Affymetrix GeneChip Custom 10K array. After excluding nine cases with call rate of <0.95, all cluster plots were checked by visual inspection by trained staff, and SNPs with ambiguous calls were excluded. Ninety-four randomly selected case samples in the first stage were re-genotyped in the second stage, and SNPs with concordance rates of <98% between two assays (Illumina and Affymetrix) were excluded from the further analysis. We used genome-wide screening data of other diseases (uterine cervical cancer, esophageal cancer, hematological cancer, pulmonary tuberculosis, ovarian cancer, uterine body cancer and keroid) as controls for the second stage. All the samples were genotyped using the Illumina HumanHap550v3 Genotyping BeadChip, and the same quality-control filters as the first screening were applied. As a result, we analyzed 9,875 SNPs in 607 cases and 1,267 controls in the second stage and found 11 SNPs ($P < 5.06 \times 10^{-6}$) to be significantly associated with chronic hepatitis B after Bonferroni correction. These first and second stages are defined as the discovery phase of the research, and the following replication studies are defined as the replication phase. In the replication analyses, we used TaqMan genotyping system (Applied Biosystems) or the multiplex PCR-based Invader assay (Third Wave Technologies).

***HLA-DPA1* and *HLA-DPB1* genotyping.** We analyzed *HLA-DP* genotypes using 607 cases (in the second stage of GWAS) and 934 controls (in the first stage of GWAS). Exon 2 of the *HLA-DPA1* and *HLA-DPB1* genes were amplified and directly sequenced according to the protocol of International Histocompatibility Workshop Group[22]. *HLA-DPA1* and *DPB1* alleles were determined based on the alignment database of dbMHC.

Statistical analysis. In the first stage of the GWAS, Fisher's exact test was applied to a two-by-two contingency table in three genetic models: an allele frequency model, a dominant-effect model and a recessive-effect model. At the second stage of GWAS and replication analyses, statistical significance of the association with each SNP was assessed using a 1-degree-of-freedom Cochrane-Armitage trend test. Significance levels after Bonferroni correction for multiple testing were $P = 5.06 \times 10^{-6}$ (0.05/9,875) in the second stage and $P = 0.025$ (0.05/2) in replication analyses. Age- and sex-adjusted odds ratios were obtained by logistic regression analysis. Odds ratios and confidence intervals were calculated using the major allele as a reference. The meta-analysis was conducted using the Mantel-Haenszel method. Heterogeneity among studies was examined by using the Breslow-Day test. To assess the association of each *HLA* allele, we used Fisher's exact tests on two-by-two contingency tables with or without each *HLA* allele. To analyze the association of haplotypes, we used R package haplo.stats. *P* values for each haplotype were given by the results of a score test, and odds ratios and 95% confidence intervals were calculated from coefficients of GLM model. Odds ratios of each haplotype were calculated relative to the second major haplotype in **Table 2**, because the most common haplotype was the disease-associated haplotype. All of these statistical values were calculated by function haplo.cc. We used Haploview software to analyze linkage disequilibrium values between *HLA-DR13* and SNPs.

Software. For general statistical analysis, we used R statistical environment version 2.6.1 or PLINK1.03 (ref. 23). To draw the LD map, we used Haploview software[24]. Estimation of haplotype frequencies and analysis of haplotype association were performed by R package haplo.stats[25]. Sequence variants in exon2 of *HLA-DPA1* and *HLA-DPB1* were analyzed by Polyphred.

URLs. The Japan Society of Hepatology, http://www.jsh.or.jp/medical/gudelines/index.html; Hiroshima Liver Study Group, http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html; PLINK1.03, http://pngu.mgh.harvard.edu/~purcell/plink/; R package haplo.stats, http://mayoresearch.mayo.edu/mayo/research/schaid_lab/software.cfm; Polyphred, http://droog.gs.washington.edu/polyphred/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS
Y.N. conceived the study; Y.N., Y.K., Y.D., M.K. and K.M. designed the study; Y.K., S.W., H.O. and N.H. performed genotyping; Y.K., T.T., M.K., N.K., Y.N. and K.M. wrote the manuscript; T.K., A.T., T.T. and N.K. performed data analysis at the genome-wide phase; Y.N., K.M. and M.K. managed DNA samples belong to BioBankJapan; K.C. and H.K. managed second replication samples; W.C., A.P. and T.S. managed third replication samples in Thailand; Y.K. summarized the whole results; Y.N. obtained funding for the study.

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1. Pungpapong, S., Kim, W. & Poterucha, J. Natural history of hepatitis B virus infection: an update for clinicians. *Mayo Clin. Proc.* **82**, 967–975 (2007).
2. Custer, B. *et al.* Global epidemiology of hepatitis B virus. *J. Clin. Gastroenterol.* **38**, S158–S168 (2004).
3. Lai, C.L., Ratziu, V., Yuen, M.F. & Poynard, T. Viral hepatitis B. *Lancet* **362**, 2089–2094 (2003).
4. Kobayashi, M. *et al.* Viral genotypes and response to interferon in patients with acute prolonged hepatitis B virus infection of adulthood in Japan. *J. Med. Virol.* **68**, 522–528 (2002).
5. Laskus, T. *et al.* Prevalence of markers of hepatitis viruses in out-patient alcoholics. *J. Hepatol.* **15**, 174–178 (1992).
6. Sheen, I., Liaw, Y., Lin, D. & Chu, C. Role of hepatitis C and delta viruses in the termination of chronic hepatitis B surface antigen carrier state: a multivariate analysis in a longitudinal follow-up study. *J. Infect. Dis.* **170**, 358–361 (1994).
7. Lin, T. *et al.* Hepatitis B virus markers in Chinese twins. *Anticancer Res.* **9**, 737–741 (1989).
8. Ben-Ari, Z. *et al.* Cytokine gene polymorphisms in patients infected with hepatitis B virus. *Am. J. Gastroenterol.* **98**, 144–150 (2003).
9. Thursz, M.R. *et al.* Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. *N. Engl. J. Med.* **332**, 1065–1069 (1995).
10. Bellamy, R. *et al.* Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J. Infect. Dis.* **179**, 721–724 (1999).
11. Deng, G. *et al.* Association of estrogen receptor polymorphisms with susceptibility to chronic hepatitis B virus infection. *Hepatology* **40**, 318–326 (2004).
12. Singh, R., Kaul, R., Kaul, A. & Khan, K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J. Gastroenterol.* **13**, 1770–1787 (2007).
13. Hohler, T. *et al.* HLA-DRB1* 1301 and* 1302 protect against chronic hepatitis B. *J. Hepatol.* **26**, 503–507 (1997).
14. Ahn, S.H. *et al.* Association between hepatitis B virus infection and HLA-DR type in Korea. *Hepatology* **31**, 1371–1373 (2000).
15. Frodsham, A. Host genetics and the outcome of hepatitis B viral infection. *Transpl. Immunol.* **14**, 183–186 (2005).
16. Frodsham, A. *et al.* Class II cytokine receptor gene cluster is a major locus for hepatitis B persistence. *Proc. Natl. Acad. Sci. USA* **103**, 9148–9153 (2006).
17. Jung, M. *et al.* Activation of a heterogeneous hepatitis B (HB) core and e antigen-specific CD4+ T-cell population during seroconversion to anti-HBe and anti-HBs in hepatitis B virus infection. *J. Virol.* **69**, 3358–3368 (1995).
18. Fontenot, A., Torres, M., Marshall, W., Newman, L. & Kotzin, B. Beryllium presentation to CD4+ T cells underlies disease-susceptibility HLA-DP alleles in chronic beryllium disease. *Proc. Natl. Acad. Sci. USA* **97**, 12717–12722 (2000).
19. Mineta, M. *et al.* Contribution of HLA class I and class II alleles to the regulation of antibody production to hepatitis B surface antigen in humans. *Int. Immunol.* **8**, 525–531 (1996).
20. Nakamura, Y. The BioBank Japan project. *Clin. Adv. Hematol. Oncol.* **5**, 696–697 (2007).
21. Saito, A. & Kamatani, N. Strategies for genome-wide association studies: optimization of study designs by the stepwise focusing method. *J. Hum. Genet.* **47**, 360–365 (2002).
22. van der Zwan, A., Griffith, B., Rozemuller, E., Williams, T. & Tilanus, M.G.J. *IHWG Technical Manual Genomic Analysis of the Human MHC: DNA-Based Typing for HLA Alleles and Linked Polymorphisms* (ed. Tilanus, M.G.J.) (Seattle, Washington, International Histocompatibility Working Group, 2002).
23. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
24. Barrett, J., Fry, B., Maller, J. & Daly, M. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265 (2005).
25. Schaid, D.J., Rowland, C.M., Tines, D.E., Jacobson, R.M. & Poland, G.A. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am. J. Hum. Genet.* **70**, 425–434 (2002).

