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。

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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

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-

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 ≥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/mm³, platelet count >80,000/mm³, 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 ≤60 kg, 800 mg for >60 kg and ≤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 ≤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
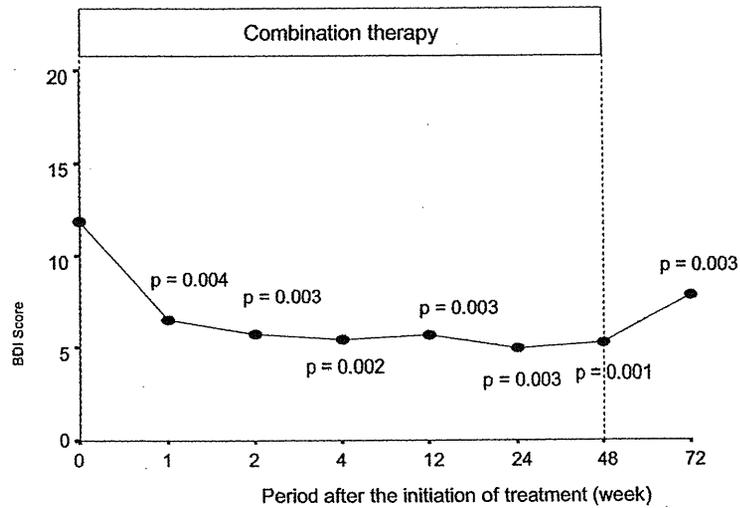
Figure 1.   The change of BDI score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the BDI-2 score at the initiation time of combination therapy by the use of Mann-Whitney U test.
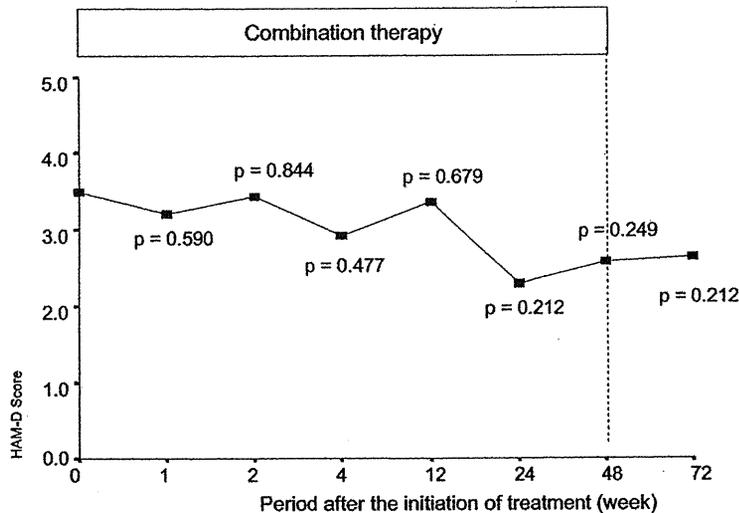

Figure 2.   The change of Ham-D score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the HAM-D score at the initiation time of combination therapy by the use of Mann-Whitney U test.

