Table 3. Adverse events developing in more than 15% of patients in either Groups A or B.

	A: T12PR24 (n = 126)		B: PR48 (n = 63)	
Anemia	115	(91.3%)	46	(73.0%)
Pyrexia	98	(77.8%)	46	(73.0%)
Leukocytopenia	86	(68.3%)	46	(73.0%)
Thrombocytopenia	81	(64.3%)	23	(36.5%)
Malaise	73	(57.9%)	30	(47.6%)
Serum uric acid increased	65	(51.6%)	5	(7.9%)
Serum hyaluronic acid increased	64	(50.8%)	25	(39.7%)
Alopecia	51	(40.5%)	29	(46.0%)
Headache	48	(38.1%)	32	(50.8%)
Skin rashes	48	(38.1%)	18	(28.6%)
Anorexia	42	(33.3%)	17	(27.0%)
Insomnia	40	(31.7%)	17	(27.0%)
Vomiting	37	(29.4%)	9	(14.3%)
Drug eruption	37	(29.4%)	2	(3.2%)
Arthralgia	36	(28.6%)	15	(23.8%)
Serum triglycerides increased	36	(28.6%)	11	(17.5%)
Dysgeusia	34	(27.0%)	10	(15.9%)
Diarrhoea	34	(27.0%)	19	(30.2%)
Nausea	32	(25.4%)	7	(11.1%)
Serum creatinine increased	32	(25.4%)	0	
Erythema at the injection site	33	(26.2%)	21	(33.3%)
Reactions at the injection site	29	(23.0%)	16	(25.4%)
Stomatitis	24	(19.0%)	12	(19.0%)
Abdominal discomfort	23	(18.3%)	12	(19.0%)
Pruritus	23	(18.3%)	13	(20.6%)
Nasopharyngitis	23	(18.3%)	18	(28.6%)
Influenza-like symptoms	22	(17.5%)	16	(25.4%)
Serum bilirubin increased	22	(17.5%)	13	(20.6%)
Back pain	21	(16.7%)	12	(19.0%)
Hyperuricemia	20	(15.9%)	2	(3.2%)
Serum phosphorus decreased	16	(12.7%)	13	(20.6%)
Constipation	14	(11.1%)	13	(20.6%)
Erythema	9	(7.1%)	13	(20.6%)

Factors influencing the treatment response are compared in Table 2. SVR was higher in Group A than B, irrespective of different genders, age ranges, or HCV RNA loads. Of note, SVR in women in Group A was higher than that in Group B (70.0% *vs.* 43.3%, $p = 0.0214$). Likewise, SVR in patients ⩾50 years was higher in Group A than B (67.1% *vs.* 42.9%, $p = 0.0125$), and that in patients with high HCV RNA loads (⩾7 $\log_{10}$ IU/ml) at the baseline was higher in Group A than B (69.2% *vs.* 27.8%, $p = 0.0132$).

Adverse events

Adverse events occurred in all patients in both Groups A and B. Adverse events with a frequency >15% in either group are listed in Table 3. Of them, frequencies of anemia, thrombocytopenia, malaise, and elevated serum levels of uric acid as well as hyaluronic acid were >10% higher in Group A than B. Most of them were mild, and severe and serious adverse events occurred in small proportions of patients (9.5% and 11.9% in Group A, respectively, and 9.5% and 9.5% in Group B). All drugs were discontinued due to adverse events comparatively frequently in Groups A and B (16.7% and 22.2%, respectively), and telaprevir alone in 19.0% of patients in Group A. The total dose of RBV was less in Group A than B (47.0% *vs.* 77.7% of the target, $p < 0.0001$). Doses of antiviral treatments were reduced or discontinued in some patients with moderate to severe adverse events, patients were taken care of by specialists, and received specific therapies when necessary. Eventually, all patients recovered from adverse events.

Hematological disorders

Anemia occurred in 91.3% and 73.0% of patients in Groups A and B, respectively. Table 4 compares the severity of anemia between Groups A and B. Combined, Grade 1 and 2 anemia developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Grade 3 anemia occurred in 11.1% in Group A only. During the follow-up, hemoglobin increased both in Groups A and B, and returned to pretreatment levels 12 weeks after the completion of therapy and thereafter (Fig. 3A). Platelet counts decreased more extensively in Group A than B (Fig. 3B). They rebounded after the completion of therapy, and then returned to pretreatment values. Decreases in neutrophil counts were milder in Group A than B (Fig. 3C). Both in Groups A and B, neutrophils started to increase immediately after the treatment completion, and returned to pretreatment levels within 12 weeks.

Skin disorders

Skin disorders were monitored at every hospital visit for severity and extent, and they were categorized into four Grades (Table 4). When skin disorders of Grades 2–4 occurred, the attendant physician was instructed to consult with a dermatologist in each institution for the diagnosis and specific cares, and telaprevir was discontinued, while PEG-IFN and RBV were reduced or discontinued, as required. Skin disorders were mainly rash, drug eruptions, and erythema. They occurred comparably frequently in Groups A and B (89.7% and 84.1%, respectively). Most skin disorders were mild and categorized into Grade 1 in 75.4% and 76.2% of patients in Groups A and B, respectively. Combined, skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). Due to skin disorders, at least one drug was discontinued in merely 9.5% and 3.2% of patients in Groups A and B, respectively, and most skin disorders were controllable by anti-histamine and/or steroid ointments.

Serious skin disorders developed in three patients in Group A, but none in Group B. Stevens–Johnson syndrome occurred in one patient 35 days after the treatment start, and led to the discontinuation of treatment. Erythema spread widely in the trunk (Fig. 4A), as well as limbs and the face. Erosion of oral mucosae, epidermal detachment, conjunctival redness, high fever to reach 39.3 °C, and lymphadenopathy were also noted. Histopathology showed the epidermal necrosis, satellite-cell necrosis, and perivascular dermatitis with infiltration of lymphocytes, neutrophils, and eosinophils in the superficial dermis (Fig. 4B). The patient was admitted and received steroids intravenously, and recovered completely within 9 weeks. Drug rash with eosinophilia and

Table 4. Decreases in hemoglobin levels and skin disorders according to the grade.

Grade	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
A Hemoglobin levels			
Grade 1 (9.5- <11.0 g/dl)	50 (39.7%)	32 (50.8%)	0.1631
Grade 2 (8.0- <9.5 g/dl)	34 (27.0%)	11 (17.5%)	0.2043
Grade 3 (<8.0 g/dl)	14 (11.1%)	0	0.0055
Total	98 (77.8%)	43 (68.3%)	0.1613
B Skin disorders			
Grade 1[a]	95 (75.4%)	48 (76.2%)	1.0000
Grade 2[b]	44 (34.9%)	12 (19.0%)	0.0282
Grade 3[c]	13 (10.3%)	3 (4.8%)	0.2709
Grade 4[d]	2 (1.6%)	0 (0.0%)	0.5532
Any grade	113 (89.7%)	53 (84.1%)	0.3451

[a]Localized skin lesions.
[b]Diffuse or multiple skin lesions.
[c]Skin lesions covering >50% of the body surface or rashes with some characteristics such as bullae, ulceration of mucous membrane, epidermal detachment, target lesion or significant systemic signs.
[d]Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS) were categorized in Grade 4.

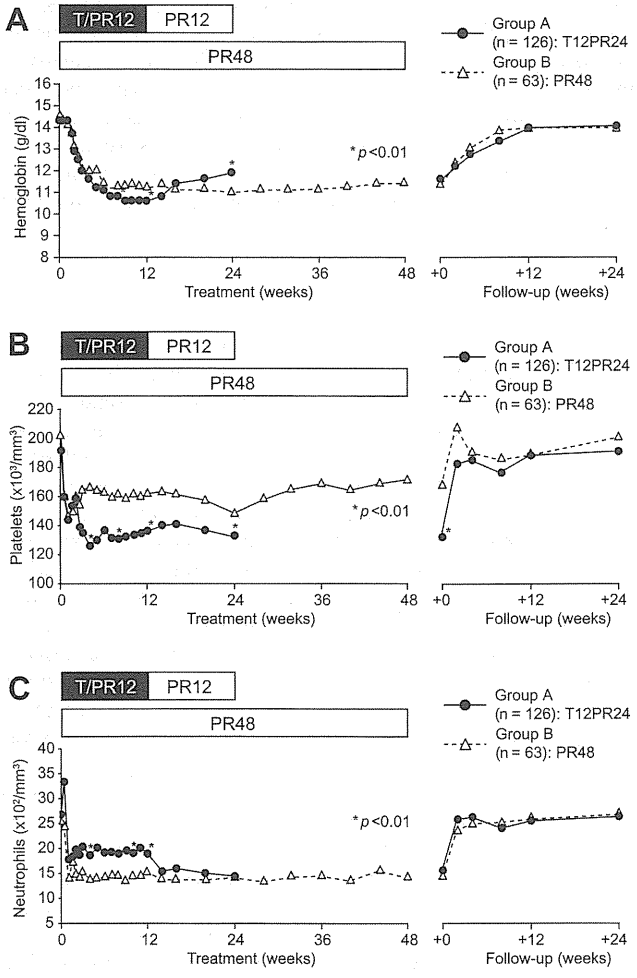

Fig. 3. Comparison of hematopoietic disorders between patients in Groups A and B. (A) Median hemoglobin levels, (B) platelet counts, and (C) neutrophil counts are plotted during treatment and follow-up. Ranges from 25% to 75% are omitted for visual clarity. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and at weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 difference.

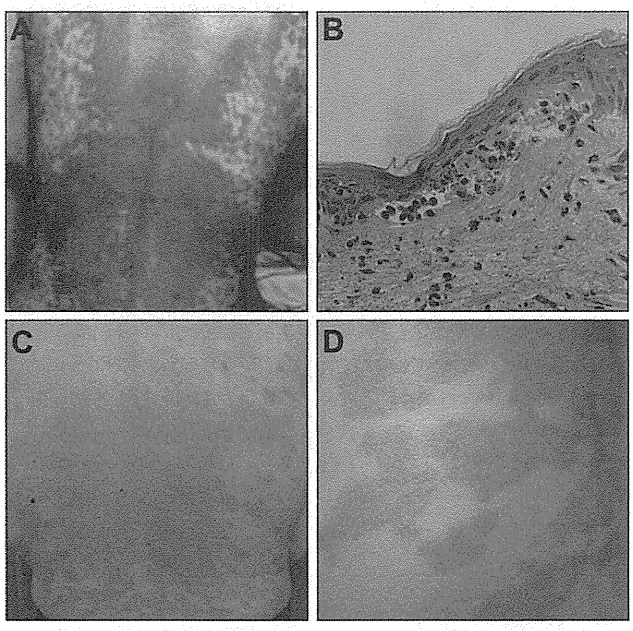

Fig. 4. Grade 4 skin regions in patients who received the triple therapy. (A) Erythema and (B) histopathology of the skin in the patient with Stevens–Johnson syndrome, as well as (C and D) generalized erythema in the patient developing drug rashes with eosinophilia and systemic symptoms (DRESS), are shown.

systemic symptoms (DRESS or drug-induced hypersensitivity syndrome) occurred in another patient. Fresh red erythema appeared on the whole body, and fresh red-colored target lesions (up to 3–4 cm in diameter) were also observed (Fig. 4C and D). Edema in the face, lymphadenopathy, fever up to 39.7 °C, and erosion of oral mucosae were noted, also. Maximum levels of white blood cells, eosinophils, and atypical lymphocytes were 46,300/mm^3, 45.7%, and 23.3%, respectively. Titers of IgG antibody to human herpes virus 6 were ×160 (29 days after the onset) and ×2560 (57 days). The remaining patient developed erythema multiforme. These two patients received steroids orally and recovered completely within 14 weeks.