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ORIGINAL ARTICLE

Antiviral activity, dose–response relationship, and safety of entecavir following 24-week oral dosing in nucleoside-naive Japanese adult patients with chronic hepatitis B: a randomized, double-blind, phase II clinical trial

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Abstract
Purpose A randomized, double-blind, multicenter study (ETV-047) was conducted to evaluate the dose–response relationship of entecavir and compare its antiviral activity and safety with lamivudine in Japanese patients with chronic hepatitis B (CHB).
Methods One hundred thirty-seven nucleoside-naive adult patients with CHB were randomized to once-daily oral doses of entecavir 0.01, 0.1, or 0.5 mg or lamivudine 100 mg for 24 weeks. The primary efficacy end point used to evaluate the dose–response relationship was mean change from baseline in serum hepatitis B virus (HBV) DNA level at week 22, as determined by polymerase chain reaction assay.
Results Entecavir demonstrated a clear dose–response relationship, with mean change from baseline in serum

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HBV DNA level of −3.11, −4.77, and −5.16 $\log_{10}$ copies/ml with entecavir 0.01, 0.1, and 0.5 mg, respectively. Entecavir 0.5 mg was superior to lamivudine 100 mg for the mean change in HBV DNA level (−5.16 vs. −4.29 $\log_{10}$ copies/ml; $P = 0.007$). The overall incidence of adverse events was comparable between treatment groups. Two patients discontinued treatment because of adverse events (one with liver cirrhosis [entecavir 0.5 mg] and one with grade 4 serum alanine aminotransferase (ALT) elevation, nausea, and malaise [lamivudine 100 mg]). Serum ALT flares were observed in four patients; flares were associated with 2 $\log_{10}$ reductions or more in HBV DNA level and resolved without dose interruption.

Conclusion Entecavir 0.01–0.5 mg is well tolerated and produces a dose-dependent reduction in viral load in nucleoside-naive Japanese patients with CHB. Compared with lamivudine 100 mg, entecavir 0.1 mg demonstrated noninferiority and entecavir 0.5 mg was superior in this population.

Keywords Chronic hepatitis B · Entecavir · Lamivudine · HBV DNA · ALT flare

Introduction

It is reported that more than 2 billion individuals worldwide have been infected with hepatitis B virus (HBV) and approximately 350 million people are long-term HBV carriers [1]. Chronic hepatitis B (CHB) is induced by chronic replication of HBV in the liver and has a poor prognosis, with 20–40% of infected individuals developing liver cirrhosis, noncompensated liver disorder, or hepatocellular carcinoma [2]. Treatment of CHB is aimed at sustained inhibition of HBV replication and remission of liver disease [3], ultimately preventing progression to liver cirrhosis or hepatocellular carcinoma [4].

Prior to the advent of the nucleoside analog lamivudine, interferon-α formed the mainstay of treatment, but this immunoregulatory cytokine requires parenteral administration and is poorly tolerated [5]. Lamivudine is well tolerated on oral administration and has been proven to be highly effective in the treatment of CHB, but the emergence of resistance mutations (including the YMDD motif) in the reverse-transcriptase domain of HBV polymerase frequently results in overt viral rebound and disease progression [6–9]. The novel nucleoside analog adefovir is effective against wild-type HBV and lamivudine-resistant strains and is well tolerated on long-term administration, but its clinical use is restricted by the need for renal monitoring in patients with impaired renal function [10].

Entecavir, a cyclopentylguanine-derived nucleoside analog and selective inhibitor of HBV replication, was approved by the U.S. Food and Drug Administration in 2005 for the treatment of CHB. Entecavir displays potent antiviral activity in the woodchuck and duck models of HBV infection [11, 12] and is reported to be 100- to 2,200-fold more potent than lamivudine and adefovir in inhibiting HBV replication in vitro [13, 14]. Phase II clinical trials of entecavir conducted in non-Japanese patients with CHB have demonstrated entecavir to be well tolerated and more effective than lamivudine [15, 16].

A global dose-finding study (ETV-005) conducted in lamivudine-naive patients with CHB compared three doses of entecavir (0.01, 0.1, and 0.5 mg once daily) with lamivudine 100 mg once daily over a 22-week treatment period. Entecavir showed a clear dose–response relationship and was well tolerated at all three dose levels; in addition, 0.1 and 0.5 mg of entecavir showed superior antiviral activity compared with 100 mg of lamivudine [15].

Phase I studies of single-dose (0.05–2.5 mg) and multiple-dose (0.1–1.0 mg daily) entecavir conducted in Japan have confirmed the drug's safety in healthy men. As in Caucasian populations, entecavir displayed linear plasma pharmacokinetics over a wide range of doses, including putative therapeutic doses (0.5 and 1.0 mg), in Japanese subjects; there was no evidence of significant ethnic differences in its pharmacokinetics and pharmacodynamics. Similar findings to those obtained in the global phase II clinical trials of entecavir might therefore be expected from corresponding studies conducted in Japanese patients.

To evaluate the dose–response relationship, the antiviral activity and safety of entecavir in Japanese CHB patients, we conducted a 24-week phase II study comparing entecavir (0.01, 0.1, and 0.5 mg daily) to lamivudine (100 mg daily).

Materials and methods

Study design

This randomized, double-blind, double-dummy study was conducted at 38 institutions in Japan from August 2003 to March 2005. Eligible patients comprised 20- to 75-year-old men and women with CHB who fulfilled the following criteria: (i) HBsAg-positive for 24 weeks or more or IgM HBcAb-negative with biopsy-confirmed CHB; (ii) HBeAg-positive or HBeAg-negative for 12 weeks or more; (iii) serum HBV DNA level 40 MEq/ml or more (143 pg/ml) by Quantiplex™ branched DNA hybridization method (bDNA assay) (≥7.6 $\log_{10}$ genome equivalent by the transcription-mediated amplification method or $\geq 10^{7.6}$ copies/ml by Roche Amplicor™ polymerase chain reaction method [PCR assay]) measured 2 weeks or more before screening and serum HBV DNA level 40 MEq/ml or more (by bDNA assay) at screening; (iv) serum alanine

aminotransferase (ALT) level 1.25–10 times the upper limit of normal (ULN); and (v) well-compensated liver disease with prothrombin time prolongation 3 s or less or international normalized ratio 1.5 or less, serum albumin level 3.0 g/dl or more, and total bilirubin 2.5 mg/dl or less (42.75 μmol/l). After a 6-week screening period, eligible patients were stratified according to HBeAg status and study site and randomized (1:1:1:1) to oral treatment with entecavir (0.01, 0.1, or 0.5 mg plus matching placebo capsule) or lamivudine (100 mg plus matching placebo tablet) once daily for 24 weeks. All doses were administered at fixed times of the day, avoiding the 2 h before and after meals. Pregnant women were excluded from the study, as were patients with liver cirrhosis, patients with a history or evidence of variceal bleeding, patients with hepatic encephalopathy or ascites requiring diuretics, or patients with paracentesis. Patients with other liver disease (e.g., autoimmune hepatitis) were excluded from the study. In addition, patients were excluded if they had a serum creatinine level more than 1.5 × ULN, hemoglobin level less than 10.0 g/dl, platelet count less than 70,000/mm^3, granulocyte count less than <1,500/mm^3 or plasma α-fetoprotein level more than 100 ng/ml, a history of allergy induced by nucleoside analog or exposure to nucleoside analogs, a recent history (previous 24 weeks) of treatment with immunosuppressives or interferon-α/β, or current treatment of CHB.

