kita Y *et al*：Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment with viral hepatitis. *Am J Gastroenterol* **102**：1939-1946, 2007
4) Nishiguchi S, Kuroki T, Nakatani S *et al*：Randomised trial of effects of interferon-α on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* **346**：1051-1055, 1995
5) Inoue A, Tsukuma H, Oshima A *et al*：Effectiveness of interferon therapy for reducing the incidence of hepatocellular carcinoma among patients with type C chronic hepatitis. *J Epidemiol* **10**：234-240, 2000
6) Yoshida H, Shiratori Y, Moriyama M *et al*：Interferon therapy reduces the risk for hepatocellular carcinoma：national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon

Therapy. *Ann Intern Med* **131** : 174-181, 1999
7) Akuta N, Suzuki F, Hirakawa M *et al* : Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* **52** : 421-429, 2010
8) Arase Y, Ikeda K, Suzuki F *et al* : Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. *J Med Virol* **79** : 1095-1102, 2007
9) Di Bisceglie AM, Shiffman ML, Everson GT *et al* : Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* **359** : 2429-2441, 2008
10) Lok AS, Everhart JE, Wright EC *et al* : Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. *Gastroenterology* **140** : 840-849, 2011
11) Arase Y, Ikeda K, Murashima N *et al* : The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* **79** : 1494-1500, 1997
12) Kato J, Miyanishi K, Kobune M *et al* : Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. *J Gastroenterol* **42** : 830-836, 2007
13) Muto Y, Sato S, Watanabe A *et al* : Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* **35** : 204-214, 2006
14) Yang HI, Lu SN, Liaw YF *et al* : Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* **347** : 168-174, 2002
15) Chen JD, Yang HI, Iloeje UH *et al* : Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. *Gastroenterology* **138** : 1747-1754, 2010.
16) Chen CJ, Yang HI, Su J *et al* : Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* **295** : 65-73, 2006
17) Liaw YF, Sung JJ, Chow WC *et al* : Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* **351** : 1521-1531, 2004
18) Matsumoto A, Tanaka E, Rokuhara A *et al* : Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B : A multicenter retrospective study of 2795 patients. *Hepatol Res* **32** : 173-184, 2005
19) Zhang BH, Yang BH, Tang ZY : Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* **130** : 417-422, 2004
20) Bolondi L, Sofia S, Siringo S *et al* : Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma : a cost effectiveness analysis. *Gut* **48** : 251-259, 2001

石川県の肝癌撲滅計画

酒井明人

金沢大学附属病院消化器内科・光学医療診療部 准教授

肝癌撲滅には背景にある肝炎ウイルスに対する治療導入が重要である。石川県では肝炎ウイルス検診初年度より協議会を設立し，陽性者をフォローアップしてきた。インターフェロン療法導入率向上を目指してさまざまな施策を講じ，導入率は30%を越えるようになった。2010年度よりかかりつけ医と専門医の連携を強化した「石川県肝炎診療連携」を新たに開始して専門医受診勧奨，抗ウイルス療法導入を図ることにより肝癌撲滅を目指している。

はじめに

2009年度人口動態統計では肝癌による死亡者数は男性で第4位，女性では第6位であり，年間3万人を越えている。肝癌の多くはウイルス性慢性肝疾患を背景に発生しており，肝癌撲滅には肝炎ウイルス感染者を早期に発見し，早期に治療することが重要である。国は2002年度より5年間で肝炎ウイルス検診を行い，肝炎ウイルス感染者の発見に努めたが，検診受診率は決して高くなく，また医療機関を受診しても適切な観察，治療導入すなわち抗ウイルス療法が行われてきたとは言い難い。本稿では肝炎ウイルス検診開始当初より石川県で取り組んできた肝炎ウイルス症例への対策について述べる。

肝炎ウイルス検診の方針 1

2002年肝炎ウイルス検診会誌当初より，石川県では肝炎協議会を設置し，県健康福祉部・医師会・保健所・検査センター・学術経験者が一体となって協力した検診体制を確立した。地域により専門医療機関の過不足があるため，精密検査は特に指定医療機関とはせず，かかりつけ医でも可とした。このため検診精度の向上と経過観察の重要性を考え，以下7つの項目を検診事業の柱とした。

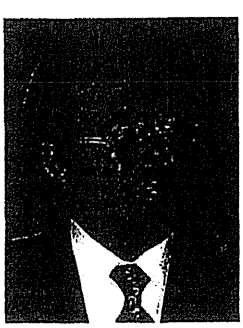
1. 検診陽性者への行政の関与することの通知と同意
2. 精密検査の全県での統一
3. 住民，担当医用の診断手引きの作成
4. 精密検査での画像検査の義務付け
5. 全症例を対象とした事例検討会
6. 前年度陽性者に対する事後調査
7. 保健師などを対象とした研修会の開催

このなかで石川県として独自性の高いと考えているものは検診陽性者を行政が継続フォローするために必要な1，6および担当医の肝炎への理解を深めた5である。毎年検診陽性者の医療機関受診・治療状況を把握することと，担当のかかりつけ医が正しく診断，治療導入することへの意識が高まるようこれら事業を継続した。

肝炎ウイルス検診の状況 2

石川県では5年間の肝炎ウイルス検診受診率は36.6～41.5%と全国平均[1]と比べると10%ほど受診率がよかったが半数には満たない。検診陽性者の精密検査受診状況は男性67.6%，女性75.0%，年齢では若年（65歳未満）66.1%，高齢（65歳以上）74.7%であった。性年齢でわけると若年男性53.4%，若年女性71.9%，高齢男性74.0%，高齢女性74.0%と若年男性で精密検査の受診率が低いことが明らかであり，仕事等で忙しく受診機会をつくりにくい状況がうかがえる。図1に性・年齢・医療圏別での精検受診状況を示す。検診自体の受診率は能登地方および南加賀で低い傾向にあった。しかし能登地方はウイルスキャリアと判明すると医療機関をきちんと受診する傾向にある。一方，南加賀ではウイルスキャリアと判明しても医療機関への受診率が悪い。能登地方ではキャリアの発掘が重要であり，南加賀ではキャリアの発掘と受診勧奨の両面が必要なことがう