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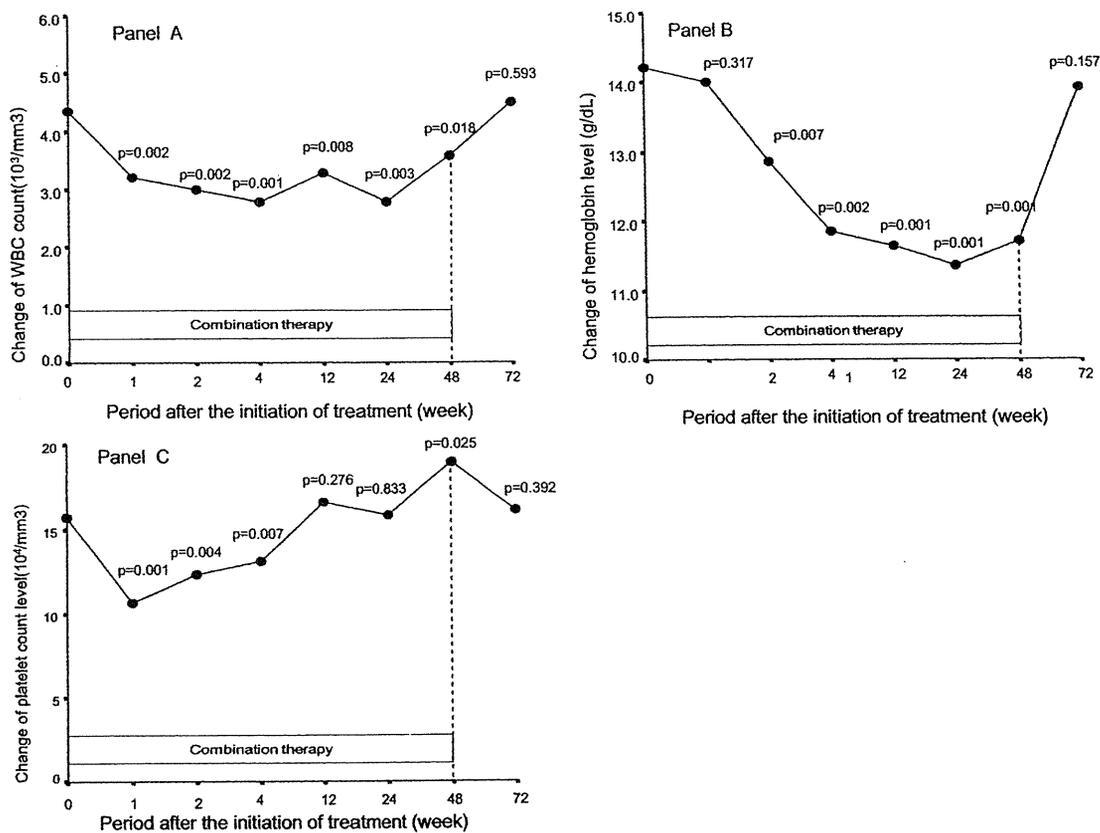
Figure 3. The change of complete blood cell count after the initiation of combination therapy. Panel A; The change of white blood cell count. Panel B; The change of hemoglobin level. Panel C; The change of platelet count.

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

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 (x10⁴/mm³)	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 AFP	Cutoff value 20 ng/mL	Sensitivity (%) 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-1 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.
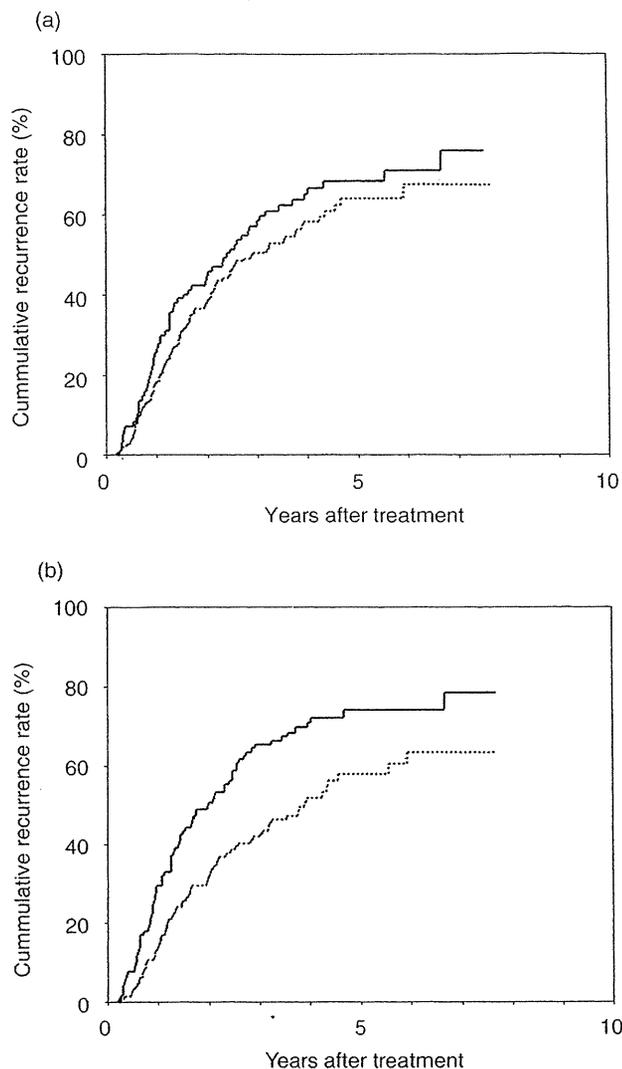
(a)