Discussion

A prospective, randomized, and treatment-controlled clinical trial was planned and conducted in Japan to compare the therapeutic efficacy and safety profiles between the triple therapy with T12PR24 and the SOC treatment with PR48. In this trial, 126 patients were assigned to receive T12PR24 (Group A) and the 63 to receive PR48 (Group B). They all were treatment-naive, and infected with HCV-1 in high viral loads ($\geqslant 5$ $\log_{10}$ IU/ml) and of genotype 1b in the great majority (98.9%). Randomization was not adopted due to ethnical concerns against giving intravenous placebo weekly for 24 weeks to patients in Group A.

Dynamics of circulating HCV RNA during treatment was quite different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, $p < 0.001$) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Accordingly, SVR was achieved more frequently in patients with T12PR24 than PR48 (73.0% *vs.* 49.2%, $p = 0.0020$), while rates of relapse (16.7% *vs.* 22.2%) and breakthrough (3.2% and 1.6%) were not different between them. Due to the higher therapeutic efficacy and shorter treatment duration, T12PR24 would be more suitable for treatment of HCV-1 patients than the standard PR48, and lessen the total economic burden of patients and the nation.

Previous clinical trials with telaprevir were conducted in Europe or the United States and combined with PEG-IFN-α2a [8–11]. In the present study, Japanese patients have responded to a triple therapy with PEG-IFN-α2b, with an efficacy of 73% in comparison with 72–75% in phase 3 clinical trials [10,11]. In a recent report, PEG-IFN-α2a and -α2b were equally effective in triple therapies in combination with telaprevir and RBV [16]. Frequency of side effects demanding the discontinuation of all drugs is comparable between patients receiving the triple therapy with PEG-IFN-α2a in phase 3 trials [10,11] and -α2b in the present study (7–17% and 17%, respectively).

In our previous report [17], the IFN-responsive C/C genotype of *IL28B* at rs12979860 was detected in 42 out of the 72 (55%) patients infected with HCV-1 in Japan; the prevalence was not much different from that in 336 out of the 769 (44%) European-Americans [18]. The susceptibility to telaprevir depends on HCV genotypes, and is higher for genotypes 1 and 2 than genotypes 4 and 5 in *in vitro* experiments [19]. Further, it may differ between 1a and 1b, due to dissimilar evolution patterns of drug-resistant mutations [14]. Nevertheless, present patients infected with HCV-1b in the great majority (98.4%) were equally responsive to the triple therapy with telaprevir as those infected with HCV-1a [8,9,11].

High efficacy of T12PR24 was accompanied by increased adverse events, of which anemia and skin lesions were worrisome. Moderate and severe anemia (<9.5 g/dl) developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Since Japanese patients with chronic hepatitis C are older by >10 years than those in Western countries, with a higher proportion of women, they are prone to develop anemia during treatment with telaprevir. Stringent precaution had to be taken, therefore, by deducting the RBV dose in patients in whom hemoglobin levels decrease <12 g/dl, higher than the conventional threshold of <10 g/dl. The total RBV dose was lower in Group A than B (47.0% *vs.* 77.7% of the target, $p < 0.0001$). However, decreased doses of RBV or PEG-IFN did not influence substantially the therapeutic efficacy of T12PR24.

Skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). It has to be noted that Grade 4 skin lesions, such as Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS), developed exclusively in patients in Group A. Since studied patients were monitored carefully and received immediate care by dermatologists, if and when skin lesions of Grades 2–4 developed, all patients eventually recovered. In the area of DAAs, potentially accompanying severe skin disorders, physicians would need close cooperation with dermatologists for the care of patients with hepatitis C.

In conclusion, this multicenter, randomized, and treatment-controlled study of T12PR24 in Japanese patients infected with HCV-1b has proven the efficacy and safety comparable to those in previous phase 3 studies [10,11]. Due to the excellence of T12PR24 over the standard PR48, we hope it will be used widely in patients with chronic hepatitis C over the world, who are expected to increase rapidly in the foreseeable future [20].

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Acknowledgments

We thank the following 41 institutions for their cooperation toward the completion of this study. Obihiro-Kosei General Hospital; Hokkaido University Hospital; Sapporo-Kosei General Hospital; Tohoku University Hospital; Yamagata University Hospital; Tokyo Medical University Ibaraki Medical Center; Saitama Red Cross Hospital; Saitama Medical University Hospital; Chiba University Hospital; Shinmatsudo Central General Hospital; Showa University Hospital; Keio University Hospital; Juntendo University Hospital; Toranomon Hospital; National Center for Global Health and Medicine; Toranomon Branch Hospital; Kanazawa University Hospital; University of Yamanashi Hospital; Shinshu University Hospital; Gifu Municipal Hospital; Gifu Prefectural General Medical Center; Ogaki Municipal Hospital; Hamamatsu University School of Medicine, University Hospital; Nagoya University Hospital; Toyohashi Municipal Hospital; Fujita Health University Hospital; University Hospital, Kyoto Prefectural University of Medicine; Osaka Red Cross Hospital; Osaka University Hospital; Ikeda Municipal Hospital; Kishiwada City Hospital; Osaka Rosai Hospital; Saiseikai Suita Hospital; National Hospital Organization Osaka National Hospital; Hiroshima University Hospital; Kagawa Prefectural Central Hospital; Shin-Kokura Hospital; Kurume University Hospital; Fukuoka University Hospital; National Hospital Organization Nagasaki Medical Center; and Kagoshima University Medical and Dental Hospital.

References

[1] World Health Organization. Hepatitis C. (Global Alert and Response, 2002). Geneva, Switzerland: World Health Organization; 2002. Updated February 2010. http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index.html.

[2] Hoofnagle JH. Course and outcome of hepatitis C. Hepatology 2002;36: S21–S29.

[3] Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002;36: S35–S46.
[4] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.
[5] Hadziyannis SJ, Sette Jr H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–355.
[6] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.
[7] Asselah T, Marcellin P. New direct-acting antivirals' combination for the treatment of chronic hepatitis C. Liver Int 2011;31:S68–S77.
[8] Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839–1850.
[9] McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827–1838.
[10] Jacobson IM, McHutchison JG, Dusheiko GM, Di Bisceglie AM, Reddy R, Bzowej NH, et al. Telaprevir in combination with peginterferon and ribavirin in genotype 1 HCV treatment-naive patients: final results of Phase 3 ADVANCE study. Hepatology 2010;52:427A, [Abstract 211].
[11] Sherman KE, Flamm SL, Afdhal NH, Nelson DR, Sulkowski MS, Everson GT, et al. Telaprevir in combination with peginterferon alfa2a and ribavirin for 24 or 48 weeks in treatment-naive genotype 1 HCV patients who achieved an extended rapid viral response: final results of Phase 3 ILLUMINATE study. Hepatology 2010;52:401A, [Abstract LB-2].
[12] Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, et al. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 2006;131:470–477.
[13] Muir AJ, Bornstein JD, Killenberg PG. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. N Engl J Med 2004;350:2265–2271.
[14] Sarrazin C, Zeuzem S. Resistance to direct antiviral agents in patients with hepatitis C virus infection. Gastroenterology 2010;138:447–462.
[15] Simmonds P, Mellor J, Sakuldamrongpanich T, Nuchaprayoon C, Tanprasert S, Holmes EC, et al. Evolutionary analysis of variants of hepatitis C virus found in South-East Asia: comparison with classifications based upon sequence similarity. J Gen Virol 1996;77:3013–3024.
[16] Marcellin P, Forns X, Goeser T, Ferenci P, Nevens F, Carosi G, et al. Telaprevir is effective given every 8 or 12 hours with ribavirin and peginterferon alfa-2a or -2b to patients with chronic hepatitis C. Gastroenterology 2011;140: 459–468.
[17] Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, et al. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 2010;52:421–429.
[18] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
[19] Imhof I, Simmonds P. Genotype differences in susceptibility and resistance development of hepatitis C virus to protease inhibitors telaprevir (VX-950) and danoprevir (ITMN-191). Hepatology 2011;53:1090–1099.
[20] Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol 2007;13:2436–2441.

JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2011 doi:10.1111/j.1365-2893.2011.01528.x

Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C

N. Hayashi,[1] T. Okanoue,[2] H. Tsubouchi,[3] J. Toyota,[4] K. Chayama[5] and H. Kumada[6]

[1]*Kansai-Rosai Hospital, Hyogo, Japan;* [2]*Department of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Osaka, Japan;* [3]*Department of Digestive and Life-style Related Disease, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan;* [4]*Department of Gastroenterology, Sapporo Kosei General Hospital, Hokkaido, Japan;* [5]*Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan; and* [6]*Department of Hepatology, Toranomon Hospital, Tokyo, Japan*

Received June 2011; accepted for publication July 2011

SUMMARY. The aims of this phase III study were to assess the efficacy and safety of telaprevir in combination with peginterferon alfa-2b (PEG-IFN) and ribavirin (RBV) for difficult-to-treat patients who had not achieved sustained virological response (SVR) to prior regimens in Japan. The subjects were 109 relapsers (median age of 57.0 years) and 32 nonresponders (median age of 57.5 years) with hepatitis C virus genotype 1. Patients received telaprevir (750 mg every 8 h) for 12 weeks and PEG-IFN/RBV for 24 weeks. The SVR rates for relapsers and nonresponders were 88.1% (96/109) and 34.4% (11/32), respectively. Specified dose modifications of RBV that differed from that for the standard of care were introduced to alleviate anaemia. RBV dose reductions were used for 139 of the 141 patients. The SVR rates for relapsers did not depend on RBV dose reduction for 20–100% of the planned dose (SVR rates 87.5–100%, $P < 0.05$). Skin disorders were observed in 82.3% (116/141). Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. The ratios of discontinuation of telaprevir only or of all the study drugs because of adverse events were 21.3% (30/141) and 16.3% (23/141), respectively. A frequent adverse event leading to discontinuation was anaemia. Telaprevir in combination with PEG-IFN/RBV led to a high SVR rate for relapsers and may offer a potential new therapy for nonresponders even with a shorter treatment period.

Keywords: direct-acting antiviral, peginterferon, ribavirin, sustained virological response, treatment failure.

