Table 2 Incidence of adverse events that occurred in two or more patients

	$N = 15$			
	Mild n (%)	Moderate n (%)	Severe n (%)	Total n (%)
Rash	5 (33.3)	3 (20.0)	0 (0.0)	8 (53.3)
Anaemia	7 (46.7)	0 (0.0)	0 (0.0)	7 (46.7)
Low-density lipoprotein increased	6 (40.0)	0 (0.0)	0 (0.0)	6 (40.0)
Blood uric acid increased	4 (26.7)	0 (0.0)	0 (0.0)	4 (26.7)
Pruritus	3 (20.0)	1 (6.7)	0 (0.0)	4 (26.7)
Anorexia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Dysgeusia	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Headache	3 (20.0)	0 (0.0)	0 (0.0)	3 (20.0)
Diarrhoea	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Pyrexia	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Thirst	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Nasopharyngitis	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood creatinine increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Blood triglycerides increased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Platelet count decreased	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Dizziness	1 (6.7)	1 (6.7)	0 (0.0)	2 (13.3)

MedDRA (Ver.12.0).

more than 25% of patients were rash (53.5%), anaemia (46.7%), low-density lipoprotein (LDL) increases (40.0%), blood uric acid increase (26.7%) and pruritus (26.7%). Two patients discontinued telaprevir treatment because of AEs (herpes zoster or rash pruritic). Except for the herpes zoster whose severity was judged as severe and serious, all the events were mild to moderate. Fifty of the 80 AEs were observed within the first 4 weeks.

In relation to skin AEs, rash, pruritus and rash pruritic were observed in 8, 4 and 1 patients, respectively. The onset day of these events is described in Fig. 2. The range of the onset day was Day 1 to Day 113, and the median was Day 15. Rash in three patients, pruritus in one patient and rash pruritic in one patient were moderate, and the others were mild. One patient discontinued telaprevir at Week 6 because of moderate rash pruritic. Most of the skin AEs were treated with oral antihistamines or topical steroids.

A decrease in haemoglobin levels was observed in all patients (Fig. 3a). Seven of 15 patients developed anaemia during and after the treatment. All anaemia events were mild and no patient needed discontinuation of telaprevir. Uric acid and LDL cholesterol increased during the treatment (Fig. 3b,c), but these changes were mild and no patient needed any medication for these AEs. There were no substantial increases in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (T-bil).

Sequence analysis at HCV NS3 protease domain

Amino acid substitutions in the NS3 protease domain were examined in 39 clones or more in each sample. Before Week 8, V36A/G, T54A and A156T/V as single substitutions, and T54A + R155K and A156T/V + V158I as multiple substitutions were observed. Among two patients who discontinued telaprevir within 2 weeks, all clones but three in one patient were wild-type variants after withdrawal of telaprevir. In three patients who discontinued at Weeks 5–7 because of viral breakthrough, predominant clones possessed A156V/T substitutions after the nadir of viral load. Predominant variants observed during and after telaprevir monotherapy in the eight patients who received telaprevir beyond 8 weeks are shown in Fig. 4 together with HCV RNA levels. In the two patients who showed the lowest HCV RNA level of on Week 4, the predominant clones detected after

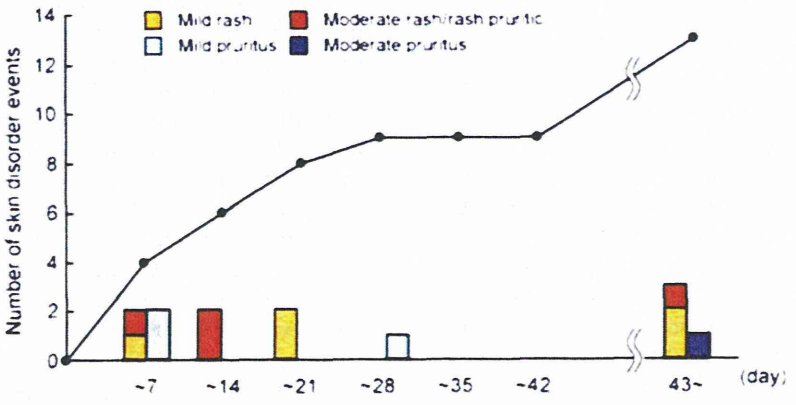

Fig. 2 Rash and pruritus occurrence.

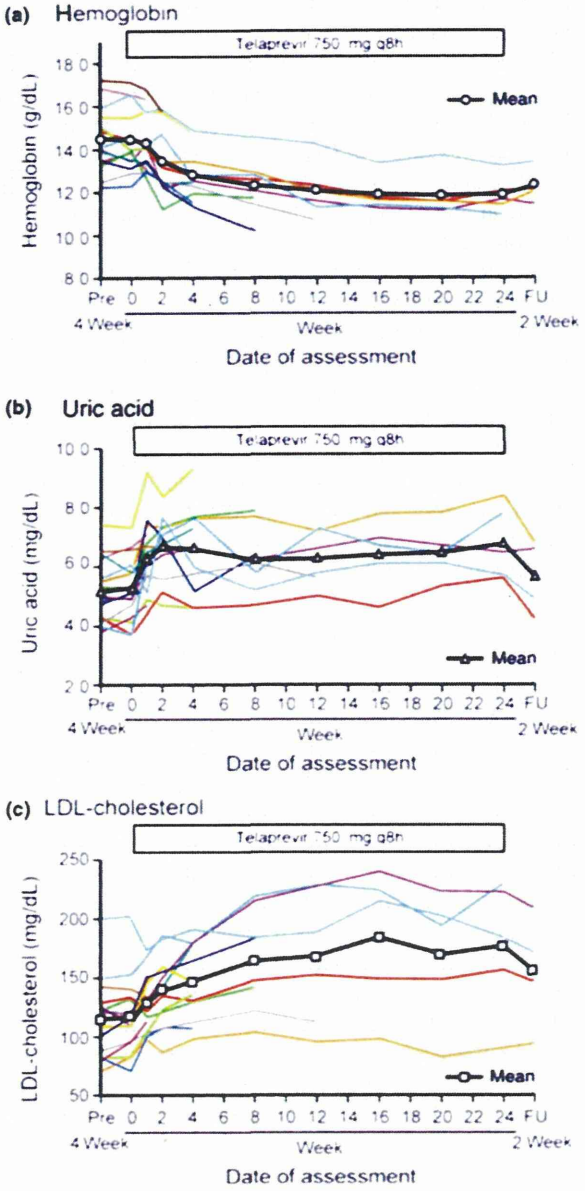

Fig. 3 Changes in (a) hemoglobin, (b) uric acid, (c) LDL-cholesterol.

viral breakthrough were A156F and T54A. One other patient with nadir HCV RNA level on Week 8 had a predominant clone of T54A + I132L after viral breakthrough. Among the five patients who completed the telaprevir treatment for 24 weeks as scheduled, two patients were HCV RNA positive at the end of treatment. One of these two patients had an A156F substitution at the end of treatment, and a A156Y substitution was also detected on Week 1 of the follow-up period. In the two patients who relapsed during the follow-up period, the predominant clone was T54A which shifted to the wild-type variant in one patient.

DISCUSSION

Although higher SVR rates and shorter duration of treatment were achieved by telaprevir in combination with PEG-IFN and RBV in US, EU and Japan [2–6], the DAA combination regimens also increased the frequency and severity of side effects usually observed in the PEG-IFN and RBV therapy. As most patients in Japan are aged people, IFN-free regimens are in urgent need because these patients are intolerant to IFN-based therapies [12–14].

In this exploratory study, one of 15 patients on telaprevir monotherapy was able to achieve SVR. A low viral load of <4 $\log_{10}$ IU/mL in this patient probably contributed to the achievement of SVR, and Suzuki *et al.* [15] published this case report in detail. Although the SVR rate obtained in the study was not beneficial enough, the telaprevir monotherapy could decrease HCV RNA levels dramatically in all cases. The severity of skin-related AEs during telaprevir monotherapy was milder than those of cases developing in the co-administration with PEG-IFN and RBV [5,6,16–18]. All the events were mild to moderate and manageable with antihistamines or topical steroids. Similarly to the skin-related events, decreases in haemoglobin levels were mild, and the incidence of anaemia was 46.7%. As all the anaemia events were mild, there was no need for discontinuation of telaprevir or use of any medications. Severe skin rash and anaemia observed in the therapy with telaprevir in combination with PEG-IFN and RBV are probably ascribable to the synergistic effect of these three drugs. Although the mechanism of uric acid and LDL cholesterol elevation during treatment with telaprevir has been established, these changes disappeared at the end of telaprevir dosing. Telaprevir was generally well tolerated in all the patients.