(b)

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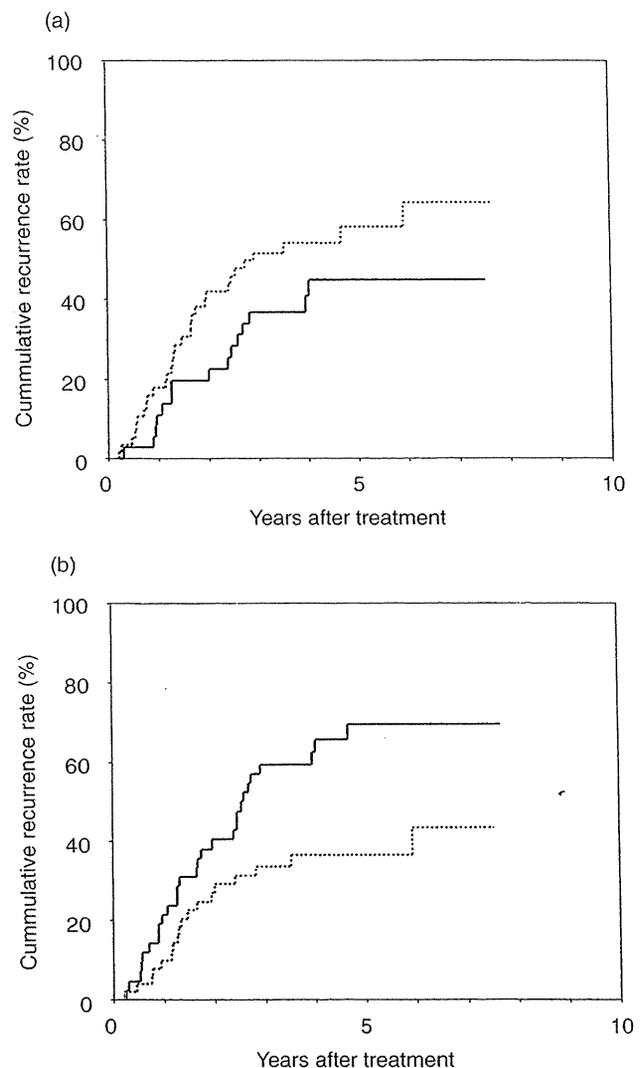
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Figure 2 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for preoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for preoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken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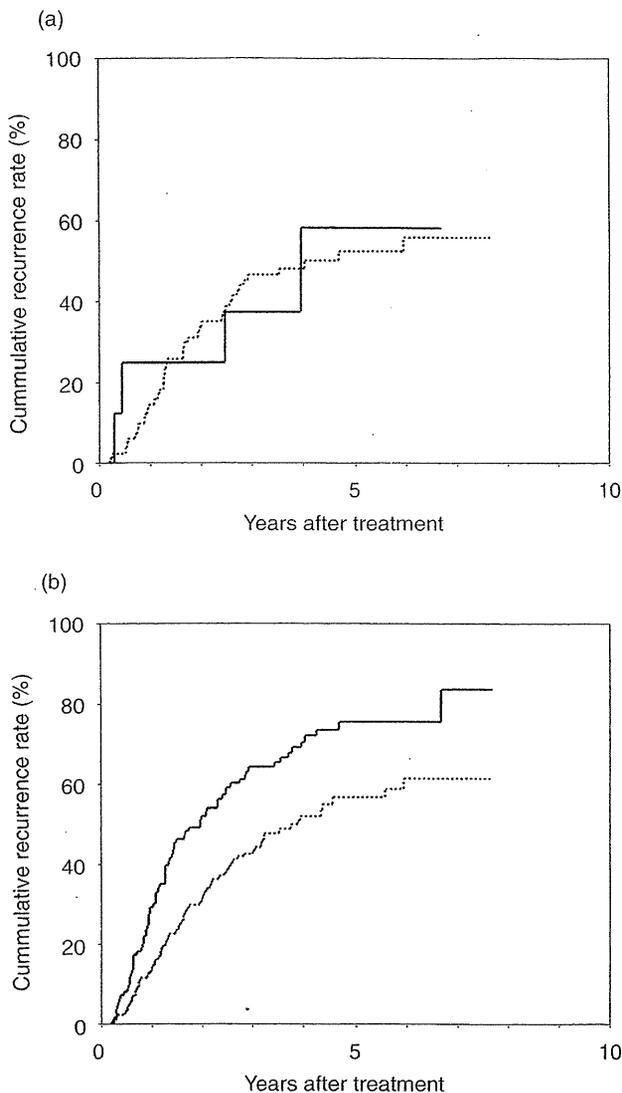
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(b)