INTRODUCTION

Hepatitis C virus (HCV) affects approximately 170 million people worldwide [1]; patients with chronic hepatitis C (CHC) eventually develop cirrhosis and hepatocellular carcinoma (HCC) [2,3]. The standard of care (SOC) with peginterferon plus ribavirin (RBV) for 48 weeks is most effective for eradicating HCV genotype 1 [4], which is a dominant genotype for CHC [1]. However, the sustained virological response (SVR) rate of SOC for the treatment of naïve patients with genotype 1 is approximately <50% [5,6]. The retreatment regimen for patients who do not achieve SVR is limited to exposure to peginterferon plus RBV with modification of dose and treatment duration. Some studies have been conducted to estimate the effectiveness of peginterferon plus RBV for 48 weeks for nonresponders to prior interferon-based combination therapy, and the SVR rates in most studies did not exceed 20% [7–9]. A large randomized study of patients who had not responded to previous treatment with peginterferon alfa-2b (PEG-IFN) plus RBV gave SVR rates for peginterferon alfa-2a 180 μg/kg plus RBV for 72 weeks that were not as high as those for 48 weeks (14%, 9%) [10]. HCV patients who had failed to achieve SVR with the combination therapy displayed high risk rates of decompensated cirrhosis, HCC and liver-related mortality [11]. Therefore, it is very important to establish new regimens to increase the SVR rate and shorten the treatment period for patients who do not achieve SVR with prior treatments.

Telaprevir, classified as a direct-acting antiviral agent, is a reversible, selective, orally bioavailable inhibitor of the nonstructural NS3/4A HCV serine protease [12]. Two phase II studies (PROVE 1 and PROVE 2) on the treatment of naïve patients with genotype 1 were conducted to assess the

Abbreviations: CHC, chronic hepatitis C; ETR, end of treatment response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PEG-IFN, peginterferon alfa-2b; RBV, ribavirin; RVR, rapid viral response; SOC, standard of care; SVR, sustained virological response.

Correspondence: Norio Hayashi, Kansai-Rosai Hospital, 3-1-69 Inabaso, Amagasaki City, Hyogo Prefecture, 660-8511 Japan.
E-mail: hayashin@kanrou.net

Reprinted from Early View
(Online Version of Record published before inclusion in an issue)

efficacy of telaprevir for 12 weeks in combination with peginterferon and RBV for 24 weeks [13,14]. These studies demonstrated that the SVR rates of the telaprevir regimen were significantly higher compared with SOC (PROVE 1: 61% *vs* 41%, $P = 0.02$, PROVE 2: 69% *vs* 46%, $P = 0.004$). A subsequent phase II study (PROVE 3) for treatment-failure patients with genotype 1 gave SVR rates for nonresponders, relapsers and breakthroughs in the telaprevir regimen of 39%, 69% and 57%, respectively [9].

In Japan, a phase III study was conducted for the treatment of naïve patients with genotype 1 to compare the efficacy and safety between the telaprevir regimen and SOC. It has demonstrated that the SVR rate for the telaprevir regimen was significantly higher than that for SOC (73.0% *vs* 49.2%, $P = 0.0020$) [15]. We decided to conduct a phase III study to assess the efficacy and safety of telaprevir in combination with PEG-IFN and RBV in relapsers and nonresponders who had not achieved SVR to a previously administered IFN-based regimen in Japan.

PATIENTS AND METHODS

Study patients

Relapsers and nonresponders were enrolled in Study 1 (ClinicalTrials.gov Identifier: NCT00780910) and Study 2 (ClinicalTrials.gov Identifier: NCT00781274), respectively. Relapsers were defined as patients who had been previously treated for CHC and had undetectable HCV RNA during interferon or peginterferon therapy (including combination with RBV). Nonresponders were defined as patients who were previously treated for CHC and had never had undetectable HCV RNA for more than 24 weeks with interferon or peginterferon therapy (including combination with RBV).

The patients were enrolled from 17 sites in Japan. Patients considered eligible were of 20–65 years of age, had CHC because of HCV genotype 1 (defined by NS5B sequence) [16] and ≥5.0 $\log_{10}$ IU/mL HCV RNA level at the screening test, had been previously treated for CHC with interferon or peginterferon therapy (including combination with RBV), had a body weight of 40 kg or more and below 120 kg, could be hospitalized for at least 2 weeks after the first administration, were not pregnant and agreed to contraception from the screening period to 24 weeks after the last dosing of the study drug. The patients were excluded if they had a haemoglobin level of <12 g/dL, neutrophil count of <1500/mm^3, platelet count of <100 000/mm^3, were positive for HBs antigen and HIV antibodies at the screening test, had chronic renal failure or creatinine clearance of ≤50 mL/min, depression, schizophrenia or its history, history of suicide attempt, decompensated cirrhosis, previous or current HCC or other malignancies, autoimmune hepatitis, alcoholic liver disease or haemochromatosis.

All patients provided written informed consent before participating in the study. These studies were approved by each site's institutional review board and conducted in accordance with good clinical practice and the Declaration of Helsinki.

Study design

All patients received PEG-IFN (PegIntron®; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg per week subcutaneously, RBV (Rebetol®; MSD) at a dose of 600 mg per day (for body weight ≤60 kg), 800 mg per day (for body weight >60 to ≤80 kg) or 1000 mg per day (for body weight >80 kg) and telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) at a dose of 750 mg every 8 h after food. The patients were treated with telaprevir, PEG-IFN and RBV for 12 weeks, followed by PEG-IFN and RBV (PEG-IFN/RBV) for 12 weeks. All patients had a 24-week follow-up period after the last dosing of study drugs to assess SVR.

Dose modification of study drugs

Specified dose modification of RBV that differed from the dose for SOC was introduced to alleviate anaemia. The initial dose of RBV was reduced by 200 mg per day in case of a haemoglobin level <13 g/dL at baseline. The RBV dose was reduced by 200 mg per day in patients receiving 600 or 800 mg per day (by 400 mg per day in those receiving 1000 mg) when the haemoglobin level was <12 g/dL and was reduced by an additional 200 mg per day when the haemoglobin level was <10 g/dL. The RBV dose was also reduced by 200 mg per day if the haemoglobin level dropped ≥1 g/dL within 1 week, and this level was <13 g/dL. Telaprevir was withdrawn when the haemoglobin level was <8.5 g/dL. PEG-IFN/RBV were withdrawn or interrupted when the haemoglobin level was <8.5 g/dL. The dose modifications of PEG-IFN were followed by SOC. Dose modification and interruption of telaprevir were not allowed. Telaprevir was withdrawn if serious adverse events appeared. The use of erythropoietin was not allowed for elevating the haemoglobin level.

Stopping rules

Patients could be discontinued from the study at any time if the investigator or sponsor determined that it was not in the interest of the patient to continue the study or the patient wished to withdraw from the study. The study drugs were discontinued if the patients had a haemoglobin level of <8.5 g/dL, white blood cell count of <1000/mm^3, neutrophil count of <500/mm^3 or platelet count of <50 000/mm^3.

In case of the following criteria for serum HCV RNA viral kinetics measured during the treatment period, discontinuation of the study drugs was decided at the investigator's discretion. (i) When the following criteria applied twice consecutively: (a) the amount of change from the lowest value for HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL and (b)

HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL after it had been confirmed to be <1.2 $\log_{10}$ IU/mL. (ii) When the serum HCV RNA level at 13 weeks after administration of study drugs did not decrease by >2.0 $\log_{10}$ IU/mL from the baseline level.

Efficacy assessments

Serum HCV RNA levels were measured using the COBAS TaqMan HCV test (Roche Diagnostics Co. Ltd., Tokyo, Japan). The linear dynamic range was 1.2–7.8 $\log_{10}$ IU/mL. Samples with undetectable HCV RNA were reported as '<1.2 $\log_{10}$ IU/mL (no detectable HCV RNA)'. Measurements were obtained at week 4 before day 1 of the screening period: at days 1 (predose), 2 and 3; weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 of the treatment period; and weeks 2, 4, 8, 12, 16, 20 and 24 of the follow-up period.

The primary endpoint was a SVR defined as an undetectable HCV RNA level 24 weeks after the end of treatment. Relapse, breakthrough, and nonresponse were defined based on AASLD Guidelines as follows [4]: 'relapse' was a state of undetectable serum HCV RNA at the end of treatment and reappearance of serum HCV RNA during the follow-up period; 'breakthrough' was a state of undetectable serum HCV RNA and reappearance of serum HCV RNA during the treatment period; and 'nonresponse' was a state of continuously detectable serum HCV RNA during the treatment period.

Safety assessments

All adverse events were recorded up to the last visit and coded using MedDRA/J version 13.0. (MedDRA Japanese Maintenance Organization, Tokyo, Japan) Measurements for chemical laboratory data were obtained at week 4 before day 1 of the screening period: at day 1 (predose); weeks 1, 2, 4, 8, 10, 12, 14, 16, 18, 20 and 24 of the treatment period; and weeks 2, 4, 8, 12 and 24 of the follow-up period. Electrocardiogram (ECG) and fundus examinations were performed once during the screening period. Adverse events, haematological and chemical laboratory data, and vital signs were assessed and summarized. The severity of rash was categorized into three grades.

Statistical analysis

Sustained virological response rates were evaluated for the full analysis set. Categorical variables were compared by Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA), and a P value < 0.05 was considered significant.

Table 1 Baseline characteristics of study patients

	Study 1 (relapsers) $N = 109$	Study 2 (nonresponders) $N = 32$
Gender – *n* (%)		
Men	66 (60.6)	17 (53.1)
Women	43 (39.4)	15 (46.9)
Age, years – median (range)	57.0 (20, 65)	57.5 (40, 65)
Weight, kg – median (range)	62.50 (41.0, 92.5)	61.30 (44.9, 92.5)
BMI, kg/m^2 – median (range)*	23.10 (18.0, 32.4)	22.60 (17.1, 31.2)
ALT (IU/L) – median (range)†	36.0 (16, 302)	48.0 (17, 190)
Haemoglobin (g/dL) – median (range)	14.70 (12.0, 17.8)	14.50 (12.3, 16.6)
White blood cell count (/mm^3)	4680.0 (2490, 15940)	4830.0 (3040, 8000)
Platelet count (×10^4/mm^3) – median (range)	17.80 (9.9, 33.8)	17.85 (9.1, 26.2)
HCV RNA ($\log_{10}$ IU/mL) – median (range)‡	6.75 (5.2, 7.6)	6.78 (6.0, 7.7)
HCV genotype 1 subtype – *n* (%)		
1a	0 (0.0)	1 (3.1)
1b	109 (100.0)	31 (96.9)
Prior therapy for chronic hepatitis C – *n* (%)		
Interferon	13 (11.9)	1 (3.1)
Interferon plus ribavirin	14 (12.8)	2 (6.3)
Peginterferon	3 (2.8)	0 (0.0)
Peginterferon plus ribavirin	79 (72.5)	29 (90.6)

HCV, hepatitis C virus.
*The body mass index (BMI) is the weight in kilograms divided by the square of the height in metres; †Alanine aminotransferase; ‡The HCV RNA level was measured using the COBAS TaqMan HCV test (Roche).