PROFILE



Akito Sakai

さかい・あきと●1991年金沢大学医学部卒業，同年金沢大学医学部第一内科入局。1999年米国国立衛生研究所肝炎ウイルス部門留学。2003年金沢大学医学部がん遺伝子治療学講座助手。2004年金沢大学医学部附属病院消化器内科助手。2005年金沢大学医学部附属病院光学医療診療部助教授。2007年金沢大学附属病院光学医療診療部准教授

【専門領域】消化器病学，肝臓学

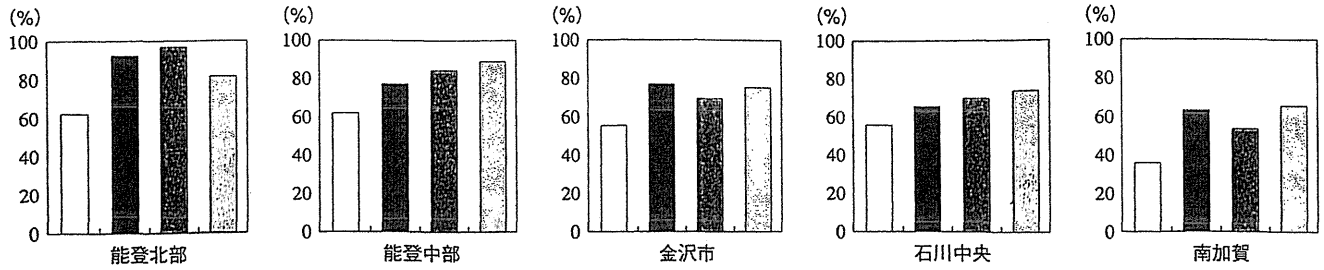


図1●検診陽性者の精密検査受診状況
□：男性・65歳未満　■：男性・65歳以上　▩：女性・65歳未満　▨：女性・65歳以上

表1●石川県精検未受診者のその後の状況

	検診初年度 精検未受診	翌年以降 医療機関受診	IFN療法 / 受診者
能登北部	18（14.8%）	12（66.7%）	3（25.0%）
能登中部	32（17.5%）	17（53.1%）	2（11.8%）
石川中央	71（31.8%）	45（63.4%）	7（15.6%）
南加賀	88（40.6%）	52（59.1%）	10（19.2%）
金沢市	147（28.1%）	39（26.5%）	2（ 5.1%）
合計	356（28.1%）	165（46.3%）	24（14.5%）

かがえる。また医療機関受診の時間がとりにくい若年男性の受診率が悪いのは地域で共通しており，受診動機を促す啓蒙活動が必要である。

フォローアップ事業の有用性 3

前述したように石川県では保健師が面談，電話，手紙などの方法で検診陽性者の状況把握に努めている。継続して医療機関で経過観察されているのはC型肝炎では48.7～63.7%であった。一方，各市町で少なくともフォロー期間（2～7年）中に1度は医療機関を受診した症例はB型肝炎ウイルス陽性者で49～100%，C型肝炎ウイルス陽性者で80～100%であった。表1に初年度精密検査未受診者のその後の状況を示す。受診勧奨を行った結果未受診者のうち能登北部66.7%，能登中部53.1%，金沢市26.5%，石川中央63.4%，南加賀59.1%がその後に医療機関を受診し，さらに受診者のうち能登北部25.0%，能登中部11.8%，金沢市5.1%，石川中央15.6%，南加賀19.2%がインターフェロン（IFN）療法を行っていた。継続した状況把握，受診勧奨が適切な医療へと結びつくことが明らかとなった。

IFN治療状況 4

肝癌撲滅という目標に対してC型肝炎であればIFN療法によりウイルスが排除されることが一番である。年齢，合併症などにより全ての症例でIFN療法を行うのは困難であるが，検診症例のIFN療法の施行率が低いことが問題となっている。厚生労働省研究班の報告では当初3年間では13.8～18.2%であった[1]。石川県でも2002年131例中5例（3.8%），2003年164例中14例（8.5%）とIFN療法施行率は低かった。特に65歳以上の高齢者ではIFN施行率は2.6%と，65歳未満の9.6%に対して有意に低かった[2]。IFN導入率が高齢者を含めて低い理由を検討するために，石川県全下で内科標榜医療機関にアンケート調査を行った。設問「一度はIFN療法を患者に説明するか（複数回答可）」に肝臓専門医の約8割は条件を問わずIFN療法について説明するが，非専門医師は約5割しか条件を問わずにIFN療法を説明していなかった。また「IFN療法を行わない理由」としては高齢であることをあげる医師が多数を占めたが，「何歳までがIFN療法の適応と考えるか」という設問では専門医は70～75歳までを適応と考えているが，非専門医はおおむね70歳以下と考えており，IFN適応年齢を非専門医は低く考えがちであることも明らかとなった[2]。このような実態を踏まえ，一例ごとの事例検討会，IFN療法をテーマにした講習会などを繰り返し行い，2004年102例中24例

表2●全国および石川県の検診C型肝炎陽性者のIFN施行率

		初年度IFN療法施行率	
		精検受診者中	慢性肝炎中
全国[1]	2002年	13.8%	
	2003年	13.3%	
	2004年	18.2%	
	2005年		
	2006年		
石川県	2002年	3.0%	3.8%
	2003年	5.7%	8.5%
	2004年	14.7%	23.5%
	2005年	24.5%	35.3%
	2006年	23.7%	31.0%

表3●「肝炎診療連携」で把握された75歳以下検診C型肝炎陽性症例のIFN治療状況

	キャリア（n：13）＋慢性肝炎（n：75） n＝88
IFN過去にあり	28（著効 6例）
現在投与中	7
投与開始	7
IFN施行数（率）	42/88（48%）
合併症不可（IP，うつなど）	4
IFN可能症例施行数（率）	42/84（50%）
IFN検討中	8