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			0.003
Resection	93	45.9	
RFA	157	58.0	
Tumor number			0.003
Single	193	50.8	
Multiple	57	62.9	
Albumin			0.001
<3.5 g/dL	105	64.9	
≥3.5 g/dL	145	45.2	
AST			0.009
<50 IU/L	131	48.3	
≥50 IU/L	119	58.7	
PLT			0.024
<10 × 10^3/mm^3	87	65.4	
≥10 × 10^3/mm^3	163	47.4	
PT			0.001
<80%	51	74.7	
≥80%	199	48.1	
Preoperative AFP-L3%			0.001
<5%	132	42.7	
≥5%	118	65.5	
Preoperative DCP			0.025
<40 mAU/mL	194	49.6	
≥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.

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Clinical Study

Oncology

Previous Chemoembolization Response after Transcatheter Arterial Chemoembolization (TACE) Can Predict the Anti-Tumor Effect of Subsequent TACE with Miriplatin in Patients with Recurrent Hepatocellular Carcinoma

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Abstract

Aim: The purpose of this retrospective study was to evaluate the efficacy and safety of transcatheter arterial chemoembolization (TACE) with miriplatin in patients with unresectable hepatocellular carcinoma (HCC). *Methods:* From 2007 to 2010, 122 consecutive patients with unresectable HCC were treated by TACE with miriplatin-lipiodol suspension in our institute. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. Ninety-eight patients (80%) had a history of TACE. *Results:* Thirty-five of the 122 treated patients (29%) showed complete response (CR). And no serious complications were observed. Patients who had shown CR after previous TACE (pre-CR) were significantly more likely to show CR in the current study compared with patients who had shown less successful responses after previous TACE (56 vs. 20%, p = 0.003). Multivariate analysis revealed that response after previous TACE (pre-CR, risk ratio: 4.76; p = 0.035), tumor multiplicity (solitary, risk ratio: 9.69; p = 0.003), and injection artery (peripheral to segmental hepatic artery, risk ratio: 5.28; p = 0.040) were significant independent predictors associated with CR after TACE using miriplatin. *Conclusion:* In repetition of TACE treatment, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown a CR after previous TACE.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide [1]. Since it is well known that more than 80% of HCC cases are associated with liver cirrhosis, a routine check-up including ultrasound for cirrhotic patients could potentially lead to the detection of early HCC [2–4]. Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation) are applicable in only 30–40% of

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HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC [5–12].

Although many chemotherapeutic agents (e.g. doxorubicin, epirubicin, mitomycin C, and cisplatin) are used with the ethyl ester of iodized fatty acids from poppy-seed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) in TACE, the best choices for first- and second-line drugs remain uncertain [13–15]. Miriplatin (cis-[((1R,2R)-1,2-cyclohexanediamine-N,N')bis(myristato)]-platinum(II)monohydrate; Dainippon Sumitomo Pharma Co., Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent [16–19]. When lipiodol is injected into an artery feeding HCC nodules, it selectively accumulates in the tumor. Accordingly, a miriplatin-lipiodol emulsion is deposited within the HCC nodules and gradually releases active platinum compounds into tumor tissues. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC, but the efficacy of TACE using miriplatin for patients with recurrent HCC after TACE has not been evaluated [20, 21]. The purpose of this retrospective study was to evaluate the efficacy and safety of TACE using miriplatin for patients with HCC.