Amino acid substitutions in the HCV NS3 protease domain were monitored during the study. The relationship between these substitutions and resistance to NS3-4A protease inhibitors has been well documented by *in vitro*, *in vivo* and clinical studies [19–22]. In the eight patients who received the telaprevir monotherapy beyond 8 weeks, the predominant breakthrough variants were T54A and A156F, which were not observed at the earlier time points (Fig. 4). Furthermore, in the clones accounting for more than 10% of each specimen, the secondary substitution of V158I and I132L was identified along with the primary resistant-associated substitution of A156T/V and T54A, respectively, and a novel substitution of A156Y was also observed. This study confirms the higher genetic barrier of HCV subtype 1b against the V36M ± R155K substitutions. Our results clearly indicate that the prolonged telaprevir monotherapy leads to the development of various variants. As the replication fitness of drug-resistant variants tends to be lower than that of wild type, the former are likely to be overtaken by the wild-type virus under drug-free conditions within 3–7 months [11,23,24]. As Ozeki *et al.* [25] reported that four patients with favourable IL28B SNP who failed to eradicate HCV with telaprevir monotherapy were

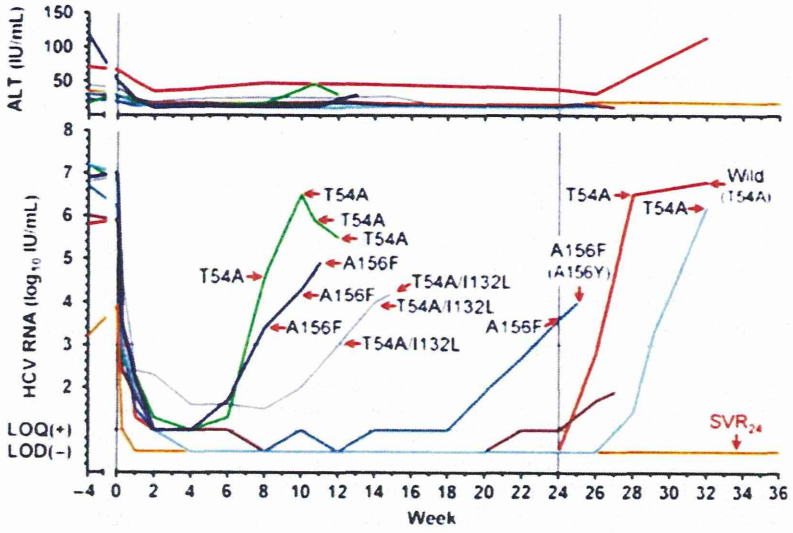

Fig. 4 Viral kinetics and predominant variants during and after telaprevir monotherapy beyond 8 weeks. Besides predominant clones, minority clones which account for 10% and more in a specimen are also summarized by brace notation. Putative secondary resistant-associated mutation is indicated by underline.

responsive to sequential therapy with PEG-IFN and RBV, the substitutions in the NS3 protease domain by the telaprevir treatment are not correlated with resistance to PEG-IFN and/or RBV directly as described previously [23,24]. Sequential therapy with PEG-IFN and RBV after relapse or viral breakthrough on telaprevir monotherapy might be a therapeutic option in some cases, including the case of low haemoglobin. By taking the error-prone nature of HCV replication into account, successful eradication with IFN-free DAA(s) regimens probably depends on how efficiently DAA can suppress various DAA-resistant variants that pre-exist and are selected under DAA pressure. The telaprevir-based combination therapy with other DAA(s) such as NS5A or NS5B polymerase inhibitors may be useful for successful treatment. Using a human chimeric liver mouse model for HCV infection, Ohara *et al.* [26] reported that the combination of telaprevir with a high-dose nucleoside analogue could successfully eradicate HCV infection. Recently, it was reported that the dual therapy with daclatasvir, an NS5A replication complex inhibitor, and asunaprevir, NS3-4A protease inhibitor, had high SVR rates in difficult-to-treat patients with subtype 1b and null responders [27,28]. These successful results are also helpful for us to consider telaprevir-based IFN-free regimens in combination with other DAAs against HCV.

In conclusion, telaprevir monotherapy was well tolerated and provided potent but temporary antiviral activity in Japanese patients with subtype 1b HCV, with an SVR rate of 7%. Most AEs were mild to moderate and much milder than those recorded in patients on combinations with PEG-IFN and RBV. As the essential characteristics of DAAs including telaprevir are substantially masked in the co-administration with other antivirals, the knowledge obtained from the long-term telaprevir monotherapy is most likely to contribute to the future HCV treatment with DAA-based regimens.

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Original Article

Changes in Plasma Vascular Endothelial Growth Factor at 8 Weeks After Sorafenib Administration as Predictors of Survival for Advanced Hepatocellular Carcinoma

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BACKGROUND: A new predictive biomarker for determining prognosis in patients with hepatocellular carcinoma (HCC) who receive sorafenib is required, because achieving a reduction in tumor size with sorafenib is rare, even in patients who have a favorable prognosis. Vascular endothelial growth factor (VEGF) receptor is a sorafenib target. In the current study, the authors examined changes in plasma VEGF concentrations during sorafenib treatment and determined the clinical significance of VEGF as a prognostic indicator in patients with HCC. **METHODS:** Plasma VEGF concentrations were serially measured in 63 patients with advanced HCC before and during sorafenib treatment. A plasma VEGF concentration that decreased >5% from the pretreatment level at 8 weeks was defined as a "VEGF decrease." An objective tumor response was determined using modified Response Evaluation Criteria in Solid Tumors 1 month after the initiation of therapy and every 3 months thereafter. **RESULTS:** Patients who had a VEGF decrease at week 8 (n = 14) had a longer median survival than those who did not have a VEGF decrease (n = 49; 30.9 months vs 14.4 months; $P = .038$). All patients who had a VEGF decrease survived for >6 months, and the patients who had both a VEGF decrease and an α-fetoprotein response (n = 6) survived during the observation period (median, 19.7 months; range, 6.5-31.0 months). In univariate analyses, a VEGF decrease, radiologic findings classified as progressive disease, and major vascular invasion were associated significantly with 1-year survival; and, in multivariate analysis, a VEGF decrease was identified as an independent factor associated significantly with survival. **CONCLUSIONS:** A plasma VEGF concentration decrease at 8 weeks after starting sorafenib treatment may predict favorable overall survival in patients with advanced HCC. ***Cancer*** **2014;120:229-37.** © 2013 The Authors. *Cancer* published by Wiley Periodicals, Inc. on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

KEYWORDS: antiangiogenic therapy, biomarker, hepatocellular carcinoma, prognosis, α-fetoprotein.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver (70%-85%) and a major cause of mortality. It is the fifth and seventh most frequent cancer and the second and sixth most frequent cause of cancer death in men and women, respectively.[1] At early stages or at Barcelona Clinic Liver Cancer stage A, a 5-year survival rate of 60% to 70% can be achieved in well selected patients with HCC who undergo surgical therapies (liver resection or transplantation) or locoregional procedures (ie, radiofrequency ablation).[2] However, treatment of advanced HCC that is not amenable to surgical or locoregional therapies remains a challenge in clinical practice.

Sorafenib is an oral, small-molecule tyrosine kinase inhibitor that blocks the synthesis of several intracellular proteins considered to be important for tumor progression, including the platelet-derived growth factor receptor beta, raf kinase, and the vascular endothelial growth factor (VEGF) receptor. VEGF is a homodimetric glycoprotein with a molecular weight of 45 kDa. The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and a structurally related molecule: placental growth factor. Three high-affinity VEGF tyrosine kinase receptors (VEGFRs) have been identified:

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VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-2 is the principal receptor that promotes the proangiogenic action of VEGF-A and has been the principal target of antiangiogenic therapies, although additional studies have underlined the importance of signaling through VEGFR-1. In 2 phase 3, placebo-controlled, randomized trials, sorafenib treatment significantly improved the time to tumor progression (TTP) and overall survival (OS) of patients with advanced HCC.[3,4] In those trials, however, no statistically significant pretreatment factors that predicted responses after patients started receiving sorafenib were identified.[5] Therefore, in clinical practice, it is extremely important to identify a predictive post-treatment biomarker that is associated with the treatment efficacy of sorafenib and the prognosis of patients after they start receiving sorafenib.

In general, the efficacy of treating solid tumors with systemic chemotherapy agents is assessed by radiologic findings. In 2010, Lencioni and Llovet published a modification of the Response Evaluation Criteria in Solid Tumors (RECIST).[6] However, the modified RECIST can be used only for typical HCC. Advanced HCCs often have atypical vascular patterns; therefore, evaluating tumor response to sorafenib is difficult with radiologic findings alone. Alternatively, α-fetoprotein (AFP) is the most popular tumor marker for HCC, and it has been reported that early AFP responses are a useful surrogate marker for predicting treatment response and prognosis in patients with advanced HCC who receive cytotoxic and antiangiogenic agents.[7-9] However, approximately 30% of patients with advanced HCC in the Sorafenib HCC Assessment Randomized Protocol (SHARP) trial had normal AFP concentrations.[10] Therefore, the identification of a new biomarker that can complementarily predict the efficacy of sorafenib and the prognosis of patients is necessary.