（23.5%），2005年68例中24例（35.3%），2006年71例中22例（31.0%）と後半2年間はIFN療法施行率が30%を超えていた（表2）。

石川県肝炎診療連携 5

年々IFN施行率は上昇してきたが，さらに向上させるには専門医が関わることが重要である。石川県では精密検査を専門医が行った症例では144例中53名（36.8%）がすぐにIFN導入され，翌年以降にさらに26例でIFN療法が施行，計79例（54.9%）でIFN療法が導入されていた。一方，かかりつけ医で診られた41症例では計8例（19.5%）のIFN導入にとどまり，IFN療法施行率をあげるには専門医がその診断，治療方針決定に関わることが重要であった。2007年にでた厚生労働省の肝炎検査後診療体制のガイドラインでも「状態に変化がなくとも年一回の専門医療機関受診が望ましい」とされており，かかりつけ医から患者を年1回の専門医に受診勧奨する「石川県肝炎診療連携」を立案した。個人情報保護の問題をクリアし，行政の保持する検診データを拠点病院と専門医療機関で構成する肝炎診療連携協議会に移行するために，行政・各市町と協議の上，患者より「石川県肝炎診療連携」への参加，データ移行に関して再同意をとり，専門医療機関を受診，順次データ移管することとなった。非同意，または返答のなかった症例は引き続き行政でフォローアップをすることとした。

2,570人の肝炎ウイルス検診陽性者に同意書・調査票が送付され494人が同意，非同意が90人，専門医療機関受診し調査票が回収されたのは328人であった。HBs抗原陽性148人，HCV抗体陽性174人であった。HBs抗原陽性では無症候性キャリアと診断されたのが79例で，そのうち5例でALT31IU/L以上の異常値であったが，4例ではHBV-DNA低値の情報が付加されており，診断が妥当であることが確認された。また核酸アナログ使用率も14%とHBs抗原陽性で治療を必要とする従来の割合と合致しているデータと考えられた。HCV抗体陽性者のうち慢性肝炎またはキャリアと診断された症例の治療方針をみると専門医がIFN療法が望ましいとしたのは全体の33%であった。一方経過観察が選択された症例では，ALT値が低いか，超高齢者が多く含まれていた。今回の専門医受診を契機にIFN療法導入が7例あり，過去のIFN歴も踏まえて現在までにIFN療法が行われたのは75歳以下の検診症例で48%であった（表3）。

おわりに

肝癌撲滅には背景となるウイルス性肝疾患への適切な経過観察，治療の導入が重要である。県下の肝炎ウイルス検診症例を専門医受診勧奨とデータ管理により早期に適切な治療導入に図りたい。

REFERENCES

1) 日野啓輔：肝炎ウイルス検診の実態と要精検者指導に対する今後の問題点．肝炎ウイルス検診の現状把握と評価及び今後のあり方に関する研究（主任研究者 吉澤浩司），厚生労働科学特別研究事業 平成18年度総括・分担報告書．p13-22，2007
2) 酒井明人，他：肝炎ウイルス検診でみる高齢者C型慢性肝炎治療の現状と高齢者IFN療法の成績．消化器科46: 408，2008

INTERNAL MEDICINE

ORIGINAL ARTICLE

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

Yasuji Arase, Yoshiyuki Suzuki, Fumitaka Suzuki, Naoki Matsumoto, Norio Akuta, Norihiro Imai, Yuya Seko, Hitomi Sezaki, Yusuke Kawamura, Masahiro Kobayashi, Tetsuya Hosaka, Satoshi Saito, Kenji Ikeda, Mariko Kobayashi and Hiromitsu Kumada

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

(Intern Med 50: 2083-2088, 2011)
(DOI: 10.2169/internalmedicine.50.5767)

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-

Department of Hepatology and Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Japan
Received for publication May 6, 2011; Accepted for publication June 7, 2011
Correspondence to Dr. Yasuji Arase, es9y-ars@asahi-net.or.jp

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

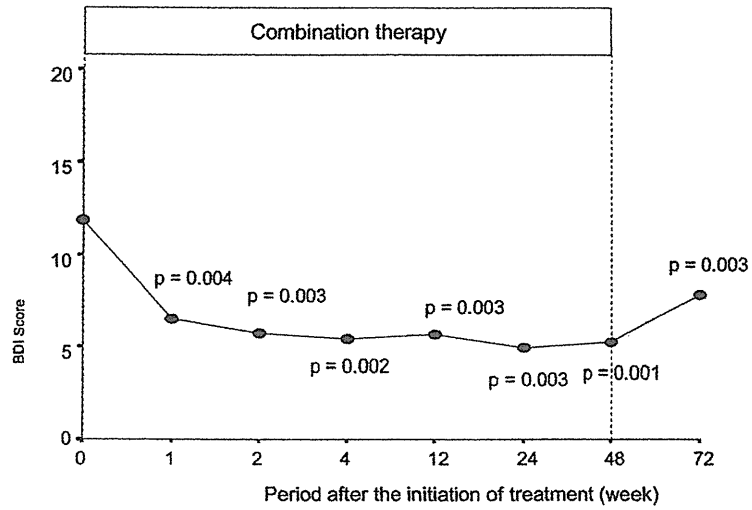


Figure 1. The change of BDI score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the BDI-2 score at the initiation time of combination therapy by the use of Mann-Whitney U test.

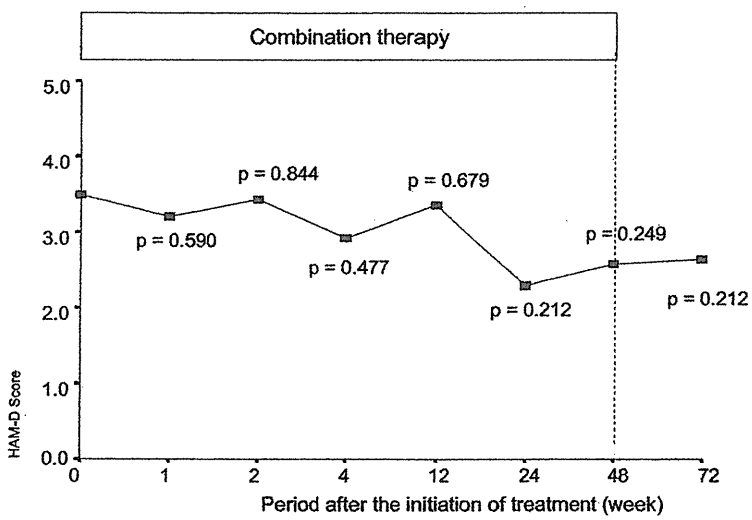


Figure 2. The change of Ham-D score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the HAM-D score at the initiation time of combination therapy by the use of Mann-Whitney U test.