Treatment efficacy was assessed after 22 weeks, and all eligible patients who completed 24 weeks of blinded therapy were given the option of enrolling in a separate entecavir trial. Patients who discontinued therapy prematurely were followed up for 24 weeks postdosing. Patients began anti-HBV therapy as recommended by their physician during the postdosing follow-up period.

Informed consent was obtained from all patients in writing prior to their inclusion in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and notifications were issued by the Ministry of Health and Labor.

Efficacy and safety assessment

The primary efficacy end point for the evaluation of the dose–response relationship of entecavir was the change from baseline in mean serum HBV DNA level at week 22, as determined by PCR assay. Secondary efficacy end points for the assessment of the noninferiority of entecavir at each dose to lamivudine included the change from baseline in mean serum HBV DNA level at week 22, as determined by PCR assay, the percentage of patients with a reduction in serum HBV DNA level 2 $\log_{10}$ copies/ml or more or a serum HBV DNA level below the limit of detection (400 copies/ml by PCR assay; 2.5 pg/ml or 0.7 MEq/ml by bDNA assay) at week 22, the percentage of patients with HBeAg loss, the percentage of patients with HBeAg seroconversion (HBeAg loss and appearance of HBe-antibody), the percentage of patients achieving ALT normalization (World Health Organization grade 0: <1.25 × ULN), and the percentage of patients achieving a protocol-defined response (HBV DNA level <0.7 MEq/ml by bDNA assay, HBeAg negativity and serum ALT level <1.25 × ULN for HBeAg-positive patients; HBV DNA level <0.7 MEq/ml by bDNA assay and serum ALT level <1.25 ULN for HBeAg-negative patients) at week 22. The incidence of genotypic drug resistance was also assessed in patients who had a 1 $\log_{10}$ copies/ml or more increase in HBV DNA by PCR from nadir while on study drug.

Based on the results of the global dose–response study of entecavir conducted in nucleoside-naive patients (ETV-005 study) [15], noninferiority of entecavir 0.1 or 0.5 mg compared with lamivudine (100 mg) was confirmed if the upper 95% confidence interval (CI) for the difference in mean HBV DNA levels at week 22 was 0.8 $\log_{10}$ copies/ml or less.

Assay methods

Serum HBV DNA level was determined by Roche Amplicor™ PCR assay (Roche Diagnostics K.K., Tokyo, Japan) and Quantiplex™ (Chiron) bDNA assay. Clinical laboratory tests, serum HBV DNA assays, and HBV serology were performed at the central clinical laboratory designated by the trial sponsor. Genotypic analysis of HBV isolates was performed using samples collected from patients on the first day of treatment. Genotypic analysis of HBV DNA polymerase was performed at SRL Inc. (Tokyo, Japan).

Statistical analysis

Numerical data were expressed by descriptive statistics. Serum HBV DNA level, a continuous variable, was analyzed after logarithmic transformation. For treatment group, comparisons of continuous variables, analysis of variance models, incorporating baseline HBV DNA level and HBeAg status as covariates were employed. For intertreatment comparisons of binary data, Cochran–Mantel–Haenszel tests were employed using baseline HBeAg status as a stratification factor. For analysis of dose–response relationships, Student's t test was applied to linear regression plots of serum HBV DNA level against log dose. A two-sided $P < 0.05$ was taken to indicate statistical significance. For analysis of dose–response relationships using efficacy data, a two-sided $P < 0.05/3$ was taken to

indicate statistical significance following Bonferroni adjustment.

Results

Study population and demographic characteristics

A total of 137 patients, including 20- to 73-year-old men and women, met the study eligibility criteria and were randomized to the following treatment groups: entecavir 0.01 mg ($n = 35$), entecavir 0.1 mg ($n = 34$), entecavir 0.5 mg ($n = 34$), and lamivudine 100 mg ($n = 34$). Three patients (two in the entecavir 0.5 mg group and one in the lamivudine 100 mg group) discontinued the study prematurely; the reasons for discontinuation were noncompliance (one patient in the entecavir 0.5 mg group) and adverse events (liver cirrhosis in one patient [entecavir 0.5 mg group] and grade 4 serum ALT elevation with nausea and malaise in one patient [lamivudine 100 mg group]). Accordingly, a total of 134 patients (entecavir 0.01 mg group, 35 patients; entecavir 0.1 mg group, 34 patients; entecavir 0.5 mg group, 32 patients; and lamivudine 100 mg group, 33 patients) completed 24 weeks of treatment and were included in the efficacy assessment.

The four treatment groups were matched with respect to gender, age, body weight, and proportion of HBeAg-positive patients (Table 1). Serum HBV DNA levels by PCR assay (mean ± SD) at baseline were 7.94 ± 0.87, 8.09 ± 1.05, 8.39 ± 0.73, and 7.94 ± 0.83 $\log_{10}$ copies/ml for the entecavir 0.01, 0.1, and 0.5 mg and lamivudine 100 mg groups, respectively. With regard to HBV genotype, 124 patients were genotype C, 6 patients were genotype A, 5 patients were genotype B, and 2 patients were genotype F. All patients were nucleos(t)ide-naive and none had been pretreated with interferon therapy.

Virologic response

Mean changes (from baseline) in serum HBV DNA level at week 22 were −3.11, −4.77, and −5.16 $\log_{10}$ copies/ml with entecavir 0.01, 0.1, and 0.5 mg, respectively (Fig 1; Table 2). Estimated differences in serum HBV DNA levels between the 0.1 and 0.5 mg entecavir groups and the low-dose entecavir group (0.01 mg) were determined after adjustment for baseline level and HBeAg status. Estimated intertreatment group differences (adjusted 95% CI) were −1.61 (−2.20 to −1.02) $\log_{10}$ copies/ml between the entecavir 0.01 and 0.1 mg groups and −1.95 (−2.53 to −1.37) $\log_{10}$ copies/ml between the entecavir 0.5 and 0.01 mg groups; both of these differences were statistically significant ($P < 0.0001$). In contrast, the difference in serum HBV DNA levels between the high-dose (0.5 mg) and medium-dose (0.1 mg) entecavir groups was not statistically significant (estimated difference [adjusted 95% CI] −0.23 [−0.69 to 0.23] $\log_{10}$ copies/ml). Taken together, these results demonstrate the superiority of high- and medium-dose entecavir (0.1 and 0.5 mg) compared with low-dose entecavir (0.01 mg) in terms of viral load reduction (Table 3). Linear regression analyses indicated a

Table 1 Baseline demographics and clinical characteristics of treated subjects

	ETV 0.01 mg ($n = 35$)	ETV 0.1 mg ($n = 34$)	ETV 0.5 mg ($n = 34$)	LVD 100 mg ($n = 34$)
Male, n (%)	25 (71.4)	23 (67.6)	23 (67.6)	28 (82.4)
Female, n (%)	10 (28.6)	11 (32.4)	11 (32.4)	6 (17.6)
Age (years), mean ± SD	42.0 ± 12.5	40.1 ± 9.8	39.8 ± 10.4	42.3 ± 12.6
Weight (kg), mean ± SD	66.2 ± 12.5	64.6 ± 11.9	65.3 ± 11.1	64.4 ± 9.0
Ethnicity Japanese, n (%)	35 (100)	34 (100)	34 (100)	34 (100)
HBV DNA ($\log_{10}$ copies/ml by PCR), mean ± SD	7.94 ± 0.87	8.09 ± 1.05	8.39 ± 0.73	7.94 ± 0.83
HBeAg positive, n (%)	30 (85.7)	30 (88.2)	30 (88.2)	31 (91.2)
ALT (IU/l), mean ± SD	150.1 ± 111.8	162.0 ± 127.1	142.4 ± 82.2	185.0 ± 130.8
AST (IU/l), mean ± SD	83.2 ± 40.0	114.3 ± 109.4	81.0 ± 43.0	121.6 ± 85.4
Total bilirubin (mg/dl), mean ± SD	0.65 ± 0.25	0.56 ± 0.15	0.66 ± 0.25	0.71 ± 0.28
HBV genotype (%)				
C	32 (91.4)	30 (88.2)	32 (94.1)	30 (88.2)
A	1 (2.86)	2 (5.88)	1 (2.94)	2 (5.88)
B	1 (2.86)	1 (2.94)	1 (2.94)	2 (5.88)
F	1 (2.86)	1 (2.94)	0	0