Patients and Methods

Study Population

From December 2007 to December 2010, 122 consecutive patients with unresectable HCC were treated by TACE with a miriplatin-lipiodol suspension at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. The study group consisted of 79 men and 43 women ranging in age from 48 to 87 years (median, 72 years). They included 11 patients (9%) positive for HBs-Ag, 103 patients (84%) positive for HCV antibody, and 8 patients (7%) negative for both. At the time of the miriplatin administration, median values were as follows: total bilirubin level = 1.1 mg/dl; serum albumin concentration = 3.3 g/dl; indocyanin-green retention rate at 15 min = 29%; prothrombin activity = 82.5%; alpha-fetoprotein (AFP) concentration = 31.2 ng/ml; and des-gamma-carboxyprothrombin (DCP) concentration = 53 AU/l. As for Child-Pugh classification, 92 patients (75%) were Class A and 30 patients (25%) were Class B. The clinical characteristics of the study group are summarized in table 1. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

Hepatocellular Carcinoma

Before treatment with miriplatin, all patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver-imaging studies (dynamic computerized tomography [dynamic CT], ultrasonography [US], digital-sub-

Table 1. Demographic characteristics and pretreatment assessments of 122 patients who underwent TACE using a miriplatin/lipiodol suspension for unresectable HCC

Number of cases	122
Age, years	72 (48–87)
Gender, male	65%
Etiology, HCV/HBV/others	103/11/8
Child-Pugh Class, A/B/C	92/30/0
ICG-R15, %	29 (4–78)
Albumin, g/dl	3.3 (2.0–4.2)
Total bilirubin, mg/dl	1.1 (0.4–4.9)
Prothrombin activity, %	82.5 (45.7–123.1)
Platelet, ×10³/μl	93 (29–282)
AFP, ng/ml	31.2 (1.8–152,800)
DCP, AU/l	53 (6–65,290)

HCV = Hepatitis C virus; HBV = hepatitis B virus; ICG-R15 = indocyanine-green retention rate at 15 min.
Variables are expressed as medians with ranges in parentheses.

Table 2. Tumor profiles, treatment history, and study drug dosages of 122 patients who underwent TACE using miriplatin for unresectable HCC

Tumor size, mm	20 (10–100)
Intrahepatic multiplicity, solitary	22 (18%)
Number of tumors	4 (1–100)
Presence of portal vein invasion	3 (2%)
History of TACE	98 (80%)
History of TACE with epirubicin	80 (66%)
History of TACE with cisplatin	37 (30%)
Median interval between previous TACE and miriplatin administration, months	4 (1–41)
Dosage of miriplatin, mg	80 (20–120)
Dosage of lipiodol, ml	3 (1–6)
Injection from peripheral to segmental branch of the hepatic artery	22 (18%)

Variables are expressed as medians with ranges in parentheses or number of cases.

traction angiography [DSA]), complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of dynamic CT, US and DSA. Patients who had extrahepatic metastasis of HCC or other malignancies were excluded.

Tumor profiles and TACE treatment history for the study group are summarized in table 2. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. The median diameter of the largest tumor was 20 mm (range 10–100 mm). Ninety-eight patients (80%) had a history of TACE. Thirty-seven patients had received cisplatin, and 80 patients had received epirubicin. Among these patients, the median number of

TACE procedures was four (range 1–13), and the median interval between previous TACE and miriplatin administration was 4 months (range 1–41 months).