Reprinted from Early View
(Online Version of Record published before inclusion in an issue)

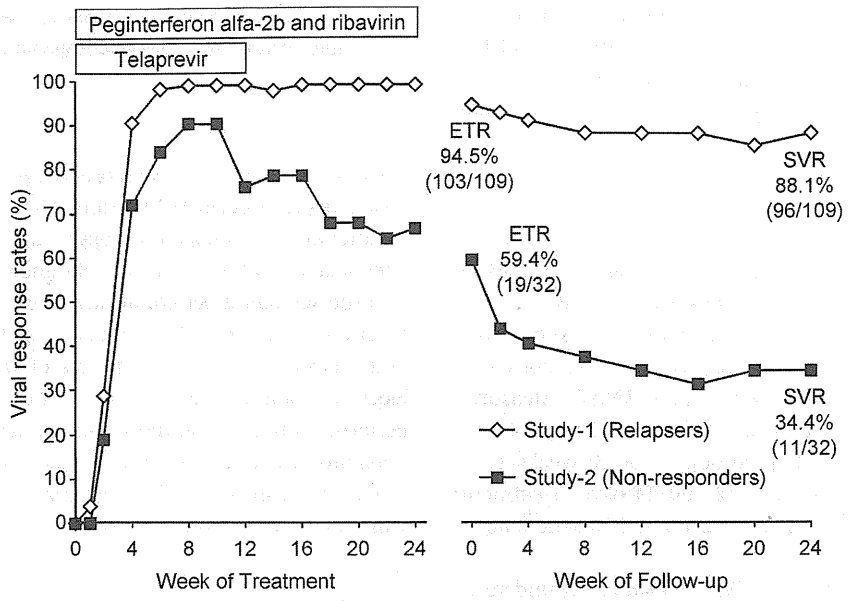

Fig. 1 Undetectable hepatitis C virus RNA rates at each measurement point. SVR, sustained virological response; ETR, end-of-treatment response.

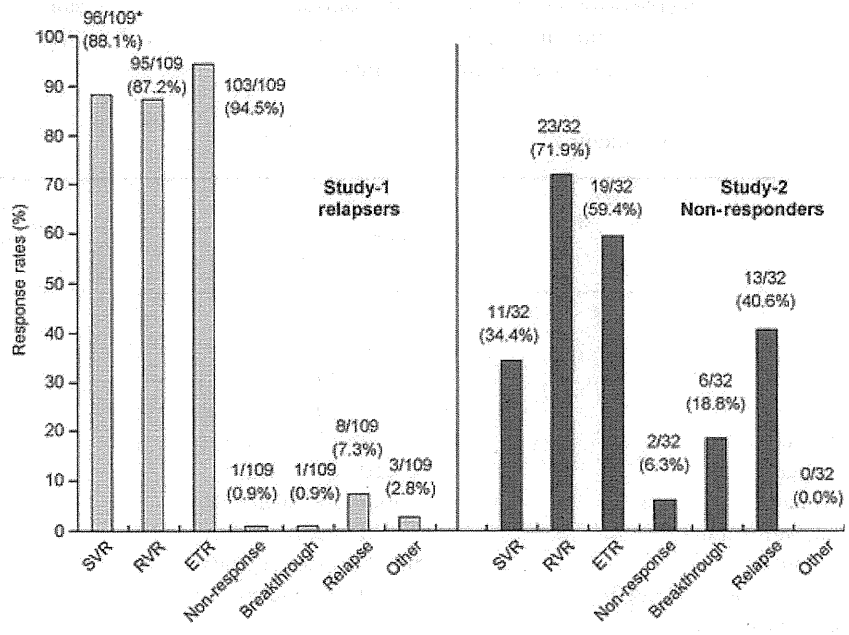

Fig. 2 Response rates of patients with virological response. *Number of patients who achieved SVR in each subgroup/*N* (%). SVR, sustained virological response; RVR, rapid viral response; ETR, end-of-treatment response.

RESULTS

Study patients

From November 2008 to August 2009, a total of 168 patients [Study 1 (*N* = 135) and Study 2 (*N* = 33)] were screened, and 141 patients [Study 1 (*N* = 109) and Study 2 (*N* = 32)] received at least one dose of a study drug. The baseline characteristics of the study patients are shown in Table 1. Patients previously treated with PEG-IFN (with or without RBV) and IFN (with or without RBV) in Study 1 and Study 2 accounted for 75.2% (82 of 109) and 24.7% (27 of 109) and 90.6% (29 of 32) and 9.4% (3 of 32), respectively. The median of age, weight, haemoglobin level, platelet count and HCV RNA level for Study 1 and Study 2 were 57.0 and 57.5 years, 62.5 and 61.3 kg, 14.7 and 14.5 g/dL, 17.8

and $17.85 \times 10^4/mm^3$, and 6.75 and 6.78 log_{10} IU/mL, respectively. Patients over 50 years of age accounted for 81.7% (89 of 109) and 81.3% (26 of 32), respectively.

Efficacy in study 1 (relapsers)

Figure 1 shows the change in the undetectable HCV RNA rates at each measurement point. The rapid viral response (RVR) rate and the end of treatment response (ETR) rate were 87.2% (95/109) and 94.5% (103/109), respectively. The SVR rate, nonresponse, breakthrough and relapse were 88.1% (96/109), 0.9% (1/109), 0.9% (1/109) and 7.3% (8/109), respectively (Fig. 2).

Factors influencing the SVR rate are compared in Table 2. The SVR rate in the patients who achieved undetectable HCV RNA at ≤week 4 was significantly higher than that in the patients who achieved undetectable HCV RNA at >week 4 (91.8% *vs* 66.7%, $P = 0.0487$). Also, the SVR rate for men was significantly higher than that for women (93.9% *vs* 79.1%, $P = 0.0316$). The SVR rate with discontinuation of all the study drugs was significantly lower than that with discontinuation of only telaprevir or no discontinuation of the study drugs (all the study drugs: 60.0%, only telaprevir: 95.0% and no discontinuation: 94.2%, $P = 0.0007$). In contrast, there was no difference in the SVR rate in relation to HCV RNA level and prior therapy for CHC. SVR rates by the ratio of the actual total RBV dose to the anticipated total RBV dose were evaluated (Fig. 3). The SVR rates did not depend on RBV dose reduction for 20–100% of the planned dose (87.5–100%, $P < 0.05$).

Efficacy in study 2 (nonresponders)

The RVR and ETR rates were 71.9% (23/32) and 59.4% (19/32), respectively (Fig. 1). The SVR rate, nonresponse, breakthrough and relapse were 34.4% (11/32), 6.3% (2/32), 18.8% (6/32) and 40.6% (13/32), respectively (Fig. 2). There was no difference in the SVR rate in relation to

Table 2 SVR rates stratified by demographic, undetectable HCV RNA and discontinuation of study drug treatment

	Study 1 (relapsers) $N = 109$	Study 2 (nonresponders) $N = 32$
Gender – *n/N* (%)		
Male	62/66 (93.9)	8/17 (47.1)
Female	34/43 (79.1)	3/15 (20.0)
P-value	0.0316	0.1475
Age – *n/N* (%)		
≤49	18/20 (90.0)	2/6 (33.3)
≥50	78/89 (87.6)	9/26 (34.6)
P-value	1.0000	1.0000
HCV RNA (log_{10} IU/mL) – *n/N* (%)		
≥7.0	26/30 (86.7)	5/10 (50.0)
<7.0	70/79 (88.6)	6/22 (27.3)
P-value	0.7498	0.2515
Prior therapy for chronic hepatitis C – *n/N* (%)		
Interferon	12/13 (92.3)	1/1 (100.0)
Interferon plus ribavirin	13/14 (92.9)	2/2 (100.0)
Peginterferon	3/3 (100.0)	– (–)
Peginterferon plus ribavirin	68/79 (86.1)	8/29 (27.6)
P-value	0.9271	0.0333
Undetectable – *n/N* (%)		
≤Week 4	90/98 (91.8)	9/23 (39.1)
>Week 4 ≤end of treatment	6/9 (66.7)	2/7 (28.6)
P-value	0.0487	1.0000
Discontinuation of study drug treatment – *n/N* (%)		
No discontinuation	65/69 (94.2)	9/20 (45.0)
Telaprevir only	19/20 (95.0)	2/7 (28.6)
All study drugs	12/20 (60.0)	0/5 (0.0)
P-value	0.0007	0.1711

SVR, sustained virological response; HCV, hepatitis C virus.
SVR was defined as an undetectable HCV RNA level 24 weeks after the end of treatment.

Reprinted from Early View
(Online Version of Record published before inclusion in an issue)

baseline characteristics, HCV RNA level and prior treatment for CHC. The SVR rates for the patients who received 40–80% RBV dose reduction were over 30% (Fig. 3).

Safety

Adverse events were observed in all the patients in Study 1 and Study 2. Adverse events observed in at least 15% of the patients in each clinical study are listed in Table 3. Adverse events were similar between Study 1 and Study 2. Most of the adverse events were mild and moderate. Serious adverse events in Study 1 and Study 2 were reported in 11.9% (13/109) and 9.4% (3/32) of the patients, respectively. The ratios of discontinuation of all the study drugs because of adverse events in Study 1 and Study 2 were 17.4% (19/109) and 12.5% (4/32), respectively. A frequent adverse event leading to discontinuation was anaemia. Discontinuation rates of all the study drugs because of anaemia in Study 1 and Study 2 were 10.1% (11/109) and 9.4% (3/32), respectively. One death was reported in Study 1. One patient in Study 1 died of pulmonary embolism. Causality of PEG-IFN and RBV was classified as 'probably related' and that of telaprevir was classified as 'possibly related'.

Adverse events related to skin disorders were observed in 82.3% (116/141) of the patients. Skin disorders reported in over 10% of the patients were rash in 39.0% (55/141), drug eruption in 24.1% (34/141), injection site reaction in 12.8% (18/141) and injection site erythema in 12.8% (18/141) of the patients. Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. Grade 3 (severe) skin disorders in Study 1 and Study 2 were reported in 6.4% (7/109) and 6.3% (2/32) of the patients, respectively. Discontinuation of all the study drugs because of skin disorders in Study 1 amounted to 3.7% (4/109). No discontinuation because of skin disorders occurred in Study 2.

Figure 4 shows the changes in haemoglobin levels, platelet counts and neutrophil counts during the treatment and follow-up periods. Changes in the haematological parameters were similar between Study 1 and Study 2. The platelet count and neutrophil count decreased sharply within 4 weeks and then gradually decreased. Despite the modification of RBV, the median haemoglobin levels in Study 1 and Study 2 decreased to 10.6 and 10.4 g/dL at week 12, respectively. No patient discontinued all the study drugs because of neutrophil decrease. The haematological parameters recovered to the baseline level at the end of the follow-up period.

DISCUSSION

This phase III study was planned and conducted to assess the efficacy and safety of telaprevir in combination with PEG-IFN/RBV for relapsers and nonresponders. Most of the patients who participated in this study had received a prior PEG-IFN/RBV regimen. Despite a shorter treatment period, the SVR rates for relapsers and nonresponders were 88.1% and 34.4%, respectively. The result indicates that the HCV RNA response to previous treatment history should be one of the diagnostic factors for predicting SVR.