ETV entecavir; *LVD* lamivudine

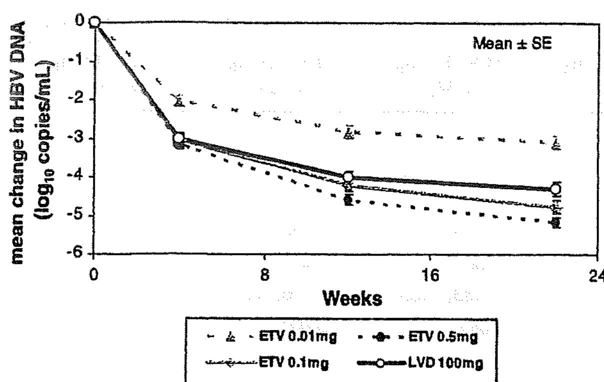

Fig. 1 Mean change from baseline in serum HBV DNA level by PCR assay through 22 weeks in patients treated with entecavir (ETV) 0.01, 0.1, and 0.5 mg and lamivudine 100 mg. Mean change in serum HBV DNA level was plotted as a function of time after the initiation of the protocol therapy (weeks). Data expressed as mean ± SE

significant dose–response relationship between $\log_{10}$ entecavir dose and reduction in $\log_{10}$ serum HBV DNA level ($P < 0.0001$).

Mean change (from baseline) in serum HBV DNA level at week 22 for the lamivudine 100 mg group was −4.29 $\log_{10}$ copies/ml (Fig. 1; Table 2). Estimated mean differences (95% CI) in serum HBV DNA level (after adjustment for baseline level and HBeAg status) were −0.39 (−0.83 to 0.05) $\log_{10}$ copies/ml between the entecavir 0.1 mg and lamivudine 100 mg groups and −0.62 (−1.06 to −0.18) $\log_{10}$ copies/ml between the entecavir 0.5 mg and lamivudine 100 mg groups, indicating the noninferiority of the entecavir 0.1 and 0.5 mg groups to the lamivudine 100 mg group and the superiority of the entecavir 0.5 mg group to the lamivudine 100 mg group ($P = 0.007$) (Table 2). In contrast, the entecavir 0.01 mg group was significantly inferior to the lamivudine 100 mg group (estimated mean difference = 1.20 [0.69–1.71]; $P < 0.0001$) (Table 2).

The secondary efficacy end point of a reduction in serum HBV DNA level 2 $\log_{10}$ copies/ml or more or HBV DNA level less than 400 copies/ml by PCR assay was achieved by 88.6% of patients in the entecavir 0.01 mg group and by 100% of patients in the entecavir 0.1 and 0.5 mg groups at week 22. Ninety-seven percent of patients in the lamivudine 100 mg group achieved this end point at week 22. HBV DNA level less than 0.7 MEq/ml by bDNA assay was achieved by 65.7%, 94.1%, and 100% of patients in the 0.01, 0.1, and 0.5 mg entecavir groups, respectively, and by 93.9% of patients in the lamivudine 100 mg treatment group.

Serologic response

Among HBeAg-positive patients, there was no significant difference between seroconversion rates at week 22 for the entecavir 0.01, 0.1, and 0.5 mg treatment groups (10.0%, 13.3%, and 3.6%, respectively) versus the lamivudine 100 mg treatment group (3.3%; Table 2). All patients who lost HBeAg also experienced HBeAg seroconversion.

Biochemical response

At baseline, elevated serum ALT levels (>1.25 × ULN) were present in more than 90% of patients in all four treatment groups. At week 22, normal serum ALT levels (World Health Organization grade 0, <1.25 × ULN) were recorded in similar proportions of patients in the entecavir 0.01, 0.1, and 0.5 mg treatment groups (75.0%, 85.3%, and 80.0% of patients, respectively) and the lamivudine treatment group (78.1% of patients), with no significant intergroup difference (Table 2).

Response

Response (HBV DNA level <0.7 MEq/ml by bDNA assay, HBeAg loss, and serum ALT level <1.25 × ULN for HBeAg-positive patients and HBV DNA level <0.7 MEq/ml by bDNA assay and serum ALT <1.25 × ULN for HBeAg-negative patients) was achieved by 14.3%, 20.6%, and 15.6% of patients in the entecavir 0.01, 0.1, and 0.5 mg

Table 2 Differences in HBV DNA levels between entecavir dose groups by PCR at week 22 in evaluable subjects

	0.1 mg ETV–0.01 mg ETV ($n = 34$, $n = 35$)	0.5 mg ETV–0.01 mg ETV ($n = 32$, $n = 35$)	0.5 mg ETV–0.1 mg ETV ($n = 32$, $n = 34$)
Estimated difference[a] ($\log_{10}$ copies/ml)	−1.61	−1.95	−0.23
Standard error	0.24	0.24	0.19
95% Confidence interval[b]	−2.20, −1.02	−2.53, −1.37	−0.69, 0.23
P-value	<0.0001	<0.0001	0.227

[a] Estimated differences are regression-adjusted for baseline serum HBV DNA and HBeAg status

[b] 95% Confidence interval is adjusted by modified Bonferroni procedures

ETV entecavir

Table 3 Virology and biochemical responses at week 22 and comparison of entecavir treatment groups with lamivudine in evaluable subjects

Response	ETV 0.01 mg ($n = 35$)	ETV 0.1 mg ($n = 34$)	ETV 0.5 mg ($n = 32$)	LVD 100 mg ($n = 33$)
HBV DNA by PCR assay				
Reduction from baseline at week 22 ($\log_{10}$ copies/ml), mean ± S.E.	−3.11 ± 0.18	−4.77 ± 0.17	−5.16 ± 0.13	−4.29 ± 0.18
HBV DNA estimated difference[a] (vs. LVD) ($\log_{10}$ copies/ml)	1.20	−0.39	−0.62	–
Standard error	0.26	0.22	0.22	–
95% Confidence interval	0.69, 1.71	−0.83, 0.05	−1.06, -0.18	–
P-value	<0.0001[b]	0.081	0.007[c]	–
HBV DNA by Roche Amplicor™ PCR assay				
Change in $\log_{10}$ HBV DNA reduction >2 or HBV DNA <400 copies/ml at week 22, *n* (%)	31 (88.6)	34 (100)	32 (100)	32 (97.0)
P-value (vs. LVD)	0.206	NR[d]	NR[d]	–
HBV DNA by Quantiplex assay				
HBV DNA <0.7 MEq/ml (2.5 pg/ml) at week 22, *n* (%)	23 (65.7)	32 (94.1)	32 (100)	31 (93.9)
P-value (vs. LVD)	0.002	1.000	NR[d]	–
Normalization of ALT levels[e]				
At week 22, *n*/*n* with abnormal baseline (%)	24/32 (75.0)	29/34 (85.3)	24/30 (80.0)	25/32 (78.1)
P-value (vs. LVD)	0.842	0.439	0.880	–
Loss of HBeAg and seroconversion at week 48[f]				
HBeAg loss, n/n HBeAg positive at baseline (%)	3/30 (10.0)	4/30 (13.3)	1/28 (3.6)	1/30 (3.3)
HBeAg seroconversion	3/30 (10.0)	4/30 (13.3)	1/28 (3.6)	1/30 (3.3)
P-value (vs. LVD)	0.605	0.350	1.000	–
Response[g] at week 22, *n* (%)	5 (14.3)	7 (20.6)	5 (15.6)	3 (9.1)
P-value (vs. LVD)	0.735	0.190	0.480	–

[a] Estimated differences are regression-adjusted for baseline HBV DNA and HBeAg status

[b] Two-sided test indicates inferiority of the entecavir 0.01 mg dose

[c] Two-sided test indicates superiority of the entecavir dose

[d] Not reported because expected counts <5

[e] WHO grade 0, ALT <1.25 × upper limit of normal

[f] Seroconversion was defined as disappearance of HBe-antigen and appearance of HBe-antibody

[g] Response was defined as HBV DNA levels <0.7 MEq/ml, HBeAg negativity and ALT <1.25 × ULN for HBeAg-positive patients and HBV DNA levels <0.7 MEq/ml and ALT <1.25 × ULN for HBeAg-negative patients

ETV entecavir

LVD lamivudine

treatment groups, respectively, and by 9.1% of patients in the lamivudine treatment group at week 22, and there were no significant differences in the rates of response between the four treatment groups (Table 2).