In a mouse model, an increase in hepatic VEGF levels was observed at 24 hours, 72 hours, and 120 hours after the administration of sorafenib,[11] suggesting that a change in VEGF levels may also occur during sorafenib therapy in humans. Therefore, we evaluated plasma VEGF changes during sorafenib treatment in patients with advanced HCC to determine whether VEGF has potential as a new biomarker for the prediction of treatment efficacy and prognosis after sorafenib administration.

MATERIALS AND METHODS

Patient Selection

Between December 2009 and August 2012, 95 consecutive patients with advanced, inoperable HCC received treatment with sorafenib at Musashino Red Cross Hospital. The diagnosis of HCC was based on guidelines established by the Liver Cancer Study Group of Japan[12] and the American Association for the Study of Liver Diseases[13] or by pathologic examination. According to these guidelines, a diagnosis of HCC is confirmed by histology or by characteristic radiologic findings, such as typical arterial enhancement of the tumor followed by a washout pattern in the images in the portal venous phase or the equilibrium phase on dynamic spiral computed tomography (CT) imaging or contrast-enhanced magnetic resonance imaging. Inclusion criteria were predefined as follows: 1) patients were alive 8 weeks after beginning treatment; and 2) patients had plasma VEGF and serum AFP concentrations evaluated at baseline, at 4 weeks, and at 8 weeks. Of 95 patients, 23 were unavailable for a week-8 VEGF measurement for the following reasons: 7 patients stopped sorafenib therapy because of erythema multiforme (grade 2-3) and started other therapies (radiation therapy or cytotoxic chemotherapy) within 1 month after starting sorafenib, 4 patients moved to another location before week 8, 5 patients refused to undergo a plasma VEGF measurement at week 8, and 7 patients were not available for obtaining VEGF concentration results. These 23 patients and 9 other patients who died within 8 weeks were excluded from the study. Hence, in total, 63 patients fulfilled the inclusion criteria. At enrollment, all patients had metastatic or locally advanced HCC that was not amenable to surgery or locoregional therapies, including transcatheter arterial chemoembolization (TACE) and local ablation. Written informed consent was obtained from all patients, and the ethics committee at Musashino Red Cross Hospital approved the study in accordance with the Declaration of Helsinki.

Sorafenib Treatment

The initial daily dose of sorafenib was 800 mg in 28 patients, 400 mg in 28 patients, and 200 mg in 7 patients. A reduced initial dose was allowed for patients who had the following factors: advanced age (≥80 years), gastrointestinal varices with a risk of bleeding, low body weight (<50 kg), and a poor performance status (≥2). In total, 60 patients underwent multiphase-multidetector CT imaging before starting sorafenib, 1 month after starting sorafenib, and every 3 months thereafter. Radiologic responses to therapy were evaluated according to modified RECIST. In all patients, serial measurements of plasma VEGF and serum AFP concentrations were performed before and after the receipt sorafenib and every month thereafter, with an allowance of ± 1 week. The endpoint of the current study was OS. In the follow-up visit after sorafenib administration, the medication was discontinued if progressive disease

(PD) was identified despite treatment, if intolerable adverse events occurred, or if inappropriate liver function was observed. Other palliative treatments or best supportive care were provided subsequently. An AFP response was defined as a decrease ≥20% in the serum AFP concentration during 8 weeks of treatment.

Plasma VEGF Measurements

Serial serum samples were collected prospectively from each patient. Venous blood samples were drawn into a serum separator tube and centrifuged at ×1800*g* for 10 minutes, and plasma samples were stored at −80°C until measurement. Plasma VEGF concentrations were measured quantitatively using an enzyme-linked immunosorbent assay kit (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions. We defined a decrease in the plasma VEGF level >5% from the pretreatment level at 8 weeks as a "VEGF decrease."

Statistical Analysis

Categorical variables were compared using the chi-square test, and continuous variables were compared using the Mann-Whitney test. All tests of significance were 2-tailed, and P values < .05 were considered statistically significant. OS curves were calculated using the Kaplan-Meier method, and differences between groups were assessed using the log-rank test. OS was determined as the interval between the date of treatment initiation and either death or the last visit. A Cox proportional-hazards model was used to determine the factors associated with OS. In univariate analyses, clinical and biologic parameters (sex, age, etiology, albumin, bilirubin concentrations, Child-Pugh class, plasma VEGF concentrations, and serum AFP concentrations) and tumor factors (vascular invasion and distant metastasis) were included. A logistic regression model was used to identify the factors associated with 1-year survival after the receipt of sorafenib. All statistical analyses were performed using StatView (version 5.0) software (Abacus Concepts, Berkeley, Calif).

RESULTS

Patient Characteristics

In total, 63 patients were enrolled in this study, and their characteristics are listed in Table 1. The diagnosis of HCC was confirmed by histology in 11 patients and by typical radiologic findings based on established guidelines in the remaining 52 patients. In all, 51 patients had previously received other therapeutic modalities, including 22 patients who previously received radiofrequency ablation, 22 who previously underwent TACE, 1 who previously received transcatheter arterial chemoinfusion, and 6 who previously underwent hepatic resection. Twelve patients had received sorafenib as initial therapy for HCC. Among the 63 enrolled patients, 33 were seropositive for hepatitis C virus antibody, 8 were seropositive for hepatitis B surface antigen, and 22 were seronegative for both hepatitis C virus antibody and hepatitis B surface antigen. Eighteen patients had evidence of extrahepatic metastasis, and 18 had major vascular invasion. No patient was lost to follow-up in this study.

TABLE 1. Characteristics of Study Patients With Advanced Hepatocellular Carcinoma (n = 63)

Characteristic	Median [Range]
Age, y	70 [40-85]
Sex: No. of men (%)	53 (84.1)
Baseline AFP, ng/mL	114 [2.0-98440]
Baseline plasma VEGF, pg/mL	288 [60-1580]
Treatment duration, mo	4.1 [0.1-28.3]
Overall survival, mo	9.3 [2.0-30.9]

Abbreviations: AFP, α-fetoprotein; VEGF: vascular endothelial growth factor.

Pretreatment Plasma VEGF Concentration and Prognosis and Extent of Hepatocellular Carcinoma

Pretreatment plasma VEGF concentrations in the 9 patients who died within 8 weeks were significantly higher than in the patients who survived beyond 8 weeks (813 ± 630 pg/mL vs 384 ± 18 pg/mL; P = .0024). Consistent with a previous study (the SHARP trial; Llovet et al[3]), our data suggested that the pretreatment plasma VEGF concentration is a useful prognostic factor for sorafenib therapy. However, there was no significant difference in OS between patients who had pretreatment plasma VEGF concentrations ≤450 pg/mL (n = 46) and those who had concentrations >450 pg/mL (n = 17; P = .731). The pretreatment plasma VEGF concentration could not predict prognosis for the patients who survived beyond 8 weeks.

We compared the size and extent of HCC between patients who had low plasma VEGF concentrations (≤450 pg/mL) and high plasma VEGF concentrations (>450 pg/mL). No difference was observed in the size or extent of HCC at baseline between patients with lower versus higher pretreatment plasma VEGF concentrations.

Association Between Changes in Plasma VEGF Concentrations and Overall Survival

The median OS assessed by the Kaplan-Meier method was 16.3 months for all 63 patients enrolled in the study

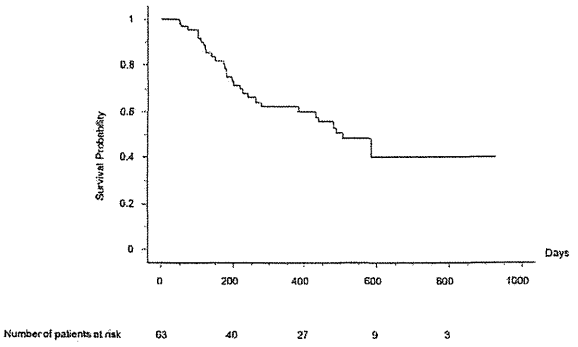

Figure 1. This Kaplan-Meier plot illustrates overall survival for all patients in the study.