The SVR rate for men was significantly higher than that for women in the relapser group (93.9% *vs* 79.1%, $P = 0.0316$). There was no significant difference in other characteristics of the patients in that group. Once the relapsers had achieved undetectable HCV RNA, this condi-

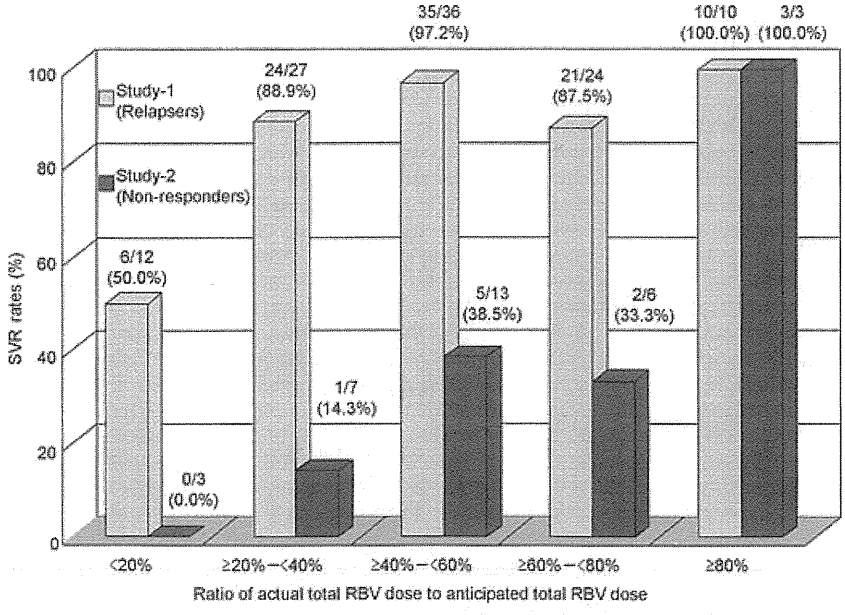

Fig. 3 Sustained virological response rates according to adherence to the ribavirin dose.

Table 3 Most common adverse events

MedDRA/J (Version.13.0) preferred term – *n* (%)	Study 1 (relapsers) *N* = 109	Study 2 (nonresponders) *N* = 32	Total *N* = 141
Anaemia	96 (88.1)	32 (100.0)	128 (90.8)
Pyrexia	90 (82.6)	30 (93.8)	120 (85.1)
White blood cell count decreased	83 (76.1)	22 (68.8)	105 (74.5)
Blood uric acid increased	72 (66.1)	25 (78.1)	97 (68.8)
Platelet count decreased	73 (67.0)	22 (68.8)	95 (67.4)
Malaise	60 (55.0)	23 (71.9)	83 (58.9)
Decreased appetite	56 (51.4)	15 (46.9)	71 (50.4)
Hyaluronic acid increased	56 (51.4)	15 (46.9)	71 (50.4)
Rash	39 (35.8)	16 (50.0)	55 (39.0)
Headache	42 (38.5)	10 (31.3)	52 (36.9)
Blood creatinine increased	36 (33.0)	12 (37.5)	48 (34.0)
Insomnia	34 (31.2)	11 (34.4)	45 (31.9)
Blood bilirubin increased	34 (31.2)	10 (31.3)	44 (31.2)
Alopecia	35 (32.1)	7 (21.9)	42 (29.8)
Diarrhoea	31 (28.4)	7 (21.9)	38 (27.0)
Dysgeusia	29 (26.6)	6 (18.8)	35 (24.8)
Vomiting	26 (23.9)	8 (25.0)	34 (24.1)
Drug eruption	24 (22.0)	10 (31.3)	34 (24.1)
Nausea	24 (22.0)	4 (12.5)	28 (19.9)
Abdominal discomfort	22 (20.2)	6 (18.8)	28 (19.9)
Blood triglycerides increased	19 (17.4)	8 (25.0)	27 (19.1)
Pruritus	20 (18.3)	2 (6.3)	22 (15.6)
Arthralgia	18 (16.5)	4 (12.5)	22 (15.6)
Nasopharyngitis	19 (17.4)	2 (6.3)	21 (14.9)
Stomatitis	13 (11.9)	6 (18.8)	19 (13.5)
Back pain	12 (11.0)	5 (15.6)	17 (12.1)
Blood phosphorus decreased	10 (9.2)	6 (18.8)	16 (11.3)

The adverse events listed are those that were reported in at least 15% of patients in each clinical study.

tion was sustained until the end of the treatment period. The patients who achieved RVR had a higher SVR rate than the patients who had no RVR in the relapser group (91.8% *vs* 66.7%, $P = 0.0487$).

In contrast, there was no significant difference related to characteristics in the nonresponder group. The SVR rates between men and women and undetectable HCV RNA were, however, slightly different. As Study 2 for the nonresponders was of a small scale, it will be necessary to evaluate a larger number of patients. The breakthrough ratio in the nonresponders during the PEG-IFN/RBV treatment period and relapse ratio were 18.8% and 40.6%, respectively. Two patients were nonresponders with high telaprevir-resistant variants; one was subtype 1a and the only patient with this characteristic in the study.

Triple therapy for 12 weeks, followed by PEG-IFN/RBV for 12 weeks for the relapsers led to a high SVR rate. In contrast to the relapsers, all breakthroughs were observed in 18.8% of nonresponder patients after the end of telaprevir treatment, and relapse were observed in 40.6% of nonresponder patients after the end of treatment period. Continuation of telaprevir over 12 weeks and PEG-IFN/RBV over 24 weeks might be needed to achieve a higher SVR rate for nonresponders.

Dose modification of RBV that differed from that for SOC was introduced to prevent anaemia in the patients [17]. Dose reductions of RBV were observed in 98.6% of the patients, and those who had 200 mg RBV per day as a minimum dose and those who discontinued it accounted for 41.8% and 29.8%, respectively. The haemoglobin level recovered to the baseline level at the end of the follow-up period. As a result of dose modification, the change in the haemoglobin level in this study was similar to that in PROVE 3 [9]. Checking the haemoglobin level once a week during the treatment period is important. The SVR rates did not depend on RBV dose reduction among the relapsers who had over 20% of the anticipated total RBV dose (87.5–100%). Thus, it is important to monitor haemoglobin levels and continue RBV dosing appropriately to achieve SVR, even with a low RBV dose.

Reprinted from Early View
(Online Version of Record published before inclusion in an issue)

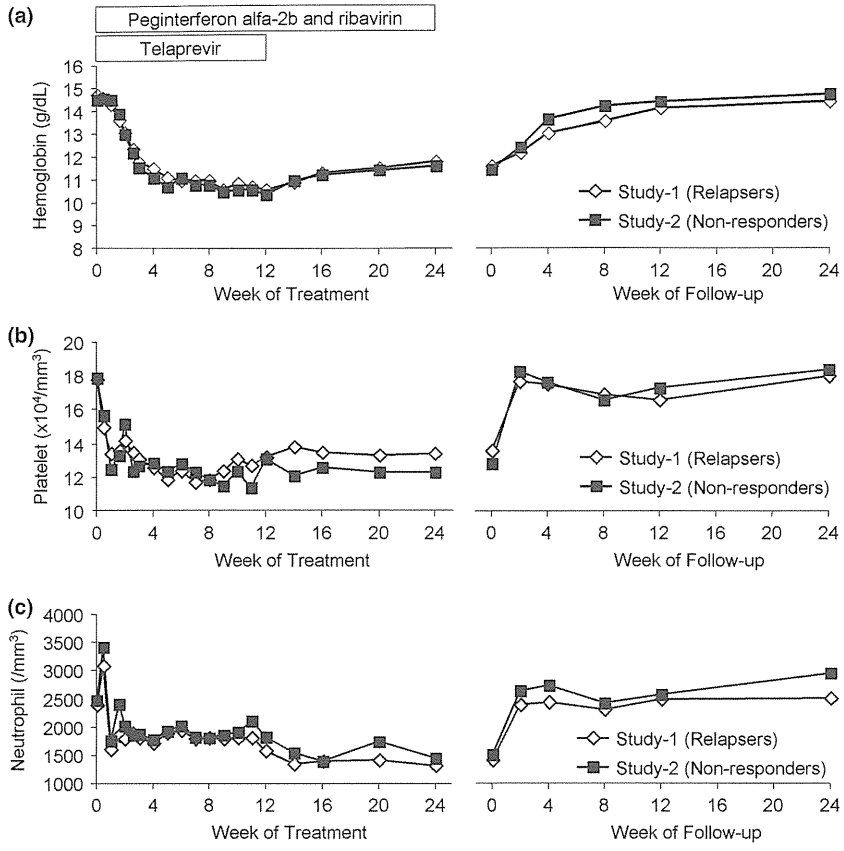

Fig. 4 Changes in hematology parameters. Median haemoglobin levels (a), median platelet counts (b) and median neutrophil counts (c) were plotted during treatment and follow-up periods.

Adverse events related to skin disorder were reported by 82.3% of the subjects. Of the nine cases of severe skin disorders, seven occurred within 8 weeks. Telaprevir was likely to be related to the occurrence of the severe skin disorders. The mechanism of skin disorders is unknown. All the patients who discontinued treatment received immediate care from dermatologists and recovered eventually. Skin disorders should be carefully monitored by physicians in collaboration with dermatologists.

The relationship between the SVR rates and the difference in SNPs in gene IL28B or near IL28B has become clear [18,19]. With genetic variation in rs8099917, SVR rates of 83.8% and 27.6% were achieved for patients with genotype TT and non-TT who were treated with telaprevir in combination with PEG-IFN/RBV, respectively [20]. Also, genetic variations in gene ITPA related to haemoglobin decrease and reduction of RBV has been discussed for patients treated with PEG-IFN/RBV [21,22]. We did not evaluate IL28B and ITPA in this study. As anaemia was the most frequent adverse event leading to the discontinuation of the study drugs in the present study, it should become a valuable pharmacogenetic diagnostic tool to optimize the triple therapy.

In conclusion, this phase III study conducted in Japan demonstrated that telaprevir in combination with PEG-IFN/RBV had a high SVR rate for relapsers and shows promise as a potential therapy for nonresponders even with a short treatment period. Prolongation of telaprevir and PEG-IFN/RBV treatment should be a better option for achieving high SVR for nonresponders. As the data demonstrated convincingly that the benefits greatly outweigh the risks, telaprevir-based regimen is at the lead for the next generation of HCV therapies.

DISCLOSURES

None to declare.

REFERENCES

1 World Health Organization. Initiative for vaccine research (IVR). [http://www.who.int/vaccine_research/diseases/viral_cancers/en/index2.html]

2 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis

C: results of a large, prospective cohort study. *Hepatology* 1998; 28: 1687–1695.
3 Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med* 1999; 340: 1228–1233.
4 Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335–1374.
5 McHutchison JG, Lawitz EJ, Shiffman ML *et al.* Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; 361: 580–593.
6 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.
7 Singal AG, Waljee AK, Shiffman M, Bacon BR, Schoenfeld PS. Meta-analysis: re-treatment of genotype I hepatitis C non-responders and relapsers after failing interferon and ribavirin combination therapy. *Aliment Pharmacol Ther* 2010; 32: 969–983.
8 Poynard T, Colombo M, Bruix J *et al.* Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009; 136: 1618–1628.
9 McHutchison JG, Manns MP, Muir AJ *et al.* Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010; 362: 1292–1303.
10 Jensen DM, Marcellin P, Freilich B *et al.* Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-α2b. *Ann Intern Med* 2009; 150: 528–540.
11 Di Bisceglie AM, Shiffman ML, Everson GT *et al.* Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; 359: 2429–2441.
12 Lin C, Kwong AD, Perni RB. Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect Disord Drug Targets* 2006; 6: 3–16.
13 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; 360: 1827–1838.
14 Hézode C, Forestier N, Dusheiko G *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; 360: 1839–1850.
15 Kumada H, Toyota J, Okanoue T *et al.* Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 2011; doi:10.1016/j.jhep.2011.07.016.
16 Simmonds P, Mellor J, Sakuldamrongpanich T *et al.* Evolutionary analysis of variants of hepatitis C virus found in South-East Asia: comparison with classifications based upon sequence similarity. *J Gen Virol* 1996; 77: 3013–3024.
17 Suzuki F, Akuta N, Suzuki Y *et al.* Rapid loss of hepatitis C virus genotype 1b from serum in patients receiving a triple treatment with telaprevir (MP-424), pegylated interferon and ribavirin for 12 weeks. *Hepatol Res* 2009; 39: 1056–1063.
18 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399–401.
19 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41: 1105–1109.
20 Akuta N, Suzuki F, Hirakawa M *et al.* Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* 2010; 52: 421–429.
21 Ochi H, Maekawa T, Abe H *et al.* ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy - a genome-wide study of Japanese HCV virus patients. *Gastroenterology* 2010; 139: 1190–1197.
22 Sakamoto N, Tanaka Y, Nakagawa M *et al.* ITPA gene variant protects against anemia induced by pegylated interferon-α and ribavirin therapy for Japanese patients with chronic hepatitis C. *Hepatol Res* 2010; 40: 1063–1071.