Resistance analysis

During the treatment period, serum HBV DNA level increased by 1 $\log_{10}$ copies/ml or more from its nadir in one patient in the entecavir 0.01 mg group and one patient in the lamivudine 100 mg group. Nucleotide sequence analysis of the DNA polymerase coding region, using viral samples collected from these two patients at day 1 and at week 22, revealed no lamivudine-resistance substitutions (rt180 and rt204 amino acid residues) [17, 18] or entecavir-resistance substitutions (rt184, rt202, and rt250 amino acid residues) [19].

Safety

During the study, adverse events were experienced by similar proportions of patients in the entecavir 0.01, 0.1, and 0.5 mg groups and the lamivudine 100 mg treatment group (97.1%, 97.1%, 91.2%, and 100.0%, respectively). Most adverse events were of mild or moderate intensity (grade 1/2) and transient. The most frequently reported adverse events (affecting ≥ 10% of patients in any one treatment group) included nasopharyngitis, headache, and

Table 4 Summary of adverse events and laboratory abnormalities during the 24-week blinded treatment phase

	ETV 0.01 mg ($n = 35$)	ETV 0.1 mg ($n = 34$)	ETV 0.5 mg ($n = 34$)	LVD 100 mg ($n = 34$)
Any adverse events	34 (97)	33 (97)	31 (91)	34 (100)
Most frequent clinical adverse events,[a] *n* (%)				
Nasopharyngitis	9 (25.7)	10 (29.4)	11 (32.4)	10 (29.4)
Headache	6 (17.1)	7 (20.6)	2 (5.9)	7 (20.6)
Diarrhea	1 (2.9)	1 (2.9)	4 (11.8)	4 (11.8)
Grade 3/4 clinical adverse events, *n* (%)	0	0	1 (2.9)	1 (2.9)
Grade 3/4 laboratory adverse events, *n* (%)	2 (5.7)	4 (11.8)	2 (5.9)	4 (11.8)
Any serious adverse events, *n* (%)	0	1 (2.9)	2 (5.9)	1 (2.9)
Discontinuations due to adverse events,[b] *n* (%)	0	0	1 (2.9)	1 (2.9)
ALT flares,[c] *n* (%)	0	1 (2.9)	1 (2.9)	2 (5.9)
Death, *n* (%)	0	0	0	0

[a] Occurring in at least 10% of patients

[b] One patient treated with ETV 0.5 mg discontinued the study drug due to hepatic cirrhosis. One patient treated with lamivudine discontinued due to increased ALT

[c] ALT flare defined ALT >2 × baseline and 10 × ULN

ETV entecavir

LVD lamivudine

diarrhea (Table 4). Grade 3/4 clinical adverse events occurred in one patient in the entecavir 0.5 mg group (colon carcinoma) and one patient in the lamivudine group (anal ulcer); neither of these events was considered to be related to the study drug. Serious adverse events were limited to the above-mentioned case of colon carcinoma, serum ALT elevation (entecavir 0.1 mg group [$n = 1$], entecavir 0.5 mg group [$n = 1$]), and serum aspartate aminotransferase (AST)/ALT elevation (lamivudine 100 mg group [$n = 1$]), but these were not considered to be causally related to the study drug and did not necessitate treatment discontinuation. Transient ALT flares (serum ALT >2 × baseline level and >10 × ULN) occurred in four patients (entecavir 0.1 mg group [$n = 1$], entecavir 0.5 mg group [$n = 1$], and lamivudine 100 mg group [$n = 2$]) and were associated with HBV DNA level decreases of 2 $\log_{10}$ copies/ml or more. None of the ALT flares were associated with hepatic decompensation and serum ALT and AST levels recovered to less than 1.25 × baseline level on continuation of the study treatment.

Discussion

The global ETV-005 study reported that entecavir was superior to lamivudine at reducing viral load in nucleoside-naive patients with CHB infection [15]. We conducted the present study, using an identical design to the ETV-005 study, to determine whether the findings from this earlier study are applicable to Japanese patients. In keeping with the previous findings, our results indicate that entecavir produces a dose-related reduction in serum HBV DNA level ($0.01 < 0.1 \leq 0.5$ mg) in nucleoside-naive Japanese patients with CHB; the log dose–response curves for the reduction in serum HBV DNA level with entecavir in the two studies were similar, with estimated regression curve slopes of –1.24 (Japanese study) and –1.32 (global study). In addition, both studies demonstrated the noninferiority of the entecavir 0.1 mg group compared with the lamivudine 100 mg group and the superiority of the entecavir 0.5 mg group compared with the lamivudine 100 mg group. The demonstration of a dose–response relationship for entecavir and the superiority of the entecavir 0.5 mg dose over lamivudine confirm that the antiviral activity of entecavir in Japanese patients is similar to that observed in study ETV-005. In a previous study, Ono et al. [14] demonstrated that the in vitro potency of entecavir was up to 2,200 times greater than that of lamivudine. The results presented here substantiate these earlier in vitro data and confirm the greater potency of entecavir over lamivudine in patients with CHB.

Serum ALT normalization rates with entecavir 0.5 mg and lamivudine 100 mg (~80%) were higher in the present study than those reported in the ETV-005 study (entecavir 0.5 mg, 69.0%; lamivudine 100 mg, 59.1%) [15]. In keeping with previous findings [20, 21], the incidence of entecavir-associated serum ALT flares in Japanese patients was low. The serum ALT flares occurred against a background of 2 $\log_{10}$ copies/ml or more reductions in serum

HBV DNA level, and serum ALT levels subsequently normalized without discontinuation of entecavir. Therefore, the serum ALT flare noted here may indicate recovery of the host's immune response arising from the reduction in HBV viral titer [22, 23]. ALT flares have been reported after the discontinuation of entecavir therapy [15, 16], thus necessitating long-term follow-up to identify possible posttreatment viral rebound.

In conclusion, the results of this dose-ranging study demonstrate a clear dose–response relationship for entecavir in terms of mean HBV DNA level reduction at week 22. Entecavir 0.5 mg was significantly more effective than lamivudine 100 mg in reducing HBV DNA levels in nucleoside-naive Japanese adult patients with CHB. At this dose level, entecavir treatment resulted in serum HBV DNA levels of less than 400 copies/ml in 100% of patients and normalization of serum ALT levels in 80% of patients after 22 weeks. Moreover, entecavir 0.5 mg once daily was well tolerated and showed a comparable safety profile to lamivudine.