Treatment Protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and the catheter was inserted superselectively into the hepatic artery that supplied the target tumor for injection of the miriplatin-lipiodol suspension and 1-mm gelatin cubes (Gelpart; Nippon Kayaku, Tokyo). The miriplatin-lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

Assessment of Therapeutic Effects

The effect of chemotherapy was evaluated by dynamic CT 1 to 3 months after TACE with miriplatin, and was based on the change in the maximum diameter of the viable target lesions (i.e. showing enhancement in the arterial phase). Response categories, according to the criteria of Modified Response Evaluation Criteria in Solid Tumors (mRECIST) [22], are as follows: complete response (CR) = disappearance of any intratumoral arterial enhancement in all target lesions; partial response (PR) = at least a 30% decrease in the sum of diameters of viable target lesions; stable disease (SD) = any cases that do not qualify for either PR or progressive disease; and progressive disease (PD) = an increase of at least 20% in the sum of the diameters of viable target lesions.

Toxicity Evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria (version 4.0). Within 2 weeks before TACE with miriplatin, and at 3 to 7 days (three times during this period) and at 1 month afterward, the following toxicity evaluations were made: hematological assessments (i.e. leukocyte and thrombocyte counts) and clinical chemistry assessments (i.e. serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], albumin, total bilirubin, serum creatine, and prothrombin activity).

Statistical Analysis

The distribution of subject characteristics was assessed by the chi-square test or the Mann-Whitney's U test, as appropriate. Multivariate logistic regression analysis was used to evaluate significant factors for CR by TACE with miriplatin. All variables are expressed as mean (range). All tests were 2-sided, and p values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., IBM, Somers, N.Y., USA).

Results

Dosing of Study Drugs

Table 2 summarizes the profiles and study drug data of 122 HCC patients who were treated with miriplatin. The median dosage of miriplatin was 80 mg (range 20–120 mg), and the median dosage of lipiodol was 3 ml
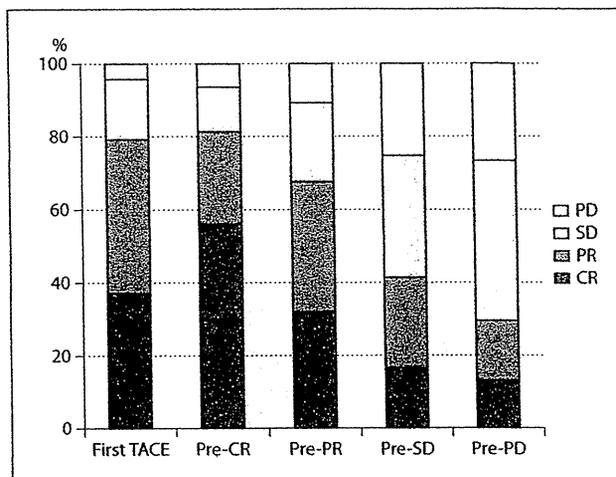

Fig. 1. The efficacy of TACE using miriplatin in patients with HCC according to response to previous TACE. Abbreviations used in the figure: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. pre-CR group (n = 16): patients who showed CR after previous TACE. pre-PR group (n = 28): patients who showed PR after previous TACE. pre-SD group (n = 24): patients who showed SD after previous TACE. pre-PD group (n = 30): patients who showed PD after previous TACE.

(range 1–6 ml). Twenty-two patients (18%) were injected with the miriplatin-lipiodol suspension from the peripheral to the segmental branch of the hepatic artery. Thirty patients (25%) were injected with the miriplatin-lipiodol suspension from the anterior or posterior segmental branch of the right hepatic artery. Sixty-six patients (54%) were injected with the miriplatin-lipiodol suspension from the right or left branch of the hepatic artery. And 4 patients (3%) were injected with the miriplatin-lipiodol suspension from the proper hepatic artery.

Treatment Effects

Thirty-five of the 122 treated patients (29%) showed CR, 35 patients (29%) showed PR, 33 patients (27%) showed SD, and 19 patients (15%) showed PD. Overall, 58% of patients showed an objective response (i.e. CR or PR).

Treatment Effects according to Previous TACE Effect

The efficacy of TACE using miriplatin according to the treatment effect of previous TACE was as follows (and is illustrated in fig. 1). For the first TACE group (patients who received TACE for the first time), 9 of 24 patients (38%) showed CR; for the pre-CR group (patients who