APPENDIX

The members of the phase III study were as follows: Sapporo Kosei General Hospital, Toranomon Hospital, Juntendo University Hospital, Musashino Red Cross Hospital, Toranomon Branch Hospital, University of Yamanashi Hospital, Shinshu University Hospital, Gifu Municipal Hospital, Ogaki Municipal Hospital, Nagoya University Hospital, Osaka University Hospital, Ikeda Municipal Hospital, Saiseikai Suita Hospital, Hiroshima University Hospital, Shin-Kokura Hospital, Kurume University Hospital and Kagoshima University Medical and Dental Hospital.

Reprinted from Early View
(Online Version of Record published before inclusion in an issue)

ORIGINAL ARTICLES—LIVER, PANCREAS, AND BILIARY TRACT

Characteristics of Patients With Nonalcoholic Steatohepatitis Who Develop Hepatocellular Carcinoma

KOHICHIROH YASUI,* ETSUKO HASHIMOTO,‡ YASUJI KOMORIZONO,§ KAZUHIKO KOIKE,‖ SHIGEKI ARII,¶ YASUHARU IMAI,# TOSHIHIDE SHIMA,** YOSHIHIRO KANBARA,** TOSHIJI SAIBARA,‡‡ TAKAHIRO MORI,§§ SUMIO KAWATA,‖‖ HIROFUMI UTO,¶¶ SHIRO TAKAMI,## YOSHIO SUMIDA,*** TOSHINARI TAKAMURA,‡‡‡ MIWA KAWANAKA,§§§ TAKESHI OKANOUE*,** and the Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan

*Department of Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto; ‡Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University, Tokyo; §Department of Hepatology, Nanpuh Hospital, Kagoshima; ‖Department of Gastroenterology, Graduate School of Medicine, University of Tokyo, Tokyo; ¶Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, Tokyo; #Department of Internal Medicine, Ikeda Municipal Hospital, Ikeda; **Center of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Suita; ‡‡Department of Gastroenterology and Hepatology, Kochi Medical School, Kochi; §§Department of Gastroenterology, Osaka Railway Hospital, Osaka; ‖‖Department of Gastroenterology, Yamagata University School of Medicine, Yamagata; ¶¶Digestive Disease and Life-style Related Disease Health Research, Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima; ##Department of Gastroenterology, Otsu Municipal Hospital, Otsu; ***Center for Digestive and Liver Diseases, Nara City Hospital, Nara; ‡‡‡Department of Disease Control and Homeostasis, Kanazawa University, Graduate School of Medical Science, Kanazawa; and §§§Center of Liver Diseases, Kawasaki Hospital, Kawasaki Medical School, Okayama, Japan

This article has an accompanying continuing medical education activity on page e50. Learning Objectives—At the end of this activity, the learner should identify the clinical features of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma and the role of hepatic fibrosis in the development of hepatocellular carcinoma.

See related article, Villanueva A et al, on page 1501 in *Gastroenterology*.

BACKGROUND & AIMS: Nonalcoholic steatohepatitis (NASH) can progress to hepatocellular carcinoma (HCC). We aimed to characterize the clinical features of NASH patients with HCC. **METHODS:** In a cross-sectional multicenter study in Japan, we examined 87 patients (median age, 72 years; 62% male) with histologically proven NASH who developed HCC. The clinical data were collected at the time HCC was diagnosed. **RESULTS:** Obesity (body mass index ≥25 kg/m^2), diabetes, dyslipidemia, and hypertension were present in 54 (62%), 51 (59%), 24 (28%), and 47 (55%) patients, respectively. In nontumor liver tissues, the degree of fibrosis was stage 1 in 10 patients (11%), stage 2 in 15 (17%), stage 3 in 18 (21%), and stage 4 (ie, liver cirrhosis) in 44 (51%). The prevalence of cirrhosis was significantly lower among male patients (21 of 54, 39%) compared with female patients (23 of 33, 70%) (P = .008). **CONCLUSIONS: Most patients with NASH who develop HCC are men; the patients have high rates of obesity, diabetes, and hypertension. Male patients appear to develop HCC at a less advanced stage of liver fibrosis than female patients.**

Keywords: Liver Cancer; Incidence; Sex; Retrospective Study.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer mortality.[1] HCC mostly occurs within an established background of chronic liver disease and cirrhosis. Although the risk factors for HCC, including infection with hepatitis B and C viruses as well as alcohol consumption, are well-defined, 5%–30% of patients with HCC lack a readily identifiable risk factor for their cancer. It has been suggested that a more severe form of nonalcoholic fatty liver disease (NAFLD), namely nonalcoholic steatohepatitis (NASH), might account for a substantial portion of cryptogenic cirrhosis and HCC cases.[2]

NAFLD is one of the most common causes of chronic liver disease in the world.[3,4] NAFLD is associated with obesity, diabetes, dyslipidemia, and insulin resistance and is recognized as a hepatic manifestation of metabolic syndrome. The spectrum of NAFLD ranges from a relatively benign accumulation of lipid (simple steatosis) to progressive NASH associated with fibrosis, necrosis, and inflammation. Despite its common occurrence and potentially serious nature, relatively little is known about the natural history or prognostic significance of NAFLD. Although prospective studies on the natural history of NAFLD and NASH with a larger cohort are awaited, these

Abbreviations used in this paper: AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CT, computed tomography; DCP, des-γ-carboxy prothrombin; γ-GTP, γ-glutamyl transpeptidase; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

1542-3565/$36.00
doi:10.1016/j.cgh.2011.01.023

studies might be limited by the long and asymptomatic clinical course of these diseases, by their high prevalence in the general population, and by the lack of serologic markers for NASH. The evidence suggesting that NASH can progress to HCC comes from (1) case reports and case series,[5-8] (2) retrospective studies,[9-12] and (3) prospective studies.[13-17] These studies generally examined limited numbers of cases and follow-ups; therefore, the incidence of HCC and risk factors for HCC in NASH patients remain unclear.

The Japan NASH Study Group (representative, Takeshi Okanoue)[18] was established in 2008 by the Ministry of Health, Labour and Welfare of Japan to address unmet research needs in the area of liver diseases. As a part of this mandate, the study group conducted a cross-sectional multicenter study to characterize the clinical features of histologically proven NASH patients who developed HCC.

Methods

Patients

We retrospectively identified and reviewed 87 Japanese patients with NASH, who developed HCC between 1993 and 2010, at 15 hepatology centers that belong to the Japan NASH Study Group[18] and their affiliated hospitals in Japan. The diagnosis of NASH was based on (1) the histologic features of steatohepatitis, (2) negligible alcohol consumption, and (3) exclusion of liver diseases of other etiology. To determine alcohol consumption as accurately as possible, we reviewed medical records in our institutions, and when patients had been transferred from other institutions, we also reviewed a summary of medical records from those institutions. According to the medical records, alcohol consumption was assessed on the basis of a detailed history that was obtained by physicians and by interviewing family members. Exclusion criteria included consumption of more than 20 g of alcohol per day, positivity for hepatitis B virus surface antigen, positivity for anti-hepatitis C virus antibody, the presence of other types of liver diseases (eg, primary biliary cirrhosis, autoimmune hepatitis, Wilson's disease, or hemochromatosis), previous treatment with drugs known to produce hepatic steatosis, and a history of gastrointestinal bypass surgery. The sections of nontumor liver tissues were reanalyzed by experienced hepatopathologists (T.O., E.H.) who were blinded to the laboratory parameters and clinical data. We excluded patients whose histologic diagnosis of NASH was not confirmed by central review and patients with insufficient or inconclusive information concerning alcohol consumption, body mass index (BMI), and laboratory data including fasting glucose and lipid.

Of the 87 patients, 14 patients had been previously diagnosed as NAFLD or NASH and had been followed at our institutions; 73 patients had been transferred from other institutions to our institutions for investigation and treatment of HCC. Most patients had been identified as having HCC during screening, which included ultrasound and/or computed tomography (CT) of the liver and alpha-fetoprotein (AFP) testing.

The diagnosis of HCC was based on liver histology and, in the absence of histology, on typical features of HCC as assessed by dynamic CT or magnetic resonance imaging (MRI) (ie, hypervascular with washout in the portal/venous phase).[19] Of the 87 patients, 49 patients were diagnosed as HCC after hepatic resection, 21 patients were diagnosed after ultrasound-guided tumor biopsy, and 17 patients were diagnosed by dynamic CT or MRI.

The Ethics Committees of each participating center approved this study. Informed consent was obtained from each patient in accordance with the Declaration of Helsinki.

Clinical Assessment and Laboratory Tests

The clinical and laboratory data were collected at the time HCC was diagnosed. BMI was calculated by using the following formula: weight in kilograms/(height in meters)^2. Obesity was defined as BMI $\geq$25 kg/m^2 according to the criteria of the Japan Society for the Study of Obesity.[20] Diabetes was defined as fasting plasma glucose concentration of $\geq$126 mg/dL or 2-hour plasma glucose concentration of $\geq$200 mg/dL during an oral glucose (75 g) tolerance test or by the use of insulin or oral hypoglycemic agents to control blood glucose.[21] Hypertension was defined as systolic blood pressure $\geq$130 mm Hg or diastolic blood pressure $\geq$85 mm Hg or by the use of antihypertensive agents.[22] Dyslipidemia was defined as serum concentrations of triglycerides $\geq$150 mg/dL or high-density lipoprotein (HDL) cholesterol $<$40 mg/dL and $<$50 mg/dL for men and women, respectively, or by the use of specific medication.[22]

Venous blood samples were taken in the morning after 12-hour overnight fast. The laboratory evaluations included blood cell count and measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), fasting plasma glucose, HbA1c, total cholesterol, HDL cholesterol, triglyceride, ferritin, hyaluronic acid, AFP, and des-γ-carboxy prothrombin (DCP). These parameters were measured by using standard clinical chemistry techniques.