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

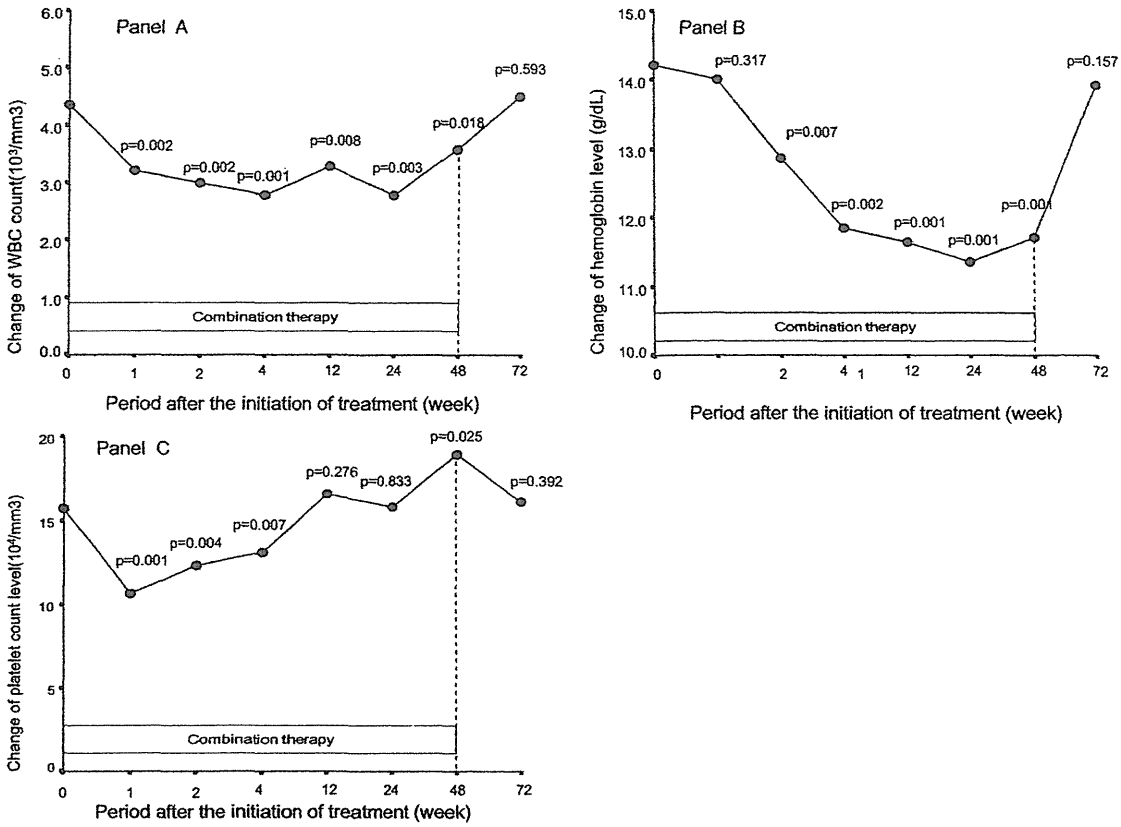


Figure 3. The change of complete blood cell count after the initiation of combination therapy. Panel A; The change of white blood cell count. Panel B; The change of hemoglobin level. Panel C; The change of platelet count.

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

The present work was supported in part by grants-in-aid from the Japanese Ministry of Health, Labour and Welfare. The authors acknowledge the editorial assistance of Thomas Hughes.