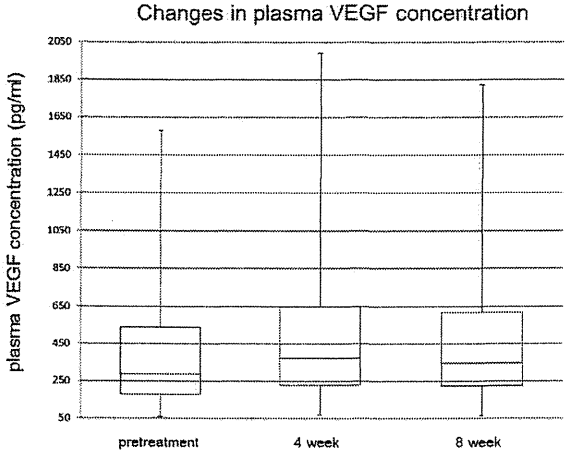

Figure 2. Changes in plasma vascular endothelial growth factor (VEGF) concentrations are illustrated.

(Fig. 1). Plasma VEGF concentrations at baseline, at 4 weeks, and at 8 weeks after the initiation of sorafenib treatment were 288 pg/mL (range, 60-1580 pg/mL), 372 pg/mL (range, 69-1990 pg/mL), and 347 pg/mL (range, 64-1840 pg/mL), respectively (Fig. 2). Plasma VEGF concentrations increased within 4 weeks after the administration of sorafenib in 47 of 63 patients (74.6%). The median survival of patients who had a decrease in their plasma VEGF concentration at week 4 (n = 16) and an increase in their plasma VEGF concentration at week 4 (n = 47) were 19.5 months and 16.8 months, respectively; and there was no significant difference in OS between changes in plasma VEGF at 4 weeks ($P = .645$). However, patients who had a VEGF decrease at week 8 (n = 14) had a longer median survival than those who did not have a VEGF decrease (n = 49; 30.9 months vs 14.4

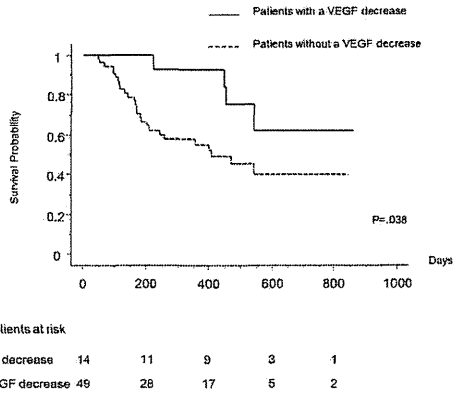

Figure 3. This Kaplan-Meier plot illustrates overall survival according to changes in vascular endothelial growth factor (VEGF) concentration.

months; $P = .038$) (Fig. 3), suggesting that a decrease in VEGF concentration 8 weeks after starting sorafenib treatment is closely associated with a favorable prognosis. The median percentage of decrease in the plasma VEGF concentration was 18.3% (range, 7%-41.7%). There were no differences in any pretreatment patient characteristics, including HCC stage and Child-Pugh score, between patients who did and did not have a VEGF decrease (Table 2).

Relation Between Radiologic Findings or Serum α-Fetoprotein Concentration and Overall Survival

The best radiologic responses to therapy assessed by modified RECIST were classified as a complete response (CR) (n = 4), a partial response (PR) (n = 16), stable disease (SD) (n = 34), and PD (n = 9). Fourteen patients had a VEGF decrease, and their best radiologic responses were a CR (n = 2), a PR (n = 2), SD (n = 9), and PD (n = 1). There was no significant difference in OS between the patients who had an objective response (CR + PR) and those with SD. The survival of patients who had PD was significantly worse than that of the patients without PD (median OS, 5.8 months and 19.4 months, respectively; $P = .0006$). There was no significant difference in OS between patients who had an AFP response and those who did not have an AFP response within the group that did not have PD (ie, those who attained a CR, a PR, or SD [the non-PD group]) (Fig. 4). There also was no significant difference ($P = .111$) between patients who did and did not have an AFP response among those in the non-PD group who had had an elevated AFP at baseline.

TABLE 2. Characteristics of Patients Categorized According to Variation in Vascular Endothelial Growth Factor Levels at 8 Weeks of Sorafenib Treatment

Characteristic	No. of Patients (%)		
	With VEGF Decrease, n = 14	Without VEGF Decrease, n = 49	*P*
Age, y	72	69	.325
Sex: Men	11 (78.6)	42 (85.7)	.679
Body weight, kg	58.3	62.3	.175
Cause of disease			.210
Hepatitis B	0 (0)	8 (16.3)	
Hepatitis C	9 (64.3)	24 (49)	
Other	5 (35.7)	17 (34.7)	
Prior treatment			.797
Yes	11 (78.6)	40 (81.6)	
No	3 (21.4)	9 (18.4)	
Baseline bilirubin, mg/dL	0.8	1.0	.375
Baseline albumin, g/dL	3.4	3.6	.190
Child-Pugh score			.178
5	7 (50)	30 (61.2)	
6	7 (50)	16 (32.7)	
7	0 (0)	3 (6.1)	
Maximum tumor size, cm			.892
≤5	8 (57.1)	22 (44.9)	
>5	6 (42.9)	27 (55.1)	
No. of tumors			.883
≤3	10 (71.4)	34 (69.4)	
>3	4 (28.6)	15 (30.6)	
Extrahepatic disease			.502
Yes	3 (21.4)	15 (30.6)	
No	11 (78.6)	34 (69.4)	
Site of metastatic disease			
Lung	1	7	
Bone	1	4	
Lymph node	1	3	
Lung and bone	0	1	
Major vascular invasion			.739
Yes	3 (21.4)	15 (30.6)	
No	11 (78.5)	34 (69.4)	

Abbreviations: VEGF: vascular endothelial growth factor.

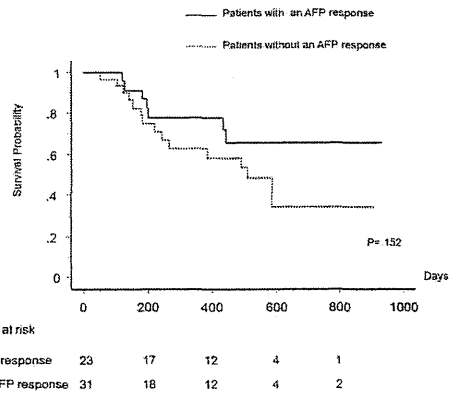

Figure 4. This Kaplan-Meier plot illustrates overall survival according to α-fetoprotein (AFP) response in patients without progressive disease (PD), classified as non-PD (ie, those who had a complete response, a partial response, or stable disease) according to modified Response Evaluation Criteria in Solid Tumors.

It is noteworthy that all patients who had a VEGF decrease and an AFP response survived during the observation period (median, 19.7 months; range, 6.5-31.0 months). In patients without a VEGF response (n = 49), there was no significant difference in OS between those who did and did not have an AFP response ($P = .147$). Of 49 patients who did not have a VEGF decrease at 8 weeks, 19 patients were able to survive beyond 1 year after starting sorafenib. Nine patients without a VEGF decrease at 8 weeks survived for >18 months.

Prognostic Factors After Sorafenib Administration

In univariate analysis, among all patients, a VEGF decease and an AFP response were associated significantly with OS after starting sorafenib. Major vascular invasion and PD, as evidenced by radiologic findings after sorafenib administration, also were significant prognostic factors. To predict which patients would have a highly favorable prognosis, the prognostic factors associated with 1-year survival after starting sorafenib were assessed in univariate and multivariate analyses. In the univariate analysis, a VEGF decrease, PD, and major vascular invasion were associated significantly with survival (Table 3). In the multivariate analysis, which was performed using those factors as covariates, a VEGF decrease was identified as an independent factor associated significantly with survival (Table 3). There was a significant difference in OS among the 3 groups (patients with a VEGF decrease and non-PD, patients without a VEGF decrease but non-PD, and patients without a VEGF decrease and PD; $P = .0013$) (Fig. 5). Only 1 patient who had a VEGF decrease was classified with PD. All 4 patients who had a VEGF decrease and an objective response (CR or PR) were able to survive during the observation period.