References

1. Maddrey WC. Hepatitis B: an important public health issue. J Med Virol 2000;61:362–366. doi:10.1002/1096-9071(200007)61:3<362::AID-JMV14>3.0.CO;2-I
2. Kao JH, Chen DS. The natural history of hepatitis B virus infection. In Lai CL, Locarnini S, editors. Hepatitis B Virus. London: International Medical Press; 2002. 161–172
3. Lok AS, McMahon BJ. American Association for the Study of Liver Diseases (AASLD) practice guidelines. Chronic Hepatitis B. Hepatology 2007;45:507–539. doi:10.1002/hep.21513
4. Rivkin A. A review of entecavir in the treatment of chronic hepatitis B infection. Curr Med Res Opin 2005;21:1845–1856. doi:10.1185/030079905X65268
5. Wong DKH, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312–323
6. Allen MI, Deslauriers M, Andrews CW, Tipples GA, Walters KA, Tyrrell DL, et al. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. Hepatology 1998;27:1670–1677. doi:10.1002/hep.510270628
7. Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. J Gastroenterol Hepatol 2004;19(11):1276–1282. doi:10.1111/j.1440-1746.2004.03428.x
8. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351(15):1521–1531. doi:10.1056/NEJMoa033364
9. Omata M. Treatment of chronic hepatitis B infection. N Engl J Med 1998;339:114. doi:10.1056/NEJM199807093390209
10. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2008;2:263–283. doi:10.1007/s12072-008-9080-3
11. Genovesi EV, Lamb L, Medina I, Taylor D, Seifer M, Innaimo S, et al. Efficacy of the carbocyclic 2′-deoxyguanosine nucleoside BMS-200475 in the woodchuck model of hepatitis B virus infection. Antimicrob Agents Chemother 1998;42:3209–3217
12. Marion PL, Salazar FH, Winters MA, Colonno RJ. Potent efficacy of entecavir (BMS-200475) in a duck model of hepatitis B virus replication. Antimicrob Agents Chemother 2002;46:82–88. doi:10.1128/AAC.46.1.82-88.2002
13. Colonno RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, et al. Entecavir resistance is rare in nucleoside naïve patients with hepatitis B. Hepatology 2006;44:1656–1665. doi:10.1002/hep.21422
14. Ono SK, Kato N, Shiratori Y, Kato J, Goto T, Schinazi RF, et al. The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. J Clin Invest 2001;107:449–455. doi:10.1172/JCI11100
15. Lai CL, Rosmawati M, Lao J, Van Vlierberghe H, Anderson FH, Thomas N, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. Gastroenterology 2002;123:1831–1838. doi:10.1053/gast.2002.37058
16. Chang TT, Gish RG, Hadziyannis SJ, Cianciara J, Rizzetto M, Schiff ER, et al. A dose-ranging study of the efficacy and tolerability of entecavir in lamivudine-refractory chronic hepatitis B patients. Gastroenterology 2005;129:1198–1209. doi:10.1053/j.gastro.2005.06.055
17. Ling R, Mutimer D, Ahmed M, Boxall EH, Elias E, Dusheiko GM, et al. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology 1996;24:711–713. doi:10.1002/hep.510240339
18. Tipples GA, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DLJ. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. Hepatology 1996;24:714–717
19. Tenney DJ, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L, et al. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. Antimicrob Agents Chemother 2004;48:3498–3507. doi:10.1128/AAC.48.9.3498-3507.2004
20. Chang TT, Gish R, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1074–1076. doi:10.1056/NEJMoa051285
21. Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw Y-F, Cianciara J, et al. Entecavir is superior to continued lamivudine for the treatment of lamivudine-refractory. HBeAg-positive chronic hepatitis B. Gastroenterology 2006;130:2039–2049
22. Boni C, Bertoletti A, Penna A, Cavalli A, Pilli M, Urbani S, et al. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J Clin Invest 1998;102:968–975. doi:10.1172/JCI3731
23. Nair S, Perrillo RP. Serum alanine aminotransferase flares during interferon treatment of chronic hepatitis B: is sustained clearance of HBV DNA dependent on levels of pretreatment viremia? Hepatology 2001;34:1021–1026. doi:10.1053/jhep.2001.28459

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Association of Amino Acid Substitution Pattern in Core Protein of Hepatitis C Virus Genotype 2a High Viral Load and Virological Response to Interferon-Ribavirin Combination Therapy

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Key Words
Hepatitis C virus · Genotype 2a · Core region · Interferon · Ribavirin · Rapid response

Abstract
Background: Substitution of amino acids (aa) 70 and 91 in the core region of HCV genotype 1b is a useful pretreatment predictor of poor response to interferon + ribavirin combination therapy, but the impacts of aa substitutions in the core region of HCV genotype 2a are still not clear. ***Methods:*** 154 consecutive Japanese adults with a high viral load (≥100 kIU/ml) of genotype 2a who could complete combination therapy for 24 weeks were evaluated. To examine the differences in virological characteristics between non-sustained virological response (non-SVR) and rapid responder (SVR patients who could achieve a HCV-RNA-negative status within 8 weeks), 86 patients could be analyzed by pretreatment substitution patterns of the core region. ***Results:*** SVR was achieved in 127 of 154 patients (83%), and rapid response in 113 of 127 (90%). In all 154 patients, multivariate analysis identified younger age, lower level of viremia, and higher level of albumin as significant determinants of SVR. As significant determinants of rapid response in 86 patients, multivariate analysis identified substitution of aa 4 (non-asparagine) in addition to the significant determinants of SVR. ***Conclusions:*** Our results suggest that the aa substitution pattern of the core region in patients with a high titer of genotype 2a may partly affect the virological response to combination therapy.
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Introduction

The response to interferon (IFN)-related therapy varies according to hepatitis C virus (HCV) genotype [1, 2]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b, and about 25% with genotype 2a [3]. Sustained virological response (SVR) to 48-week IFN + ribavirin combination therapy is about 50% in genotype 1b infection, and SVR to 24-week combination therapy is more than 80% in genotype 2 infection [4–9].

IFN + ribavirin combination therapy carries potential serious side effects and is costly, especially when used

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long enough to achieve a high SVR. For these reasons, especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (≤16 weeks) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. Furthermore, we also sometimes encounter treatment-resistant patients infected with genotype 2a [3, 10]. The underlying mechanism(s) of the different virological responses to treatment in patients infected with genotype 2a is still unclear. Hence, the pretreatment predictors of the efficacy of IFN + ribavirin combination therapy were investigated in the present study.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of infected patients with genotype 1b and a high viral load (≥100 kIU/ml) are predictors of poor virological response to 48- and 72-week pegylated IFN (PEG-IFN) + ribavirin combination therapies [11–15], and also affect clinical outcomes, including insulin resistance and hepatocarcinogenesis [16–18]. However, it is unknown whether the aa substitutions of the core region in patients infected with genotype 2a who have high viral load might also be as useful as the pretreatment virological predictive factors apart from the genotype and viral load.

The present study included 154 Japanese adults with genotype 2a and a high viral load, who received combination therapy for 24 weeks. The aim of the study was to investigate the treatment efficacy and pretreatment predictive factors including virological features.

Materials and Methods

Study Population

A total of 190 HCV genotype 2a-infected Japanese adult patients were consecutively recruited into the study protocol of the combination therapy with IFN (PEG-IFNα-2b or IFNα-2b) + ribavirin for 24 weeks between March 2002 and February 2008 at Toranomon Hospital, Tokyo, Japan. Among these, 154 patients, who could complete a total of 24 weeks of combination therapy, were enrolled in this retrospective study and fulfilled the following criteria: (1) They were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, Calif., USA), and positive for HCV-RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Calif., USA). (2) They were naive to ribavirin therapy. (3) They were infected with HCV genotype 2a alone. (4) Each had a high viral load (≥100 kIU/ml) by quantitative analysis of HCV-RNA with PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc., Pleasanton, Calif., USA) within the preceding 2 months of enrolment. (5) They had no hepatocellular carcinoma. (6) Their body weight was >40 kg. (7) All were free of coinfection with human immunodeficiency virus. (8) None had been treated with antiviral or immunosuppressive agents within the preceding 3 months of enrolment. (9) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (10) None had other forms of hepatitis, such as hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (11) None of the females were pregnant or lactating mothers. (12) All patients had completed a 24-week follow-up program after cessation of treatment, and SVR could be evaluated. (13) Each signed a consent form of the study protocol that had been approved by the Human Ethics Review Committee.

Table 1 summarizes the profiles and data of the 154 patients at the commencement of combination therapy with IFN + ribavirin. The study included 92 men and 62 women, aged 20–70 years (median 53). In all patients, the total duration of treatment was 24 weeks. In 43 of the 154 (27.9%) patients, the dose of ribavirin was reduced during treatment due to a fall in Hb concentration.