References

1. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet **358**: 958-965, 2001.
2. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med **347**: 975-982, 2002.
3. McHutchison JG, Manns M, Patel K, et al; International Hepatitis Interventional Therapy Group. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology **123**: 1061-1069, 2002.
4. Hadziyannis SJ, Sette H, Morgan TR, et al; PEGASYS International Study Group. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med **140**: 346-355, 2004.
5. Shiffman ML, Ghany MG, Morgan TR, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology **132**: 103-112, 2007.
6. Iwasaki Y, Ikeda H, Araki Y, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology **43**: 54-63, 2006.
7. Arase Y, Suzuki F, Suzuki Y, et al. Side effects of combination therapy of peginterferon and ribavirin for chronic hepatitis-C. Intern Med **46**: 1827-1832, 2007.
8. Festi D, Sandri L, Mazzella G, et al. Safety of interferon beta treatment for chronic HCV hepatitis. World J Gastroenterol **10**: 12-16, 2004.
9. Katamura Y, Suzuki F, Akuta N, et al. Natural human interferon beta plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus and a high viral load. Intern Med **47**: 1827-1834, 2008.
10. Arase Y, Suzuki F, Suzuki Y, et al. The efficacy of interferon-beta monotherapy for elderly patients with type C hepatitis of genotype 2. Intern Med **48**: 1337-1342, 2009.
11. Kainuma M, Ogata N, Kogure T, et al. The efficacy of a herbal medicine (Mao-to) in combination with intravenous natural interferon-beta for patients with chronic hepatitis C, genotype 1b and high viral load: a pilot study. Phytomedicine **9**: 365-372, 2002.
12. Kurosaki M, Enomoto N, Murakami T, et al. Analysis of genotypes and amino acid residues 2209 to 2248 of the NS5A region of hepatitis C virus in relation to the response to interferon-beta therapy. Hepatology **25**: 750-753, 1997.
13. Enomoto M, Tamori A, Kawada N, et al. Interferon-beta plus ribavirin for patients with hepatitis C virus genotype 1: a randomized pilot trial. Gut **55**: 139-140, 2006.
14. Arase Y, Suzuki F, Akuta N, et al. Efficacy and safety of combination therapy of natural human interferon beta and ribavirin in chronic hepatitis C patients with genotype 1b and high virus load. Intern Med **49**: 957-963, 2010.
15. Arase Y, Suzuki F, Akuta N, et al. Efficacy and safety of combination therapy of natural human interferon Beta and ribavirin in chronic hepatitis C patients with genotype 2 and high virus load. Intern Med **49**: 965-970, 2010.
16. Beck AT. Comparison of Beck Depression Inventories-IA and -II in psychiatric outpatients. J Pers Assess **67**: 588-597, 1996.
17. Hamilton M. A rating scale for depression. J Nerol Neurosurg Psychiat **23**: 56-62, 1960.
18. Doglio A, Laffont C, Caroli-Bosc FX, Rochet P, Lefebre J. Second generation of the automated Cobas Amplicor HCV assay improves sensitivity of hepatitis C virus RNA detection and yields results that are more clinically relevant. J Clin Microbiol **37**: 1567-1569, 1999.
19. Albadalejo J, Alonso R, Antinozzi R, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. J Clin Microbiol **36**: 862-865, 1998.
20. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology **19**: 13-18, 1994.
21. Akuta N, Suzuki F, Sezaki H, et al. Association of amino acid substitution pattern in core protein of hepatitis C virusgenotype1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology **48**: 372-380, 2005.
22. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature **461**: 399-401, 2009.
23. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet **41**: 1105-1109, 2009.
24. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet **41**: 1100-1104, 2009.
25. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28 B and spontaneous clearance of hepatitis C virus. Nature **461**: 798-801, 2009.
26. Rauch A, Kutalik Z, Descombes P, et al; Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. Swiss Hepatitis C and HIV Cohort Studies. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure—a genome-wide association study. Gastroenterology **138**: 1338-1345, 2010.
27. Kearns NP, Cruickshank CA, McGuigan KJ. A comparison of depression rating scales. Br J Psychiatry **141**: 5-49, 1982.

Hepatology Research 2011; 41: 1036–1045 doi: 10.1111/j.1872-034X.2011.00858.x

27

Original Article

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

Masahiro Kobayashi, Tetsuya Hosaka, Kenji Ikeda, Yuya Seko, Yusuke Kawamura, Hitomi Sezaki, Norio Akuta, Fumitaka Suzuki, Yoshiyuki Suzuki, Satoshi Saitoh, Yasuji Arase and Hiromitsu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

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

Correspondence: Dr Masahiro Kobayashi, Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: mshkobayashi@toranomon.gr.jp
Supported in part by a Research Grant from the Japanese Ministry of Health, Labor and Welfare, and Okinaka Memorial Foundation of Toranomon Hospital.
Received 29 March 2011; revision 10 June 2011; accepted 13 June 2011.

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.

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

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.

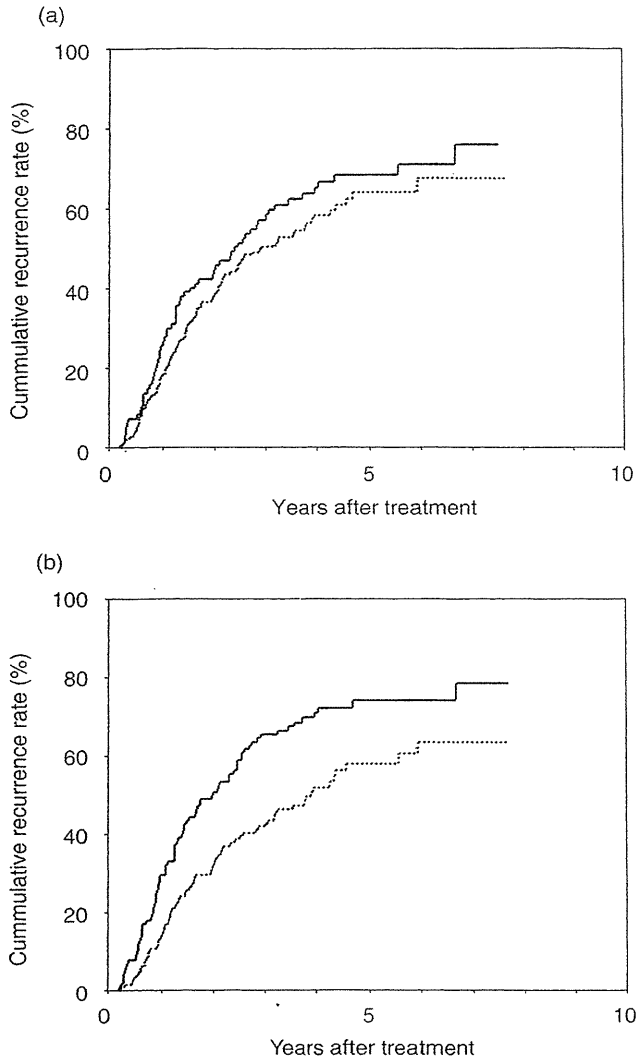


Figure 1 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for α-fetoprotein (AFP) and AFP-L3% in all patients. (a) Recurrence rate for AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for AFP-L3%: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