Adverse Events During Sorafenib Treatment

The overall incidence of treatment-related adverse events was 100%. The rate of discontinuation of sorafenib as a result of adverse events was 22.2%. Adverse events that led to the discontinuation of sorafenib treatment were liver dysfunction (63.6%), hand-foot skin reaction (18.2%), interstitial pneumonia (9.1%), and rash (9.1%). Dose reductions because of adverse events occurred in 62 patients. The most frequent adverse event leading to dose reductions was liver dysfunction (33.9%). In addition,

TABLE 3. Prognostic Factors Associated With 1-Year Survival After Sorafenib Administration

Risk Factor	OR (95% CI)[a]	*P*
Univariate analysis		
Age, by every 10 y	1.47 (0.75-2.87)	.266
Sex		
Women	1.00	
Men	0.26 (0.50-1.39)	.116
HBV infection		
Negative	1.00	
Positive	0.33 (0.06-2.02)	.231
HCV infection		
Negative	1.00	
Positive	1.23 (0.41-3.74)	.714
Albumin, by every 1 g/dL,	1.34 (0.45-3.99)	.604
Total bilirubin, by every 1 mg/dL	0.79 (0.28-2.25)	.656
Pre-AFP, by every 10 ng/mL	1.00 (1.00-1.00)	.161
Tumor size, cm		
<5	1.00	
≥5	0.42 (0.14-1.32)	.147
No. of tumors		
≤3	1.00	
≥4	0.26 (0.06-1.08)	.064
Major vascular invasion		
Yes	1.00	
No	4.00 (1.12-14.4)	.034
Extrahepatic metastasis		
Yes	1	
No	1.82 (0.56-5.90)	.320
5% VEGF decrease at wk 8		
No	1.00	
Yes	11.1 (1.29-94.6)	.028
PD		
No	1.00	
Yes	0.16 (0.29-0.86)	.033
Objective response: CR + PR		
No	1.00	
Yes	1.63 (0.49-5.42)	.426
AFP response		
No	1.00	
Yes	2.76 (0.80-9.52)	.107
Multivariate analysis[b]		
5% VEGF decrease at wk 8		
No	1.00	
Yes	10.0 (1.02-91.3)	.041
PD		
No	1.00	
Yes	0.20 (0.29-1.39)	.104
Major vascular invasion		
Yes	1.00	
No	3.03 (0.71-12.9)	.134

Abbreviations: AFP, α-fetoprotein; CI, confidence interval; CR, complete response; HBV, hepatitis B virus; HCV, hepatitis C virus; PD, progressive disease; PR, partial response; VEGF, vascular endothelial growth factor.
[a] The ORs for 1-year survival were calculated using logistic regression analysis.
[b] In the multivariate logistic analysis, a 5% VEGF decrease, PD, and portal invasion were included as covariates.

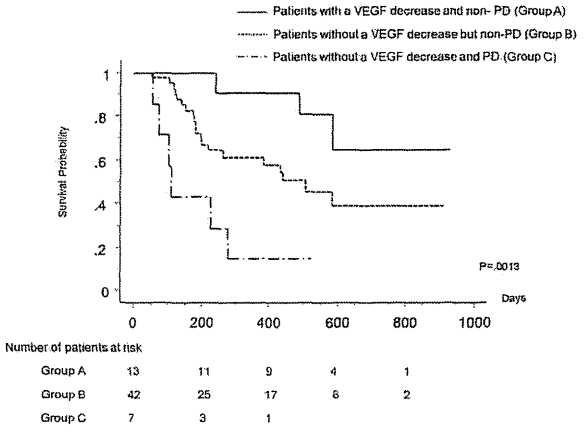

Figure 5. This Kaplan-Meier plot illustrates overall survival according to the combination of vascular endothelial growth factor (VEGF) changes and radiologic findings classified by modified Response Evaluation Criteria in Solid Tumors. Non-PD indicates patients who did not have progressive disease (PD) (ie, those who had a complete response, a partial response, or stable disease).

the incidence of adverse events was not related to plasma VEGF concentrations.

DISCUSSION

In the current study, we demonstrated that plasma VEGF concentrations change dynamically during sorafenib therapy, and changes in VEGF concentration are closely associated with OS in patients who receive treatment with sorafenib. VEGF is the major mediator of angiogenesis in HCC, and several studies have correlated VEGF concentrations with the prognosis of patients who have advanced HCC.[5,14-21]

Recently, a new staging system was proposed that includes the plasma VEGF concentration along with the Cancer of the Liver Italian Program (CLIP) score; this new system—known as the V-CLIP score—classifies patients with advanced HCC more appropriately into a homogeneous prognostic group.[22] Therefore, the concentration of circulating VEGF is included as a candidate prognostic marker for HCC, especially in patients with advanced disease. The objective of our study was to elucidate the important question of whether an on-treatment change in VEGF is a potentially useful new biomarker for predicting prognosis in patients who survive beyond 8 weeks, because such an on-treatment predictor among patients who have relatively longer survival has not yet been elucidated. In this study, plasma VEGF concentrations increased from pretreatment levels within 4 weeks of starting sorafenib in 47 of 63 patients (74.6%). This was followed by a decrease in plasma VEGF levels at 8 weeks in 68.1% of patients. A possible mechanism of this transient increase in VEGF after starting sorafenib may be related to a reactive increase against the inhibition of VEGF activity or hypoxia induced by sorafenib. This

hypothesis is supported by the demonstration that plasma VEGF concentrations increased shortly after treatment with TACE.[24-26] It is believed that these increases in plasma VEGF concentration are related to the induction of tissue hypoxia.[27] However, the peak time point of VEGF elevation during sorafenib administration was different from that previously reported in TACE, in which a transient elevation of VEGF was observed within 7 days after TACE.[24-26] This observed difference may be related to the continuous induction of hypoxia by sorafenib administration.

It is noteworthy that, in our study, decreases in plasma VEGF observed within 8 weeks of sorafenib administration were associated with better OS. One possible reason for this association may be that the decrease in VEGF concentrations reflects a decrease in the number of tumor cells secreting VEGF. An association between changes in VEGF concentrations and disease progression was observed in a previous study of an anti-VEGF antibody, bevacizumab, in patients with advanced HCC.[23] In that study, plasma VEGF-A concentrations decreased from baseline in all patients after 8 weeks of bevacizumab therapy and increased to near baseline levels in 5 of 6 patients at the time of disease progression. Unfortunately, plasma VEGF-A levels after 8 weeks of bevacizumab in that study were available for only 8 of 46 patients who were enrolled the study, and plasma VEGF-A levels after 4 weeks were not evaluated. In our study, all patients were evaluated before and every 4 weeks after starting sorafenib. Moreover, we demonstrated the usefulness of plasma VEGF concentrations at 8 weeks and not at 4 weeks. Zhu et al[28] reported that plasma levels of VEGF and placental growth factor increased after cediranib, a pan-VEGFR tyrosine kinase inhibitor monotherapy for advanced HCC. In that study, progression-free survival was correlated inversely with baseline levels of VEGF, soluble VEGFR2 (sVEGFR2), and basic fibroblast growth factor and with on-treatment levels of basic fibroblast growth factor and insulin-like growth factor-1; and progression-free survival was directly associated with on-treatment levels of interferon-γ. Because changes of VEGF concentrations during therapy were not identified as a prognostic factor in the study by Zhu et al, biomarkers that predict prognosis may be different among different types of tyrosine kinase inhibitors. Jayson et al[29] reported that plasma VEGF-A in patients who received bevacizumab was potentially predictive and prognostic in metastatic breast, gastric, and pancreatic cancers; however, it was only prognostic (and not predictive) in metastatic colorectal cancer, nonsmall cell lung cancer, and renal cell carcinoma. In our study, we measured plasma VEGF concentrations and not plasma VEGF-A concentrations. Sorafenib is a multikinase inhibitor, whereas bevacizumab is a humanized monoclonal antibody that recognizes and blocks VEGF-A expression. Further studies to evaluate the clinical usefulness of determining VEGF and VEGF-A concentrations during sorafenib therapy are necessary in various cancers. Although the precise mechanism underlying the association between serial changes in VEGF and disease progression is unclear, the findings of the current study are extremely valuable for clinical practice in predicting the prognosis of patients who receive treatment with sorafenib.

Llovet et al[5] studied plasma biomarkers as predictors of outcome in patients with advanced HCC. They measured plasma biomarkers in 491 patients at baseline and in 305 patients after 12 weeks in a phase 3, randomized, controlled trial (the SHARP trial). Those authors concluded that angiopoietin-2 and VEGF were independent predictors of survival in patients with advanced HCC and that none of the tested biomarkers significantly predicted response to sorafenib. In our study, by measuring plasma VEGF monthly, we demonstrated that the changes 8 weeks after starting sorafenib were important for predicting OS.

It has been reported that modified RECIST guidelines are useful for predicting efficacy and prognosis after patients with advanced HCC receive treatment with sorafenib.[30] However, modified RECIST can only be used for typical hypervascular HCC, and not for atypical HCC, including poorly differentiated HCC and diffuse-type HCC. Moreover, the percentage of patients in our study who had PD was only 11.1% (9 of 63 patients), and the objective response rate (CR + PR vs SD) could not predict OS, suggesting that using only modified RECIST guidelines was insufficient for predicting OS in most patients who received sorafenib (non-PD patients). Therefore, it is important to identify a predictive biomarker for those patients who can expect long survival during sorafenib therapy, although their radiologic findings may not be categorized as objective responses.