Histopathologic Examination

Nontumor liver tissues were obtained from all 87 patients to diagnose the background liver tissue at the time HCC was diagnosed. In 49 patients who underwent hepatic resection for HCC, we examined nontumor liver tissues that were surgically resected. In 21 patients who underwent ultrasound-guided tumor biopsy, nontumor liver tissues far from HCC tumors were biopsied separately. In 17 patients who were diagnosed as HCC by dynamic CT or MRI and did not undergo either hepatic resection or tumor biopsy, only nontumor liver tissues far from HCC tumors were obtained by ultrasound-guided biopsy.

The specimens were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin, with Masson trichrome, and by silver impregnation. NASH was defined as steatosis with lobular inflammation, hepatocellular ballooning, and Mallory's hyaline (Mallory's body) or fibrosis.[23-25] The necroinflammatory grade and the degree of fibrosis were evaluated and scored according to the criteria proposed by Brunt et al.[26]

Statistical Analysis

Results are presented as numbers with percentages in parentheses for qualitative data or as the medians and ranges (25th-75th percentiles) for quantitative data. Comparisons were made by using a χ^2 test for qualitative factors or a Mann-

Table 1. Patient Characteristics

Characteristic	Total (n = 87)	Male (n = 54)	Female (n = 33)	*P* value[a]
Age (*y*)	72 (69–75)	72 (69–75)	72 (68–75)	.52
BMI (kg/m^2)	26.0 (23.8–28.3)	26.0 (23.8–28.8)	26.2 (23.9–27.7)	.54
Obesity	54 (62%)	35 (65%)	19 (58%)	.50
Diabetes	51 (59%)	31 (57%)	20 (61%)	.77
Dyslipidemia	24 (28%)	13 (24%)	11 (33%)	.35
Hypertension	47 (54%)	22 (41%)	25 (76%)	.001
Platelet count ($\times 10^4/\mu L$)	13.9 (10.1–18.0)	14.5 (11.7–18.0)	10.9 (7.8–18.0)	.05
AST (*IU/L*)	47 (30–59)	46 (27–60)	47 (35–58)	.45
ALT (*IU/L*)	36 (26–55)	43 (26–69)	34 (26–42)	.11
γ-GTP (*IU/L*)	75 (40–115)	68 (36–177)	75 (40–115)	.90
Fasting glucose (*mg/dL*)	114 (99–145)	112 (99–144)	120 (97–152)	.59
HbA1c (*%*)	6.1 (5.4–7.1)	5.9 (5.4–7.0)	6.3 (5.2–7.1)	.78
Total cholesterol (*mg/dL*)	169 (147–202)	169 (147–202)	169 (147–202)	.62
HDL cholesterol (*mg/dL*)	50 (41–60)	45 (41–58)	55 (50–73)	.03
Triglyceride (*mg/dL*)	100 (76–138)	118 (80–147)	96 (74–116)	.06
Ferritin (*ng/dL*)[b]	197 (74–401)	273 (154–703)	98 (23–172)	.005
Hyaluronic acid (*ng/mL*)[c]	166 (67–241)	151 (69–244)	174 (61–332)	.85
AFP (*ng/mL*)	7.1 (5.0–18.0)	6.0 (4.0–14.7)	10.8 (5.9–18.0)	.02
DCP (*mAU/mL*)	66 (22–298)	48 (22–243)	81 (21–942)	.42
HCC tumor size (*cm*)	3.0 (2.0–4.0)	3.1 (2.2–4.5)	2.6 (1.9–4.0)	.18
Number of HCC tumors				.78
1	65 (75%)	39 (72%)	26 (79%)	
2 or 3	16 (18%)	11 (20%)	5 (15%)	
≥4	6 (7%)	4 (8%)	2 (6%)	
Background liver tissue				
Steatosis grade				.64
0: <5%	1 (1%)	1 (2%)	0 (0%)	
1: 5%–33%	60 (69%)	36 (67%)	24 (73%)	
2: 34%–66%	19 (22%)	11 (20%)	8 (24%)	
3: >66%	7 (8%)	6 (11%)	1 (3%)	
Necroinflammatory grade[d]				.22
1: mild	31 (35%)	22 (41%)	9 (27%)	
2: moderate	45 (52%)	26 (48%)	19 (58%)	
3: severe	11 (13%)	6 (11%)	5 (15%)	
Fibrosis stage[d]				.003
1	10 (11%)	10 (18%)	0 (0%)	
2	15 (17%)	10 (18%)	5 (15%)	
3	18 (21%)	13 (25%)	5 (15%)	
4	44 (51%)	21 (39%)	23 (70%)	

NOTE. Values are medians (25th–75th percentiles) or numbers (%). Where no other unit is specified, values refer to number of patients.
[a]χ^2 test or Mann–Whitney *U* test.
[b]Missing data for 27 patients.
[c]Missing data for 29 patients.
[d]According to reference 26.

Whitney *U* test on ranks for quantitative factors with non-equal variance. *P* values less than .05 from two-sided tests were considered to be significant. All statistical analyses were performed by using SPSS 15.0 software (SPSS Inc, Chicago, IL).

Results

The characteristics of the 87 NASH patients who developed HCC are summarized in Table 1. The median age was 72 years (25th percentile, 69; 75th percentile, 75); the mean age (standard deviation) was 71.2 (6.7) years. There were 54 male patients (62%) and 33 female patients (38%); the male:female ratio was 1.6:1. The median BMI was 26.0 kg/m^2, and 54 patients (62%) were obese (BMI ≥25 kg/m^2). Diabetes, dyslipidemia, and hypertension were present in 51 (59%), 24 (28%), and 47 (55%) patients, respectively.

The diagnosis of NASH was proved by histologic examination of nontumor liver tissues at the time HCC was diagnosed. The degree of steatosis was grade 1 (5%–33%) in 60 patients (69%), grade 2 (34%–66%) in 19 (22%), and grade 3 (>66%) in 7 (8%). One patient who showed less than 5% steatosis was diagnosed as "burn-out" NASH, because a previous liver biopsy that was performed before development of HCC had demonstrated typical histologic features of NASH. The necroinflammatory grade was mild (grade 1) in 31 patients (35%), moderate (grade 2) in 45 (52%), and severe (grade 3) in 11 (13%). The degree of fibrosis was stage 1 in 10 patients (11%), stage 2 in 15 (17%), stage 3 in 18 (21%), and stage 4 (ie, liver cirrhosis) in 44 (51%).

The median diameter of HCC tumors was 3.0 cm (25th percentile, 2.0; 75th percentile, 4.0). A single HCC lesion was present in 65 of 87 patients (75%).

Data were stratified according to sex (Table 1). Compared with female patients, male patients had significantly less hypertension, lower HDL cholesterol and AFP, higher ferritin, and a less advanced stage of fibrosis. The prevalence of cirrhosis was significantly lower in male patients (21 of 54, 39%) than in female patients (23 of 33, 70%) (P = .008).

Discussion

In this cross-sectional multicenter study in Japan, we showed the clinical features of a relatively large number (n = 87) of NASH patients with HCC. The male:female ratio was 1.6:1. Men have higher HCC rates than women in almost all populations, with male:female ratios usually averaging between 2:1 and 4:1.[2] In the latest nationwide survey of HCC in Japan,[27] this ratio was 2.5:1. The reasons underlying higher rates of HCC in men might relate to sex-specific differences in exposure to risk factors. Men are more likely to be infected with hepatitis B and C viruses, consume alcohol, smoke cigarettes, and have increased iron stores.[2] Moreover, androgens are considered to influence the development of HCC. With regard to the male: female ratio of HCC associated with NASH, a male:female ratio of 1.3:1 was reported in a summary of 16 published cases of HCC associated with NASH.[28] Ratios of 2.8:1 and 0.67:1 were reported in 2 retrospective studies of HCC arising from cryptogenic cirrhosis in Italy (n = 44)[10] and the United States (n = 30),[9] respectively, and a ratio of 1.6:1 was reported for 36 cases of NASH-associated HCC from a single center in Japan.[15] Overall, NASH patients with HCC are more often men. However, these male:female ratios might be lower than the ratios for HCC of other etiologies, including viral hepatitis and alcohol consumption.

Although it is well-known that male gender is a risk factor for HCC in patients infected with hepatitis B and C viruses,[2] it remains unclear whether male gender is a factor associated with the development of HCC in NASH patients. It is now suspected that there is an even distribution of NASH among men and women.[29] In another study by our group,[30] the male:female ratio was 0.85:1 in 342 NASH patients without cirrhosis and HCC. The male:female ratio (1.6:1) of NASH patients with HCC in the present study is higher than this ratio. In agreement with our observations, a case-control study showed that the male: female ratio was 1.6:1 in 34 NASH patients with HCC, whereas the ratio was 0.69:1 in 348 NASH patients without HCC.[15] A recent prospective study indicated that older age and alcohol consumption were independent risk factors for the development of HCC in patients with NASH-cirrhosis and that male gender tended to be associated with the development of HCC, although this trend did not reach statistical significance.[17]

The median age of our patients was 72 years. There was no significant difference in age between men and women. Although the global age distribution of HCC varies by geographic region, sex, and etiology, in almost all areas the peak female age group in HCC patients is 5 years older than in male HCC patients.[2] In a nationwide survey of HCC in Japan,[27] the mean ages were 65.5 years for men and 69.4 years for women. The male patients in the present study are slightly older than the mean ages reported in these previous studies.

Consistent with the literature,[9-12] more than half of our patients displayed obesity, diabetes, and hypertension. Obesity constitutes a significant risk factor for cancer mortality in general and is an increasingly recognized risk factor for HCC in particular.[31,32] In the present study, body weight was measured at the time HCC was diagnosed. Because advanced HCC might cause weight loss, it is likely that our patients were obese before the development of HCC. Diabetes has also been proposed as a risk factor for HCC.[2] Thus, HCC shares 2 major risk factors, obesity and diabetes, with NASH.

Once cirrhosis and HCC are established, it is difficult to identify pathologic features of NASH. As NASH progresses to cirrhosis, steatosis tends to disappear, so-called burn-out NASH.[5] As expected, the grade of steatosis was mild in most of our cases. It was possible to diagnose 1 case without steatosis as burn-out NASH, because a previous liver biopsy specimen (liver biopsy was performed 25 years prior) was preserved and available. It is likely that many cases of NASH-associated HCC might have been missed because of loss of the telltale sign of steatosis.

Most HCC arises on a background of cirrhosis. It is less clear whether cirrhosis is a necessary predisposition for the development of HCC in patients with NASH. Case reports of HCC arising from NAFLD and NASH patients without fibrosis or cirrhosis have been accumulating.[33-36] Cirrhosis (fibrosis stage 4) was present in 51% of cases, and advanced stages of fibrosis (stage 3 or 4) were found in 72% of cases in the present study. Indeed, cirrhosis or advanced fibrosis appeared to be the predominant risk factors for HCC development. However, in the remaining 28% of cases, HCC developed in patients with less fibrosis (stage 1 or 2). Interestingly, male patients developed HCC at a less advanced stage of fibrosis than female patients, and the prevalence of cirrhosis was significantly lower in men (39%) than in women (70%). Although the reason for the sex differences is unclear, these findings indicate that screening for HCC is needed not only in NASH patients with advanced fibrosis but also in those with less fibrosis, particularly if they are men. Further studies are needed to confirm this potentially important observation. Paradis et al[37] reported that in patients whose only risk factors for chronic liver disease are features of metabolic syndrome, HCC usually occurs in the absence of significant liver fibrosis. In addition, they found that some of these HCCs developed on preexisting liver cell adenomas. However, no preexisting adenomas were observed in the present cases.