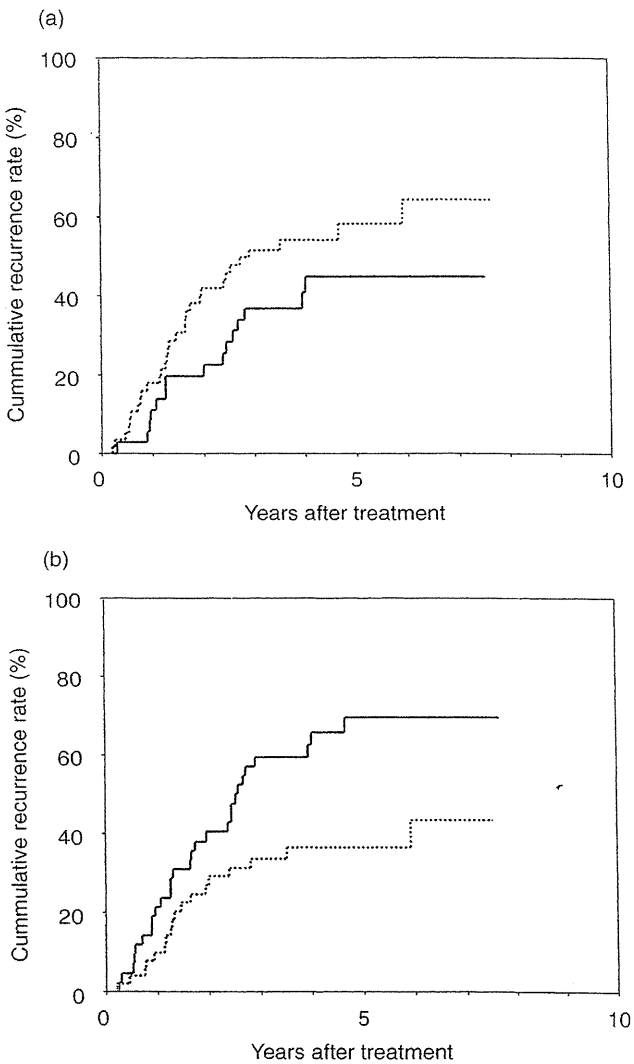


Figure 2 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for preoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for preoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL.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

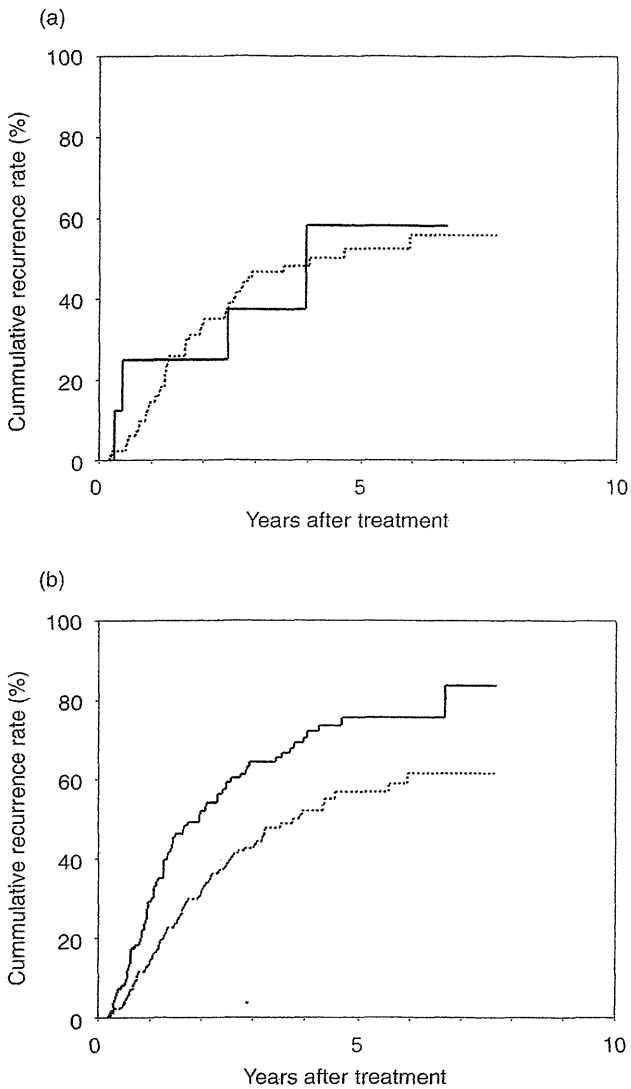


Figure 3 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for postoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for postoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for postoperative AFP-L3%: solid line, recurrence rate in patients with AFP-L3 ≥ 5%; broken line, recurrence rate in patients with AFP < 5%.

Prognostic factors for HCC recurrence

Factors related to HCC recurrence were analyzed by the Kaplan–Meier method and multivariate analysis (Table 4). Potential risk factors for recurrence included the following 15 variables: age, gender, etiology of background liver disease, amount of alcohol intake, albumin, bilirubin, aspartate aminotransferase (AST), platelet count (PLT), prothrombin time (PT), preoperative AFP, AFP-L3%, DCP, tumor size, tumor number, and treatment procedure (resection or ablation). In all of the patients ($n = 250$), factors that were significantly related to HCC recurrence were RFA therapy, multiple tumors, albumin <3.5 g/dL, AST ≥ 50 IU/L, platelets <10 × 104/μL, prothrombin time <80%, preoperative AFP-L3% ≥ 5%, and preoperative DCP ≥ 40 mAU/mL by the Kaplan–Meier method (Table 4A). On multivariate analysis, the following were significant prognostic factors: multiple tumors ($P = 0.004$), preoperative AFP-L3% ≥ 5% ($P = 0.003$), albumin <3.5 g/dL ($P = 0.008$), and RFA ($P = 0.003$) (Table 4B).

In the 93 resected patients, on multivariate analysis, factors contributing to HCC recurrence were tumor number and preoperative AFP-L3% ($P = 0.003$ and 0.019, respectively). In the 157 RFA patients, similarly the four factors of age, preoperative AFP, AFP-L3%, and albumin were identified ($P = 0.003$, 0.006, 0.009, and 0.011, respectively) (data not shown).

Histological features and serum AFP, AFP-L3%, and DCP levels

From the 93 patients who underwent resection, we were able to obtain 85 specimens and assess their histological features. Ten nodules were well-differentiated HCCs; 69, moderately differentiated HCCs; and the remaining six, poorly differentiated HCCs. The nodules were macroscopically classified: four nodules were of small nodular type with indistinct margin (SNIM); 50, of simple nodular type (SN); 24, of simple nodular type with extranodular growth (SNEG); and seven, of confluent multinodular type (CM). Microscopic vascular invasion was observed in 14 (16.5%) nodules, and microscopic intrahepatic metastasis was observed in four (4.7%) nodules.