From this point of view, decreases in VEGF observed in non-PD patients at week 8 may identify patients who have a favorable prognosis. According to our results, the median survival of patients who had a VEGF decrease was extremely good at 31.0 months, and we demonstrated that a VEGF decrease, but not modified RECIST or AFP, was the only significant post-therapeutic factor associated with favorable survival after sorafenib administration (Table 3). In our study, all

patients who had both a VEGF decrease and an AFP response survived during the observation period (median, 19.7 months). Taken together, the combination of a plasma VEGF decrease, an AFP response, and modified RECIST is useful for predicting an extremely favorable prognosis.

This study had a few limitations. The first was our subanalysis of consecutive patients. However, the median survival for the 23 excluded patients who were available for estimation was equivalent to that of the included patients (16.8 months); therefore, it is unlikely that selection bias affected our results. The second limitation is that we measured only plasma VEGF concentrations. In previous studies, many factors, including VEGF-A, short VEGF-A isoform, sVEGFR1, sVEGFR2, sVEGFR3, angiopoietin-2, and insulin-like growth factor-2, were evaluated as biomarkers. However, to our knowledge, this is the first clinical study to demonstrate the early dynamic changes in plasma VEGF concentrations in patients who received sorafenib. Finally, the number of patients in this study was relatively small to make recommendations to physicians. Our results indicated that patients who have decreased VEGF concentrations at 8 weeks have a favorable prognosis, regardless of their radiologic findings. However, further studies with a larger number of patients will be necessary to propose new recommendations.

In conclusion, changes in plasma VEGF concentrations during sorafenib treatment are dynamic in patients with advanced HCC, and an observed decrease in the plasma VEGF concentration 8 weeks after starting sorafenib is associated significantly with favorable OS. Today, because many clinical trials of new molecular-targeted agents for HCC are being conducted, it is necessary for hepatologists and oncologists to determine the time when alternative agents should be started as a second or third line of treatment. Our results have potentially important clinical implications for physicians and may influence their decisions regarding a treatment strategy for advanced HCC in individual patients.

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CONFLICT OF INTEREST DISCLOSURES

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Hepatology Research

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Original Article

Impaired brain activity in cirrhotic patients with minimal hepatic encephalopathy: Evaluation by near-infrared spectroscopy

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Aim: Near-infrared spectroscopy (NIRS) is a tool that could non-invasively measure the regional cerebral oxygenated hemoglobin (oxy-Hb) concentration with high time resolution. The aim of the present study is to reveal the time-dependent regional cerebral oxy-Hb concentration change coupled with brain activity during task performance in patients with minimal hepatic encephalopathy (MHE).

Methods: Cerebral oxy-Hb concentration was measured by using NIRS in 29 cirrhotic patients without overt hepatic encephalopathy (HE). Of those, 16 patients who had abnormal electroencephalography findings were defined as having MHE. Responsive increase in oxy-Hb during a word-fluency task was compared between MHE and non-MHE patients.

Results: There was no difference in the maximum value of oxy-Hb increase between patients with and without MHE (0.26 ± 0.12 vs 0.32 ± 0.22 mM·mm, $P = 0.37$). However, the pattern of the time course changes of oxy-Hb was different between the two groups. The MHE group was characterized by a gradual increase of oxy-Hb throughout the task compared to steep and repetitive increase in the non-MHE group. Increase in oxy-Hb concentration at 5 s after starting the task was significantly small in the MHE group compared to the non-MHE (0.03 ± 0.05 vs 0.11 ± 0.09 mM·mm, $P = 0.006$).

Conclusion: The cerebral oxygen concentration is poorly reactive in response to tasks among cirrhotic patients without overt HE but having abnormal electroencephalography findings. These impaired responses in regional cerebral oxy-Hb concentration may be related to the latent impairment of brain activity seen in MHE.

Key words: hepatic encephalopathy, near-infrared spectroscopy

INTRODUCTION

HEPATIC ENCEPHALOPATHY (HE) is a major complication of liver cirrhosis. Apart from clinically overt HE (OHE), minimal HE (MHE) is troublesome because it is associated with reduced quality of life (QOL), reduced cognitive function, lowered work efficiency, higher risk of progression to OHE and may be a cause of traffic accidents.[1–3] MHE treatment can improve QOL, driving capability and progression of OHE.[4–6] Adequate diagnosis of MHE and early therapeutic intervention are precluded by the lack of reliable diagnostic standards, and HE is usually diagnosed only after the presentation of overt symptoms. For the diagnosis of MHE, neuropsychological function tests, such as number connection test, light/sound reaction time, inhibitory control test, Wechsler adult intelligence scale (WAIS) or electro-psychological tests

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Conflict of interest: The authors who participated in this study have had no affiliation with the manufacturers of the drugs involved either in the past or at present, and have not received funding from the manufacturers to conduct this research.

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including electroencephalography (EEG), cerebral evoked potential, p300 event-related potential, psychometric hepatic encephalopathy score (PHES) and critical flicker test[7–15] have been employed. Diagnostic specificity can be improved by combining these tests, but complexity becomes a major disadvantage.

Recent advances in diagnostic imaging, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), made it possible to map brain function in tomographic images with high space and time resolutions. Recent study using PET[16] revealed that the primary event in the pathogenesis of OHE is inhibition of cerebral energy metabolism evidenced by reduced cerebral oxygen consumption and reduced cerebral blood flow. Whether the same mechanism could be applied to MHE is not known. Near-infrared spectroscopy (NIRS) is a tool that could non-invasively measure the cerebral blood volume as an oxygenated hemoglobin (oxy-Hb) concentration. The space and time resolution of NIRS is equivalent or higher than that of PET and fMRI. Moreover, NIRS is highly portable, does not have any restriction in the posture and flexible in setting tasks. Therefore it is possible to perform tests in a natural environment and to evaluate brain function as reflected by the dynamic changes in regional cerebral oxy-Hb concentration in response to a given task. The latter may be especially important to disclose a latent abnormality of brain function.

Recent study suggested that astrocytes regulate the cerebral blood flow and provide the oxy-Hb to the activation site of the brain.[17–19] In hepatic encephalopathy patients, function of astrocyte is impaired which may lead to cerebral oxygen consumption and blood flow.[16,20–22] We hypothesized that clinically latent abnormality of brain function in MHE also may be linked to the impairment of adequate increase in cerebral energy metabolism in response to the stimulation for activating the brain due to impaired function of astrocytes. In the present study, we used NIRS to evaluate the latent abnormality of brain function in patients with MHE, by measuring the increase of regional cerebral oxy-Hb concentration in response to task stimulation.

METHODS

Patients

A TOTAL OF 29 liver cirrhosis patients without OHE were enrolled. The underlying etiology of liver disease was hepatitis C virus infection in 19 patients, hepatitis B virus infection in two, alcoholic liver disease in five and other liver disease in three. All participants were examined by two psychiatrists to exclude mental disorders. No patient had any history of taking antidepressants or other psychotropic drugs. Subjects were examined by brain MRI or brain CT and they had no apparent brain structural disease including brain infarction. The study was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of Musashino Red Cross Hospital and National Center of Neurology and Psychiatry. Informed consent was obtained from each subject. MHE was defined as those who had abnormal EEG findings. According to this definition, 16 patients were assigned to the MHE group and 13 were assigned to the non-MHE group. Table 1 shows the clinical characteristics of patients. The age and sex ratio did not differ between groups.

NIRS measurements

Concentration of oxy-Hb was measured by a 52-channel NIRS machine (Hitachi ETG4000; Hitachi Medical,

Table 1 Patient characteristics

	MHE ($n = 16$)	Non-MHE ($n = 13$)	*P*-value
Age	67.9 ± 8.9	70.1 ± 10.2	0.53
Sex (M/F)	7/9	7/6	0.72
Albumin (g/dL)	2.68 ± 0.39	3.63 ± 0.47	<0.0001
T-Bil (mg/dL)	1.83 ± 1.22	0.88 ± 0.34	0.011
PT%	64.5 ± 10.8	85.2 ± 12.7	<0.0001
Child–Pugh (A/B/C)	0/9/7	11/2/0	<0.0001
Etiology (HC/HB/Alc/Others)	8/2/4/2	11/0/1/1	0.28
NH3 (mmol/L)	90.1 ± 64.3	40.1 ± 18.3	0.012

Alc, alcoholic liver disease; HB, hepatitis B; HC, hepatitis C; MHE, minimal hepatic encephalopathy; PT%, prothrombin time percentage; T-Bil, total bilirubin.