Compared with female patients, male patients had significantly higher serum ferritin value. The normal value for ferritin varies according to the age and gender of the individual. Adult men have serum ferritin values averaging approximately 100 ng/mL (range, 75–250), whereas adult women have levels averaging approximately 30 ng/mL (range, 20–75).[38] Thus, normal men have higher ferritin levels than women. Elevation of ferritin levels is associated with NASH.[39] Because we excluded patients with alcohol consumption as rigorously as possible, we believe that alcohol consumption did not contribute to the elevation of ferritin levels in our patients.

The median diameter of the HCCs in the present study was 3.0 cm, which is equal to or smaller than the size of previously reported HCCs.[9,10,12,28,37] This is probably because most of our patients had been identified as having HCC during screening. A single HCC lesion was present in 75% of patients. For early detection of NASH-associated HCC, vigilant screening is important,[9] and the development of serologic markers for NASH is necessary.

The mechanisms of carcinogenesis in NASH remain to be elucidated. Possible mechanisms include hyperinsulinemia

caused by insulin resistance in NASH, increased levels of insulin-like growth factor that promotes tumor growth, increased susceptibility of the steatotic liver to lipid peroxidation, production of reactive oxygen species and subsequent DNA mutations, disordered energy and hormonal regulation in obesity, and aberrations in regenerative processes occurring in cirrhosis.[25]

Certain limitations should be considered in the interpretation of our findings. First, the cross-sectional study design hinders the ability to draw inferences regarding the causality of NASH in HCC. Second, the study did not include a control group of HCC patients with other liver diseases. Third, there might be a bias in patient selection, because patients were retrospectively identified as having NASH-associated HCC. Finally, although our patients were negative for hepatitis B virus surface antigen, it is still possible that occult hepatitis B virus infection might be associated with the development of HCC in some of our cases.

In summary, we showed the clinical features of NASH patients with HCC. NASH patients with HCC were more often men and frequently displayed obesity, diabetes, and hypertension. Our results suggest that male patients might develop HCC at a less advanced stage of fibrosis than female patients. Further prospective studies with a longer follow-up time and larger cohorts are needed to determine the causal association of NASH with HCC and to identify risk factors for the development of HCC in NASH patients.

References

1. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533–543.
2. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557–2576.
3. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43:S99–S112.
4. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346:1221–1231.
5. Powell EE, Cooksley WG, Hanson R, et al. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 1990;11:74–80.
6. Cotrim HP, Paraná R, Braga E, et al. Nonalcoholic steatohepatitis and hepatocellular carcinoma: natural history? Am J Gastroenterol 2000;95:3018–3019.
7. Zen Y, Katayanagi K, Tsuneyama K, et al. Hepatocellular carcinoma arising in non-alcoholic steatohepatitis. Pathol Int 2001;51:127–131.
8. Shimada M, Hashimoto E, Taniai M, et al. Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2002;37:154–160.
9. Marrero JA, Fontana RJ, Su GL, et al. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. Hepatology 2002;36:1349–1354.
10. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123: 134–140.
11. Ratziu V, Bonyhay L, Di Martino V, et al. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. Hepatology 2002;35:1485–1493.
12. Regimbeau JM, Colombat M, Mognol P, et al. Obesity and diabetes as a risk factor for hepatocellular carcinoma. Liver Transpl 2004;10:S69–S73.
13. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–121.
14. Sanyal AJ, Banas C, Sargeant C, et al. Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. Hepatology 2006;43:682–689.
15. Hashimoto E, Yatsuji S, Tobari M, et al. Hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. J Gastroenterol 2009;44:89–95.
16. Yatsuji S, Hashimoto E, Tobari M, et al. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. J Gastroenterol Hepatol 2009;24:248–254.
17. Ascha MS, Hanouneh IA, Lopez R, et al. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology 2010;51:1972–1978.
18. Okanoue T, Umemura A, Yasui K, et al. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in Japan. J Gastroenterol Hepatol 2011;26(Suppl 1):153–162.
19. Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.
20. Japan Society for the Study of Obesity. New criteria of obesity (in Japanese). J Jpn Soc Study Obes 2000;6:18–28.
21. Kuzuya T, Nakagawa S, Satoh J, et al. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. Diabetes Res Clin Pract 2002;55:65–85.
22. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–2497.
23. Matteoni CA, Younossi ZM, Gramlich T, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–1419.
24. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
25. Brunt EM. Non-alcoholic fatty liver disease. In: Burt AD, Portmann BC, Ferrell LD, eds. MacSween's pathology of the liver. 5th ed. London: Churchill Livingstone, 2006:367–397.
26. Brunt EM, Janney CG, Di Bisceglie AM, et al. Non-alcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–2474.
27. Ikai I, Arii S, Okazaki M, et al. Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. Hepatol Res 2007;37:676–691.
28. Bugianesi E. Non-alcoholic steatohepatitis and cancer. Clin Liver Dis 2007;11:191–207.
29. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. Hepatology 2003;37:1202–1219.
30. Sumida Y, Yoneda M, Hyogo H, et al. A simple clinical scoring system using ferritin, fasting insulin and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. J Gastroenterol 2011;46:257–268.
31. Calle EE, Rodriguez C, Walker-Thurmond K, et al. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–1638.
32. Caldwell S, Park SH. The epidemiology of hepatocellular cancer: from the perspectives of public health problem to tumor biology. J Gastroenterol 2009;44:96–101.
33. Bullock RE, Zaitoun AM, Aithal GP, et al. Association of non-alcoholic steatohepatitis without significant fibrosis with hepatocellular carcinoma. J Hepatol 2004;41:685–686.
34. Ichikawa T, Yanagi K, Motoyoshi Y, et al. Two cases of non-alcoholic steatohepatitis with development of hepatocellular carcinoma without cirrhosis. J Gastroenterol Hepatol 2006;21:1865–1866.
35. Guzman G, Brunt EM, Petrovic LM, et al. Does nonalcoholic fatty liver disease predispose patients to hepatocellular carcinoma in the absence of cirrhosis? Arch Pathol Lab Med 2008;132:1761–1766.

36. Kawada N, Imanaka K, Kawaguchi T, et al. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. J Gastroenterol 2009;44:1190–1194.
37. Paradis V, Zalinski S, Chelbi E, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology 2009; 49:851–859.
38. Adamson JW. Hematopoietic disorders. In: Fauci AS, Braunwald E, Kasper DL, et al, eds. Harrison's principles of internal medicine. 17th ed. New York: McGraw-Hill Companies, 2008:628–634.
39. Bonkovsky HL, Jawaid Q, Tortorelli K, et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. J Hepatol 1999;31:421–429.

Reprint requests

Address requests for reprints to: Takeshi Okanoue, MD, PhD, Director, Center of Gastroenterology and Hepatology, Saiseikai Suita Hospital, 1-2 Kawazono-cho, Suita 5640013, Japan. e-mail: okanoue@suita.saiseikai.or.jp; fax: +81-6-6382-1524.

Conflicts of interest

The authors disclose no conflicts.

Funding

This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan (H20-hepatitis-008 to Takeshi Okanoue).

doi:10.1111/j.1440-1746.2010.06547.x

REVIEW

Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in Japan

Takeshi Okanoue,*,† Atsushi Umemura,* Kohichiroh Yasui† and Yoshito Itoh†

*Center of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Osaka and †Department of Gastroenterology, Faculty of Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan

Key words
diabetes mellitus, obesity, metabolic syndrome.

Correspondence
Dr. Takeshi Okanoue, Center of Gastroenterology and Hepatology, Saiseikai Suita Hospital, 1-2 Kawazono-cho Suita, Osaka 564-0013, Japan. Email: okanoue@suita.saiseikai.or.jp

Conflict of interest
The authors do not have any conflicts of interest to disclose.

Abstract

During the past 20 to 30 years, the frequency of patients presenting with nonalcoholic fatty liver diseases (NAFLD) has increased gradually in Japan in proportion to the increase in the population with life-style related diseases. We describe here the current status of the clinical and basic aspects of research into NAFLD in Japan.

The increase in the incidence of life-style-related diseases has resulted in an increase in NAFLD throughout the past 20 to 30 years. The rate of obesity in the population is not high compared to western countries but the incidence of NAFLD is similar to those countries. In 2008 we started a nationwide study of NAFLD which has been supported by the Ministry of Labor and Welfare Japan. In this project, we planned to investigate the epidemiology, genetic backgrounds and biochemical markers, and liver injury in patients with diabetes mellitus (DM) and hepatocellular carcinoma in NASH, and treatment of NASH. Approximately 20 to 25% of DM patients showed NAFLD in which the prevalence of NASH might be more than 30 to 40%. Fortunately, we have been able to obtain very interesting results from our group studies, including single necleotide polymorphisms (SNPs) which will be published in the near future.

Introduction

In 1980, Ludwig *et al.* proposed a new disease concept called nonalcoholic steatohepatitis (NASH), a condition which may progress to cirrhosis and hepatocellular carcinoma (HCC). In Japan, much attention has been paid over the past few decades to patients infected with hepatitis B virus (HBV) and hepatitis C virus (HCV), because the rates of carriage of these viruses are high and most cases of cirrhosis and hepatocellular carcinoma (HCC) in Japan are associated with persistent HBV and HCV infection. In recent years, however, with the westernization of the Japanese lifestyle, public interest in lifestyle-related diseases has increased rapidly metabolic syndrome has attracted attention for its association with underlying insulin resistance, and the risk of dyslipidemia, and hypertension even in non-obese Japanese. In 2007, the Japan Society of Diabetes Mellitus reported that, among the causes of death for 18 385 individuals with diabetes, liver cancer was the leading cause (8.6%), while death from liver cirrhosis also was very common (4.7%). Altogether, 13.3% of death among diabetes patients were attributable to liver disease (Fig. 1);[1] however, the prevalence of hepatitis virus infections and heavy alcohol drinking were not analyzed in that paper.

Seventy to seventy-five percent of HCC in Japan is associated with HCV infection, approximately 15% of patients are positive for hepatitis B surface B antigen (HBsAg), and the remaining 10–15% are so-called non-B non-C HCC. The proportion of non-B non-C HCC increased from 6.8% in 1992 to almost twice that during the subsequent ten years. Total alcohol consumption in Japan has not increased in the past 15 years, the possibility arises that NASH is responsible for this apparent increase in non-B non-C HCC (Fig. 2).

Most Japanese are not obese but nonalcoholic fatty liver disease (NAFLD) is becoming more common. At present, NASH is one of the most important liver diseases in Japan. In 2008, the Japan NASH Study Group (of which Takeshi Okanoue is a member) was founded, supported by the Ministry of Labor and Welfare, Japan. The purpose was to elucidate the epidemiology, pathophysiology, genetic backgrounds and long-term prognosis of NAFLD. Other objectives are to establish biochemical markers for differential diagnosis between simple steatosis (SS) and NASH, and devise treatment guidelines based on the individual pathophysiology of NASH.

Epidemiology

In the last two decades, patients diagnosed with fatty liver by image analysis and with elevated serum alanine aminotransferase (ALT) increased in number in proportion to the increase in