The median (25–75 percentile) preoperative DCP level in moderately/poorly differentiated HCCs was 25 (15–113) AU/L, whereas that of the well-differentiated HCCs was 18 (14–20) AU/L, and this difference was statistically significant ($P = 0.041$). Similarly, a significant difference was observed in the preoperative AFP-L3% between groups: the median AFP-L3% in the SNEG/CM group was 6.4 (2.5–18.9), whereas in the SNIM/SN group, it was 2.5 (≤0.5–7.4) ($P = 0.032$).

DISCUSSION

IN THE PRESENT study, AFP-L3% assayed by the μTAS method was detected with high clinical sensitivity

Table 4 Prognostic factors of hepatocellular carcinoma (HCC) recurrence. (A) Cumulative recurrence rate by variable and (B) Multivariate analysis

(A) Cumulative recurrence rate by variable

Variables	*n*	3-year Recurrence (%)	*P*-value
Treatment			
Resection	93	45.9	0.003
RFA	157	58.0	
Tumor number			
Single	193	50.8	0.003
Multiple	57	62.9	
Albumin			
<3.5 g/dL	105	64.9	0.001
≥3.5 g/dL	145	45.2	
AST			
<50 IU/L	131	48.3	0.009
≥50 IU/L	119	58.7	
PLT			
$<10 \times 10^4/mm^3$	87	65.4	0.024
$\geq 10 \times 10^4/mm^3$	163	47.4	
PT			
<80%	51	74.7	0.001
≥80%	199	48.1	
Preoperative AFP-L3%			
<5%	132	42.7	0.001
≥5%	118	65.5	
Preoperative DCP			
<40 mAU/mL	194	49.6	0.025
≥40 mAU/mL	56	67.0	

(B) Multivariate analysis

Variables		Hazard ratio (95% CI)	*P*-value
Tumor number	(multiple/single)	1.70 (1.19–2.43)	0.004
Preoperative AFP-L3%	(≥5%/<5%)	1.63 (1.18–2.26)	0.003
Albumin	(<3.5/≥3.5 g/dL)	1.55 (1.12–2.14)	0.008
Treatment	(RFA/resection)	1.09 (1.03–1.16)	0.003

AST, aspartate aminotransferase; CI, confidence interval; PLT, platelet count; PT, prothrombin time; RFA, radiofrequency ablation.

even in cases of HCC at a relatively early stage, which can be potentially cured by hepatic resection or RFA. It is worth noting that the sensitivity for HCC was as high as 47.2% when the cutoff value of AFP-L3% was set to 5%, compared to the sensitivity of 38.0% for total AFP. In addition, using a cutoff value of 10%, the sensitivity was 18.8%, which is comparable to that reported with the conventional method in patients whose HCC was curatively treated.[17–19]

One of the advantages of the highly sensitive μTAS method is measurement of AFP at low concentrations. Previously, the conventional method was unable to accurately determine AFP-L3% when total AFP concentration was less than 20 ng/mL, while in the present study detection of AFP-L3% was possible in 40.3%, 24.0%, and 12.3% of patients with AFP values less than 20 ng/mL when using the cutoff value for the AFP-L3% was set to 5%, 7%, and 10%, respectively. In our previous study of prognostic factors in patients that underwent hepatic resection or RFA with HCC of size less than 3 cm and not more than three tumors, it was reported that DCP was a significant prognostic factor in RFA

patients, while both AFP and DCP were not in resected patients.[27] During that study, we could not measure the highly sensitive AFP-L3%, and we measured the conventional AFP-L3% in only about half the patients. Therefore, we did not include the results of the AFP-L3% levels in that study. In the present study using the highly sensitive μTAS method to assay AFP-L3%, multivariate analysis revealed the AFP-L3% is a predictive factor for HCC recurrence with statistical significance both in the group of overall study population and surgically resected patients. These results showed that this highly sensitive assay method can increase clinical sensitivity and predict recurrence, suggesting that it is of additional clinical utility.

Toyoda *et al.*[24] assayed AFP-L3% in 270 patients with AFP less than 20 ng/mL and 396 patients with chronic liver diseases using the same μTAS method as in the present study, and reported that the AFP-L3% assayed by this method was useful for differential diagnosis of HCC and benign liver diseases with a sensitivity of 41.5% and specificity of 85.1% with the AFP-L3% cutoff value of 5%. He also found AFP-L3% to be related to survival rate. In the present study, the sensitivity was similar to that reported by Toyoda *et al.*,[24] although it was not possible to compare specificity, since in this study we included only HCC patients.

Similarly, Tamura *et al.*[25] reported a sensitivity of 60%, specificity of 90.3%, accuracy of 76.4%, positive predictive value (PPV) of 83.9%, and negative predictive value (NPV) of 72.8% at a cutoff value of 7% in 295 HCC patients and 350 patients with benign liver diseases. Comparison of cutoff values showed that the 7% was most clinically useful. Compared with the sensitivity of 60% reported by Tamura *et al.*, the sensitivity at 31.6% was relatively low in the present study with cutoff value at 7%. This appears to reflect differences in some fundamental patient characteristics between the two studies: for example, Stage III and IV HCC accounted for 50.2% of patients (148 of 295) in the report by Tamura *et al.* and 10.8% (27 of 250) in the present study.

The optimal cutoff value of a marker depends on the target disease under study and its intended use. We believed that the cutoff value for differential diagnosis between HCC and benign liver disease should achieve high specificity, preferably using receiver-operating characteristic (ROC) curve analysis. The purpose of the present study was to identify recurrence-predictive factors in a patient population with curatively treatable HCC at a relatively early stage; we determined that 5% AFP-L3% was most useful.