Tokyo, Japan). NIRS detects changes in brain activity by capturing increases in regional cerebral blood flow caused by neural activity. For each channel, an optic fiber device is connected to an application probe that is placed on the subject's scalp. The 52 channels cover the frontal lobe, upper temporal lobe and anterior parietal lobe of the brain (Fig. 1). The near-infrared light penetrates the scalp and skull, passes through the brain tissue, and is partially absorbed by oxy-Hb. The reflected light is detected by a probe positioned 30 mm away from the application probe. The changes in concentration of oxy-Hb can be calculated by measuring reflected light.[23] In this study, the results measured by the seven channels which were previously reported to be diagnostic for mental disorders; (channels 36–38 and 46–49)[24–26] were selected for the analysis. The time-dependent changes in oxy-Hb concentration in each of these seven channels were compared between MHE and non-MHE patients. The sum of increase in oxy-Hb concentration in these seven channels was calculated and compared between MHE and non-MHE patients. For this analysis, increase of oxy-Hb at 5 s and maximum increase were used.

Activation task

A word-fluency task was used to stimulate frontal lobe activity. Subjects were instructed to generate as many words as possible with a given letter. For example, with a task involving "naming words starting with the letter 'T' ", subjects were given 20 s to say as many words as they could starting with the letter "T", such as "tomato", "tail" and "tea". Three tasks were presented for a total of 60 s. During the word-fluency test, the real-time changes in the oxy-Hb concentration were measured at each channel. Data are expressed as a wave form as well as in the form of topographic images.

Statistical analysis

The SPSS software package ver. 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Categorical data were analyzed using Fisher's exact test. Continuous variables were compared with Student's *t*-test. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

THE NUMBER OF words generated by the word-fluency task did not differ significantly between the MHE and non-MHE groups (10.8 ± 3.4 vs 10.7 ± 2.5 words, $P = 0.93$). Figure 2 shows the time-dependent changes in the oxy-Hb concentration during the task in the representative seven channels. The average value of the seven channels (36–38 and 46–49) is shown in Figure 2. These changes reflected frontal lobe activation by the word-fluency test and correspondingly elevated cerebral blood flow in the frontal lobe. In the non-MHE

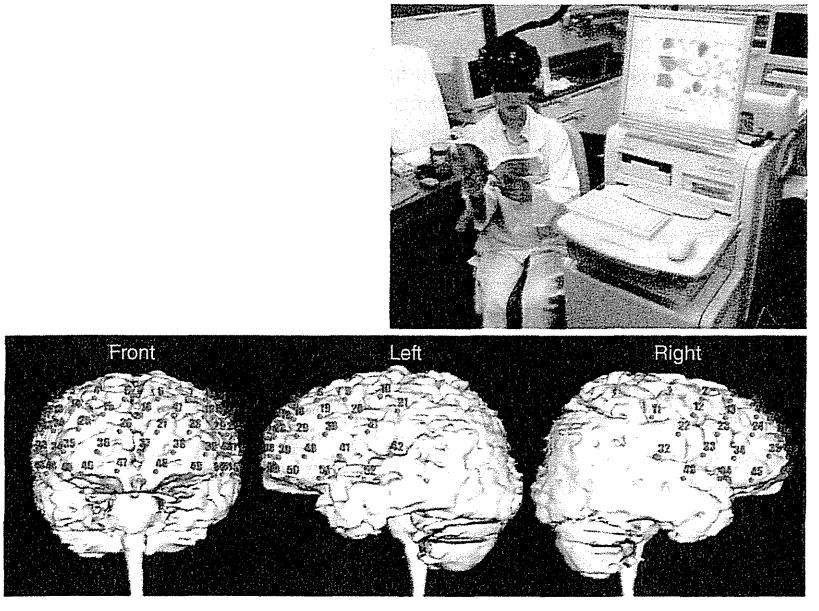

Figure 1 Near-infrared spectroscopy. An optic fiber device connected to a probe is placed on the subject's scalp covering the frontal to temporal regions. The relative concentration of oxygenated hemoglobin (oxy-Hb) was measured every 0.1 s during word-fluency testing.

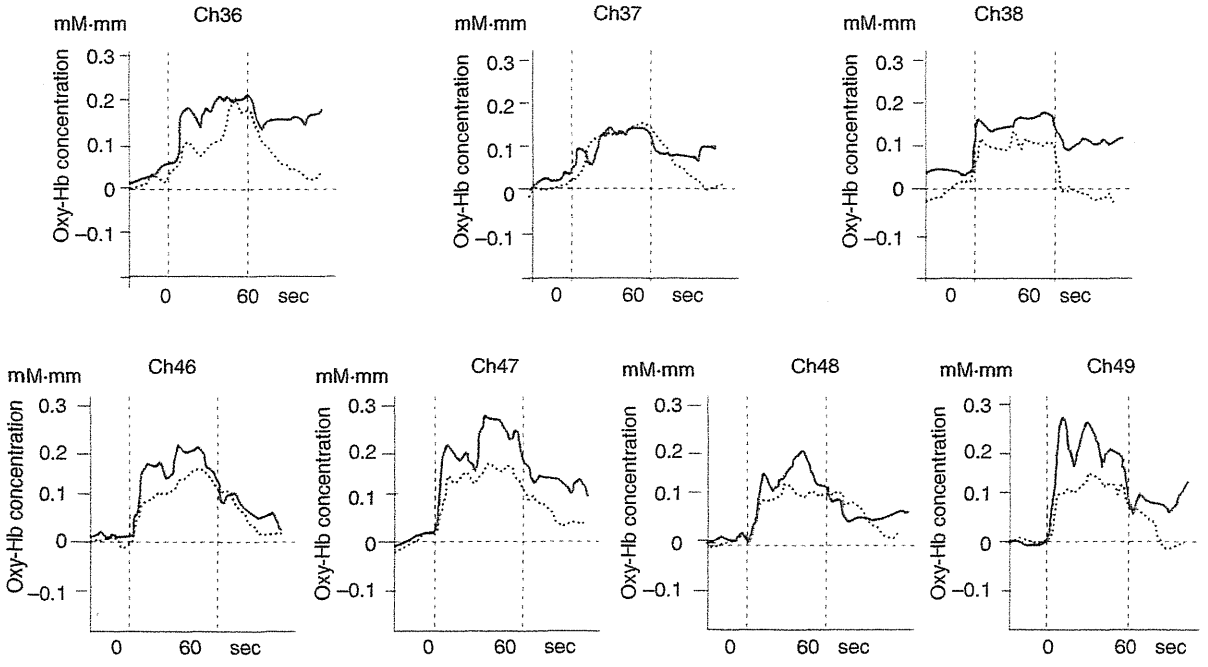

Figure 2 Time-dependent changes in oxygenated hemoglobin (oxy-Hb) concentration in response to tasks. The average waveforms of time-dependent changes in oxy-Hb concentration in representative channels (Ch) are shown. The solid and broken line represents non-minimal hepatic encephalopathy (MHE) and MHE groups, respectively. The area between the two vertical lines corresponds to the 60 s of the word-fluency test.

group, the oxy-Hb concentration increased immediately after the start of the task, remained high with repetitive steep peaks during the task, and decreased after the end of the task. In contrast, the time course of oxy-Hb changes was somewhat different in the MHE group, characterized by a slow increase of oxy-Hb throughout the task, gradually reaching a plateau at the end of the task (Fig. 2). These differences in the degree of oxy-Hb changes also could be visualized by the topographic presentation. In the topographic image, increase of oxy-Hb concentration is expressed as a deepening of the red shading. Figure 3 shows a topographic image showing the increase in oxy-Hb concentration in response to a task. The image in Figure 3 is the average value (arithmetic mean topographic image) of all patients. The concentration of oxy-Hb is small in the MHE group, as reflected by blue or green color, compared to the non-MHE group, as reflected by orange or red color.

When the average value of the seven channels were calculated, the maximum value of oxy-Hb increase was smaller in MHE compared to non-MHE patients but it did not reach statistical significance (0.26 ± 0.12 vs 0.32 ± 0.22 mM·mm, $P = 0.37$) (Fig. 4). On the other hand, increase in oxy-Hb concentration at 5 s after starting the task was significantly small in MHE compared to non-MHE patients (0.03 ± 0.05 vs

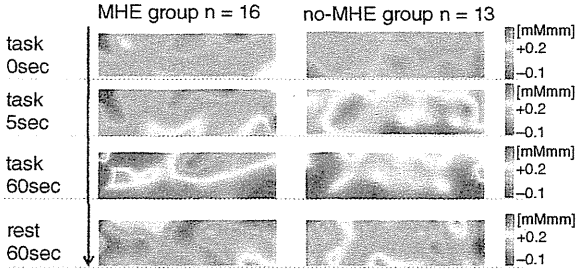

Figure 3 Topographic image showing cumulative increase in oxygenated hemoglobin (oxy-Hb) concentration. Increase in oxy-Hb concentration is shown by deepening of the red shading. The concentration of oxy-Hb is small in the minimal hepatic encephalopathy (MHE) group, as reflected by the blue or green color compared to the non-MHE group as reflected by orange or red color.