With regard to the treatment protocol, 104 (67.5%) patients received PEG-IFNα-2b + ribavirin for 24 weeks, and the remaining 50 (32.5%) patients received IFNα-2b + ribavirin for 24 weeks. They received PEG-IFNα-2b at a median dose of 1.5 μg/kg (range 1.0–1.8) subcutaneously each week, or IFNα-2b at a median dose of 6 million units (range 3–6) intramuscularly each day (7 times per week for the initial 2 weeks, followed by 3 times per week for 22 weeks). They also received oral ribavirin at a median dose of 11.2 mg/kg (range 5.4–14.1) daily.

The treatment efficacy was evaluated by HCV-RNA positivity based on qualitative PCR analysis at the end of treatment (nonvirological response; NVR), and by HCV-RNA negativity based on qualitative PCR analysis at 24 weeks after the completion of therapy (SVR). Furthermore, rapid responders were defined as SVR patients who could achieve a negative status within 8 weeks after the start of treatment, based on qualitative PCR analysis.

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase (ALT) and HCV-RNA levels. The serum samples were frozen at –80° within 4 h of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region [19]. HCV-RNA levels were measured by quantitative PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc.) at least once every month before, during, and after therapy. The dynamic range of the assay was 5–5,000 kIU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA (<5 kIU/ml) were checked by qualitative PCR (Amplicor HCV v2.0, Roche Molecular Systems, Inc.), which has a higher sensitivity than quantitative analysis, and the results were expressed as positive or negative. The lower limit of the assay was 50 IU/ml.

Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens

Table 1. Profile and laboratory data of 154 patients infected with HCV genotype 2a

Demographic data	
Number of patients	154
Sex, M/F	92/62
Age, years*	53 (20–70)
History of blood transfusion	43 (27.9%)
Family history of liver disease	26 (16.9%)
Body mass index*	22.7 (17.9–31.8)
Laboratory data*	
Serum aspartate aminotransferase, IU/l	43 (7–404)
Serum alanine aminotransferase, IU/l	54 (8–651)
Serum albumin, g/dl	3.9 (3.2–4.7)
γ-Glutamyl transpeptidase, IU/l	38 (9–406)
Leukocytes, /mm^3	4,800 (2,200–9,000)
Hemoglobin, g/dl	14.4 (9.9–17.8)
Platelet count, ×104/mm^3	17.9 (6.1–32.9)
Indocyanine green retention rate at 15 min, %	13 (4–35)
Serum iron, μg/dl	136 (22–336)
Serum ferritin, μg/l	124 (<10–820)
Level of viremia, kIU/ml	720 (5–>5,000)
α-Fetoprotein, μg/l	4 (2–103)
Total cholesterol, mg/dl	174 (107–275)
High-density lipoprotein cholesterol, mg/dl	47 (15–109)
Low-density lipoprotein cholesterol, mg/dl	105 (48–201)
Triglycerides, mg/dl	98 (36–449)
Uric acid, mg/dl	5.6 (2.5–9.4)
Fasting plasma glucose, mg/dl	93 (75–187)
Histological findings	
Stage of fibrosis (F1/F2/F3/ND)	58/23/16/57
Grade of activity (A1/A2/ND)	57/40/57
Hepatocyte steatosis (<5% (absent)/≥5% (present)/ND)	35/52/67
Treatment	
PEG-IFNα-2b/IFNα-2b	104/50
Ribavirin dose, mg/kg*	11.2 (5.4–14.1)
Total duration of treatment, weeks	24
Past history of IFN monotherapy	56 (36.4%)

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values. ND = Not determined.

for examinations contained ≥6 portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H. K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histological assessment according to the scoring system of Desmet et al. [20].

Nucleotide Sequencing of the Core Region

We determined the sequences of aa 1–191 in the core region by the direct sequencing method using pretreatment sera of patients who could be analyzed due to adequate serum samples obtained at the start of combination treatment. These sequences were compared with the consensus sequences of genotype 2a, which were determined by comparing the sequences obtained in this study and prototype sequence (HCV J6) [21]. HCV-RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by nested PCR using the following primers. Nucleotide sequences of the core region: the first-round PCR was performed with 2ACF5 (sense, 5′-GCA AGA CTG CTA GCC GAG TA-3′) and 2ACR6 (antisense, 5′-ATC TGA GCT GCG AGC ATC AC-3′) primers, and the second-round PCR with 2ACF3N (sense, 5′-CCT TGT GGT ACT GCC TGA TA-3′) and 2ACR8 (antisense, 5′-CCA GGT GAT GCT GTC ATT AG-3′) primers. All samples were initially denatured at 95° for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 s at 95°, annealing of primers for 30 s at 55°, and extension for 1 min at 72° with an additional 7 min for extension. Then 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR prim-

Table 2. Factors associated with sustained virological response to combination therapy with IFN + ribavirin for 24 weeks in 154 patients infected with HCV genotype 2a, identified by multivariate analysis

Factor	Category	Odds ratio (95% CI)	p
Age, years	1: ≥50	1	
	2: <50	6.37 (1.76–23.3)	0.005
Serum albumin, g/dl	1: <3.9	1	
	2: ≥3.9	3.19 (1.17–8.73)	0.024
Level of viremia, kIU/ml	1: ≥1,000	1	
	2: <1,000	2.86 (1.11–7.41)	0.030

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 3. Amino acid substitutions in the core region in non-SVR and rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a

	Non-SVR (n = 25)	Rapid response (n = 61)	p*
Presence of substitution site			
aa 4	1 (4.0%)	15 (24.6%)	0.032
aa 23	2 (8.0%)	0 (0%)	0.082
aa 70	1 (4.0%)	0 (0%)	NS
aa 91	0 (0%)	4 (6.6%)	NS
aa 110	11 (44.0%)	34 (55.7%)	NS

* Non-SVR vs. rapid response (Fisher's exact probability test; statistical significance ($p < 0.05$), marginal significance ($p < 0.10$)). aa = Amino acid; SVR = sustained virological response; NS = not significant.

ers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo, Japan).

To avoid false-positive results, the procedures recommended by Kwok and Higuchi [22] to prevent contamination were strictly applied to these PCR assays. No false-positive results were observed in this study.

Statistical Analysis

Non-parametric tests were used to analyze the aa substitutions in HCV core between the groups, including the Mann-Whitney U test, χ^2 test and Fisher's exact probability test. Uni- and multivariate logistic regression analyses were used to determine the factors that significantly contributed to SVR and rapid response. We also calculated the odds ratios and 95% confidence intervals (CI). All p values <0.05 calculated by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors associated with SVR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), ALT, albumin, γ-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelets, indocyanine green retention rate at 15 min (ICG R15), iron, ferritin, level of viremia, α-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, fasting blood glucose, type of IFN, ribavirin dose/body weight, and past history of IFN monotherapy. Furthermore, in addition to potential predictive factors associated with SVR, potential predictive factors associated with a rapid response also included aa substitution in the core region. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, Ill., USA).

Results

Virological Response Rates to Combination Therapy

The virological response could be evaluated in all 154 patients. SVR was achieved in 127 of 154 (82.5%) patients, and rapid response in 113 of 127 (90.0%). Only 5 of 154 (3.2%) patients were considered NVR.

Predictive Factors Associated with SVR in Multivariate Analysis

We then analyzed the data of all 154 patients to determine those factors that could predict SVR. Univariate analysis identified 5 parameters associated with SVR that achieved statistical significance or marginal significance. These included age (<50 years; $p < 0.001$), serum albumin (≥3.9 g/dl; $p = 0.003$), level of viremia (<1,000 kIU/ml; $p = 0.049$), history of blood transfusion (absent; $p = 0.064$), and ALT (≥30 IU/l; $p = 0.088$).

Multivariate analysis identified 3 parameters that independently influenced SVR, including age (<50 years; $p = 0.005$), serum albumin (≥3.9 g/dl; $p = 0.024$), and level of viremia (<1,000 kIU/ml; $p = 0.030$) (table 2).