The relationships of postoperative AFP and AFP-L3% with HCC recurrence were also investigated in the present study. Notably, postoperative AFP-L3% remaining elevated greater than 5% was indicative of risk of HCC recurrence. Furthermore, it is noted that total AFP turned negative in 78.4% of patients after curative treatment, while AFP-L3% did in only 38.1% of patients (5% cutoff). Included in the present study of recurrence were all resected patients in whom radical cure was histologically confirmed. Therefore, all remnants of HCC should have been surgically removed. We speculate that lack of reduction in AFP-L3% after curative treatment appears was due to intra-hepatic multi-centric carcinogenesis or intra-hepatic micrometastasis. Miyaaki *et al.*,[28] who assayed AFP-L3% and protein induced by vitamin K absence-II (PIVKA-II), also known as DCP, by the conventional method in 110 resected patients, reported more cases of infiltrative growth-type HCC and poorly differentiated-type HCC in patients with postoperative AFP-L3% greater than 10%. Tada *et al.*[29] also reported a high rate of infiltrative growth, capsule infiltration, septum formation, portal vein invasion, and hepatic invasion in 111 patients with HCC with a high level of AFP-L3%. Regrettably, however, subsequent HCC recurrence was not followed. In our patients, the preoperative DCP level was related to the histological grade of the tumor, and a preoperative AFP-L3% greater than 5% was related to the macroscopic type of the nodule. In contrast, no relationship was observed between the postoperative markers and histological features in the current study. Unfortunately, we cannot clearly explain the discrepancies between the results of Tada *et al.* and this study; further examination with a larger number of patients is required to determine the relationship between highly sensitive AFP-L3% and the histological features of the tumors. In any case, patients with high level of AFP-L3% either before or after curative treatment should be followed closely.

The present study shows the high clinical sensitivity in diagnosis of HCC using μTAS AFP-L3% in patients with curative treatment of HCC. With a cutoff value of 5%, sensitivity was optimal in AFP less than 20 ng/mL where the conventional method was unable to determine the AFP-L3% value. Furthermore, both pre- and postoperative AFP-L3% were determined as prognostic factors of HCC recurrence. Since the high recurrence rate of HCC after even curative treatment is reported, it is of great importance to be able to predict such recurrence. Our study showed that the highly sensitive AFP-L3% is expected to be of clinical utility in predicting recurrence after curative treatments.

REFERENCES

1 Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2008. *CA Cancer J Clin* 2008; 58: 71–96.
2 Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994; 19: 61–6.
3 Daniele B, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterology* 2004; 127: S108–12.
4 Marrero JA, Feng Z, Wang Y *et al.* Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; 137: 110–18.
5 Saitoh S, Ikeda K, Koida I *et al.* Diagnosis of hepatocellular carcinoma by concanavalin A affinity electrophoresis of serum alpha-fetoprotein. *Cancer* 1995; 76: 1139–44.
6 Bruix J, Sherman M, Llovet JM *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. *J Hepatol* 2001; 35: 421–30.
7 Omata M, Lesmana LA, Tateishi R *et al.* Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010; 4: 439–74.
8 Makuuchi M, Kokudo N. Surveillance algorithm and diagnostic algorithm for hepatocellular carcinoma. *Hepatology Research* 2010; 40: 6–7.
9 Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; 53: 1020–2.
10 Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC. High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus infection, age, p53 and beta-catenin mutations. *Int J Cancer* 2004; 112: 44–50.
11 Imamura H, Matsuyama Y, Miyagawa Y *et al.* Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. *Br J Surg* 1999; 86: 1032–8.
12 Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. *Cancer* 1989; 64: 2117–20.
13 Liaw YF, Tai DI, Chen TJ, Chu CM, Huang MJ. Alpha-fetoprotein changes in the course of chronic hepatitis: relation to bridging hepatic necrosis and hepatocellular carcinoma. *Liver* 1986; 6: 133–7.
14 Chu CW, Hwang SJ, Luo JC *et al.* Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. *J Clin Gastroenterol* 2001; 32: 240–4.
15 Aoyagi Y, Isemura M, Suzuki Y *et al.* Fucosylated alpha-fetoprotein as marker of early hepatocellular carcinoma. *Lancet* 1985; 2: 1353–4.
16 Taketa K, Sekiya C, Namiki M *et al.* Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. *Gastroenterology* 1990; 99: 508–18.
17 Toyoda H, Kumada T, Kaneoka Y *et al.* Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. *J Hepatol* 2008; 49: 223–32.
18 Tamura Y, Igarashi M, Suda T *et al.* Fucosylated fraction of alpha-fetoprotein as a predictor of prognosis in patients with hepatocellular carcinoma after curative treatment. *Dig Dis Sci* 2010; 55: 2095–101.
19 Tateishi R, Shiina S, Yoshida H *et al.* Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. *Hepatology* 2006; 44: 1518–27.
20 Sterling RK, Jeffers L, Gordon F *et al.* Clinical utility of AFP-L3% measurement in North American patients with HCV-related cirrhosis. *Am J Gastroenterol* 2007; 102: 2196–205.
21 Shimizu K, Taniichi T, Satomura S, Matsuura S, Taga H, Taketa K. Establishment of assay kits for the determination of microheterogeneities of alpha-fetoprotein using lectin-affinity electrophoresis. *Clin Chim Acta* 1993; 214: 3–12.
22 Yamagata Y, Katoh H, Nakamura K, Tanaka T, Satomura S, Matsuura S. Determination of alpha-fetoprotein concentration based on liquid-phase binding assay using anion exchange chromatography and sulfated peptide introduced antibody. *J Immunol Methods* 1998; 212: 161–8.
23 Kagebayashi C, Yamaguchi I, Akinaga A *et al.* Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. *Anal Biochem* 2009; 388: 306–11.
24 Toyoda H, Kumada T, Tada T *et al.* Clinical utility of high sensitive lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein less than 20 ng/mL. *Cancer Sci* 2011; 102: 1025–31. [Epub ahead of print].
25 Tamura Y, Igarashi M, Kawai H, Suda T, Satomura S, Aoyagi Y. Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. *Dig Dis Sci* 2010; 55: 3576–83.
26 Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer. English Edn.* Tokyo: Kanehara, 2003.
27 Kobayashi M, Ikeda K, Kawamura Y *et al.* High serum des-gamma-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. *Cancer* 2009; 115: 571–80.