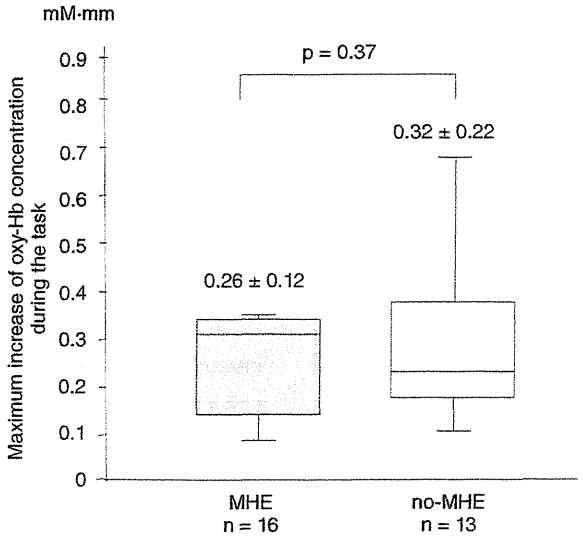

Figure 4 Comparison of maximum increase in oxygenated hemoglobin (oxy-Hb) concentration between patients with and without minimal hepatic encephalopathy (MHE). The average value of maximum increase in oxy-Hb did not differ significantly between the MHE and non-MHE groups.

0.11 ± 0.09 mM · mm, $P = 0.006$) (Fig. 5). For the diagnosis of MHE, the receiver–operator curve analysis identified an optimal cut-off of 0.05 mM · mm for the oxy-Hb concentration at 5 s after starting the task. The area under the curve was 0.774 ($P = 0.012$; 95% confidence interval, 0.60–0.95), sensitivity and specificity of NIRS for the diagnosis of MHE was 69% and 77%, respectively. The positive predictive value was 79% and negative predictive value was 67%.

DISCUSSION

USING NIRS, WHICH can detect changes in regional cerebral oxy-Hb concentration with an extremely high level of sensitivity, we found that increase in cerebral oxy-Hb concentration in response to tasks was slow and small among cirrhotic patients without OHE but having abnormal electroencephalography findings. The impairment of response was most significant at an early time point after the start of the task. These findings indicated that cerebral oxygen metabolism is poorly reactive in response to tasks among patients with MHE and that this impaired cerebral oxygen metabolism may be related to the pathogenesis of latent impairment of brain activity seen in MHE. To the best of our knowledge, our study appears to be the first evaluating MHE with NIRS. The non-invasiveness and high time resolution of NIRS give it potential as a valuable research tool for the examination of brain function in HE, as well as a clinically useful tool for the diagnosis of MHE.

Hepatic encephalopathy in its early stage, such as latent or minimal HE, can reduce cognitive function, lower work efficiency, reduce QOL[27,28] or impair driving skill.[1,2,29,30] Although there are several practical requirements for the diagnosis of MHE, adequate diagnosis of MHE is difficult due to the lack of reliable diagnostic standards.[31,32] Several diagnostic methods such as neuropsychological function tests, number connection test, light/sound reaction time, inhibitory control test, WAIS or electro-psychological tests including EEG, spectral EEG, and cerebral evoked potential, PHES, critical flicker test and computer-aided quantitative neuropsychological function test system (NP-test)[7–15] have been proposed,[32–36] but there is no ideal test for MHE as yet. Because these tests are developed for the screening of MHE, these are not diagnostic. Establishment of a reliable diagnostic method for MHE is imperative. We

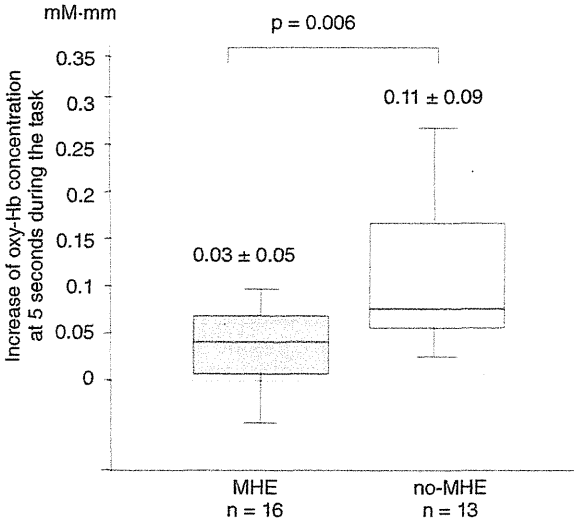

Figure 5 Comparison of increase in oxygenated hemoglobin (oxy-Hb) concentration at 5 s after the start of task between patients with and without minimal hepatic encephalopathy (MHE). The average value of increase in oxy-Hb was compared between the MHE and non-MHE groups at 5 s after starting the word-fluency task. The increase in the oxy-Hb concentration was significantly lower in patients with MHE compared to non-MHE ($P = 0.006$).

have some cases in which NIRS results improved with lactulose and branched-chain amino acid. A prospective study is ongoing to evaluate the effect of treatment by NIRS. The major advantage of NIRS over "paper and pencil tests" is the absence of learning effect which is generally seen in other neuropsychological function tests[37] and NIRS could also discriminate other mental disorders.[24,25]

Neuroimaging using MRI, magnetic resonance spectroscopy and PET has made it possible to non-invasively assess hepatic encephalopathy.[38–47] However, these tests require extensive equipment and are therefore costly. NIRS is a new methodology for brain research and brain function testing, and has applications in various areas of medicine, being used not only in research, but also in clinical medicine.[23–25,48] NIRS has been approved for identifying the language-dominant hemisphere before brain surgery and measuring epileptic foci.[49] In human studies comparing NIRS and fMRI,[50–52] a correlation was seen between blood-oxygen-level-dependent signal and oxy-Hb concentration as measured by NIRS. In brain function analysis, the detection sensitivity of NIRS is comparable to that of fMRI, but the time resolution of NIRS is greater. Furthermore, the advantages of NIRS are convenience, bedside analysis, non-invasiveness, free task setting and low cost.

Here, we used multichannel NIRS to measure the changes in oxy-Hb concentration during task performance from the frontal to temporal regions of the cortex in MHE patients and compared the results with those of liver cirrhosis without MHE. In all subjects, oxy-Hb increased during task performance and gradually decreased after the completion of task performance. However, the time-dependent changes in the degree of increase in oxy-Hb concentration differed between patients with and without MHE. The degree of increase in oxy-Hb concentration during task performance was smaller and more gradual in MHE compared to non-MHE patients. The increase of the oxy-Hb concentration reflects the increase of cerebral blood volume in the area of the brain activated by the task. Iversen *et al.* found that the cerebral oxygen consumption and blood flow were both reduced in cirrhotic patients with an acute episode of OHE[16]and that the oxygen delivery was approximately twice the oxygen consumption, indicating that oxygen delivery or blood flow was not a limiting factor for the oxygen consumption. Consequently, cerebral blood flow seems to be reduced as a result of diminished cerebral oxygen requirement during HE, and not vice versa.[16] It is reported that neuron-to-astrocyte signaling is a key mechanism in functional hyperemia,[17–19,53,54] and that function of astrocytes is impaired in hepatic encephalopathy patients.[20–22] Therefore, impaired astrocyte-mediated control of cerebral microcirculation can result in slow increase of cerebral blood flow during task performance in MHE patients. Thus, the sluggish increase in cerebral blood flow seen in MHE in the present study may reflect the impaired brain activity and dysfunction of astrocytes and impaired cerebral oxygen metabolism in these patients.

There are several limitations in the present study. The number of patients was not enough to make a comparison stratified by Child grade. We would like to analyze this important point in a future study. It may be possible that cerebral oxy-Hb may change due to aging or by the arteriosclerotic changes. In the present study, age was not related to NIRS results. All patients were examined by brain MRI or brain CT and they had no apparent brain structural disease including brain infarction. However, it was not possible to evaluate the arteriosclerotic changes. This may be another limitation of this study. Many neuropsychological function tests, such as number connection test, light/sound reaction time, inhibitory control test, WAIS or electro-psychological tests including EEG, cerebral evoked potential, p300 event-related potential, PHES and critical flicker test have been employed for the diagnosis of MHE. In Japan, Kato and colleagues established the computer-aided quantitative neuropsychological function test system called NP-test.[7] However, these tests were not simultaneously measured in the present study. Because we recognize the importance of comparing NIRS with other tests, we would like to solve this issue in future study.

In conclusion, NIRS, with its high degree of time resolution, enabled us to identify the characteristic time course of oxy-Hb concentration changes during tasks in MHE. The observations imply that cerebral oxygen supply and metabolism is poorly reactive in MHE, which may be related to the pathogenesis of latent impairment of brain activity.

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