Fig. 1. Sequences of aa 1–30 and aa 61–110 in the core region at the commencement of combination therapy in 86 patients infected with high HCV viral load genotype 2a. Dashes indicate aa identical to the consensus sequence of genotype 2a, and substituted aa are shown by standard single-letter codes. The aa patterns at positions that are probably associated with sensitivity to therapy are shown in boldface characters. NSR = Non-SVR; RR = rapid response.

```
Consensus         10         20         30            70         80         90        100        110
HCJ6  MSTNPKPQRK TKRNTNRRPQ DVKFPGGGQI//RRQPIPKDRR STGKSWGKPG YPWPLYGNEG LGWAGWLLSP RGSRPSWGPT Efficacy
      ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N
Case 1 ---------- ---------- ----------//---------- -------R-- ---------- ---------- ---------N NSR
   2  ---------- ---------- ----------//---------- -------R-- ---------- ---------- ---------N NSR
   3  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
   4  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------S NSR
   5  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N NSR
   6  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
   7  ---T------ ---------- ----------//------R--H ---------- ---------- ---------- ---------- NSR
   8  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
   9  ---------- ---------- --R-------//---------- ---------- ---------- ---------- ---------- NSR
  10  ---------- ---------- ----------//-------H-- ---------- ---------- ---------- ---H------ NSR
  11  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N NSR
  12  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N NSR
  13  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------S NSR
  14  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N NSR
  15  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
  16  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
  17  ---------- ---------- --R-------//---------- ---------- ---------- ---------- ---------- NSR
  18  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
  19  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
  20  ---------- SN-------- ----------//---------- ---------- ---------- ---------- ---------- NSR
  21  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N NSR
  22  ---------Q ----I----- ----------//---H------ P--------- ---------- ---------- Q--------N NSR
  23  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
  24  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- NSR
  25  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N NSR
  26  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  27  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  28  ---IS----- -H-------- ----------//---------- ---------- ---------- ---------- ---------N RR
  29  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------S RR
  30  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  31  ---------Q -Q-------- ----------//---------- ---R------ ---------- ---------- ---------- RR
  32  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  33  ---V------ ---------- ----------//---------- ---------- ---------- ---------- ---------S RR
  34  ---I------ ---------- ----------//---------- T--------- ---------- ---------- ---------N RR
  35  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------S RR
  36  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  37  ---------- ---------- ----------//-------SQ- -S-R------ ---------- ---------- ---------N RR
  38  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---C------ RR
  39  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  40  ---D------ ---------- ----------//-------H-- ---------- ---------- ---------- -----C---- RR
  41  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  42  ---I------ ----I----- ----------//---------- ---------- ---------- C--------- ---------N RR
  43  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  44  ---------- -----I---- ----------//---------- ---------- ---------- ---------- ---------N RR
  45  ---------- ---------- ----------//---H------ -P-------- ---------- ---------- ---------- RR
  46  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------S RR
  47  ---------- ---------- ----------//---------- ---R---R-- ---------- ---------- ---------N RR
  48  ---Y------ -Q-------- ----------//---------- P------R-- ---------- ---------- ---------N RR
  49  ---I------ ---------- ----------//------RV-- ---------- ----F----- ---------- G--------- RR
  50  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  51  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  52  ---------- ---------- --------R-//---------- ---------- ---------- ---------- ---------- RR
  53  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  54  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  55  ---D------ ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  56  ---------I ---------- ----------//---------- ---------- ---------- C----Y---- -----T---- RR
  57  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
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  60  ---------- ---------- ----------//---------- ---------- ---------- ---------- -----N---S RR
  61  ---------I ---------- ----------//------R--- ---------- ---------- ---------- ---------N RR
  62  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  63  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  64  ---T-----I -----Y---- N---------//---------- P--------- ---------- ---------- ---------- RR
  65  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  66  ---D-----I ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  67  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  68  ---------- ---------- ----------//---------- -------R-- ---------- ---------- ---------N RR
  69  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  70  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  71  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  72  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  73  ---I------ ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  74  ---------Q ---------- ----------//-------H-- ---------- ---------- ---------- ---------N RR
  75  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------N RR
  76  ---D------ ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  77  ---------- ---------- ----------//---------- ---------- ---------- ---------- -----N---S RR
  78  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------- RR
  79  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------S RR
  80  ---I------ ---------- ----------//---------- ---R---R-- ---------- ---------- ---------N RR
  81  ---------- ---------- ----------//---------- ---------- ---------- C--------- ---------- RR
  82  ---I------ ---------- ----------//---------- P--------- ---------- ---------- ---------S RR
  83  ---------- -H-------- ----------//--------Q- ---------- ---------- ---------- ---------- RR
  84  ---------- ---------- ----------//---------- ---------- ---------- ---------- ---------S RR
  85  ---------- ---------- ----------//---------- -A-------- ---------- ---------- ---------S RR
  86  ---T------ ---------- ----------//---------- ---------- ---------- C--------- ---------N RR
```

Table 4. Patient profile and laboratory data of non-SVR and rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a, who could be analyzed by the nucleotide sequences of the core region

	Non-SVR	Rapid response	p[a]
Demographic data			
Number of patients	25	61	
Sex, M/F	12/13	39/22	NS
Age, years*	58 (34–64)	51 (20–66)	0.006
History of blood transfusion	10 (40.0%)	15 (24.6%)	NS
Family history of liver disease	4 (16.0%)	10 (16.4%)	NS
Body mass index*	23.1 (19.5–30.0)	22.7 (17.9–31.1)	NS
Laboratory data*			
Serum aspartate aminotransferase, IU/l	31 (19–200)	42 (7–125)	NS
Serum alanine aminotransferase, IU/l	44 (14–357)	53 (8–280)	NS
Serum albumin, g/dl	3.8 (3.2–4.2)	3.9 (3.2–4.5)	0.005
γ-Glutamyl transpeptidase, IU/l	38 (14–141)	39 (10–406)	NS
Leukocytes, /mm^3	4,600 (3,100–8,000)	4,800 (2,400–9,000)	NS
Hemoglobin, g/dl	14.2 (12.5–17.8)	14.7 (11.1–17.2)	NS
Platelet count, $\times 10^4$/mm^3	16.3 (8.0–32.9)	18.0 (10.6–30.6)	NS
Indocyanine green retention rate at 15 min, %	16 (5–26)	12 (6–35)	NS
Serum iron, μg/dl	152 (30–284)	144 (26–304)	NS
Serum ferritin, μg/l	133 (16–756)	123 (10–820)	NS
Level of viremia, kIU/ml	1,200 (93–>5,000)	680 (5–4,600)	0.053
α-Fetoprotein, μg/l	5 (2–103)	5 (2–48)	NS
Total cholesterol, mg/dl	172 (117–236)	184 (137–264)	NS
High-density lipoprotein cholesterol, mg/dl	49 (27–82)	49 (15–101)	NS
Low-density lipoprotein cholesterol, mg/dl	102 (48–150)	109 (73–198)	NS
Triglycerides, mg/dl	108 (55–276)	97 (39–418)	NS
Uric acid, mg/dl	5.2 (3.2–8.7)	5.7 (2.5–8.7)	NS
Fasting blood glucose, mg/dl	92 (80–126)	93 (77–109)	NS
Treatment			
Ribavirin dose, mg/kg*	11.1 (8.0–12.9)	11.2 (7.3–14.0)	NS
Past history of IFN monotherapy	9 (36.0%)	19 (31.1%)	NS

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

[a] Non-SVR vs. rapid responder (Mann-Whitney U test or χ^2 test; statistical significance ($p < 0.05$), marginal significance ($p < 0.10$)). SVR = Sustained virological response; NS = not significant.

Treatment Efficacy according to Substitution Patterns in Amino Acids of the HCV Core Region

To examine the differences in virological characteristics between non-SVR and rapid response, 86 patients (25 of 27 non-SVR patients and 61 of 113 rapid responders) could be analyzed by the nucleotide sequences of HCV core region due to adequate serum samples obtained at the start of combination treatment. Figure 1 shows the sequences of aa 1–30 and aa 61–110 of the core region in 86 patients at the commencement of combination therapy. Substitutions at aa 4 (non-asparagine) of HCV core were significantly more frequent in rapid response (n = 15, 24.6%) than non-SVR (n = 1, 4.0%) patients (p = 0.032). Inversely, substitutions at aa 23 (non-lysine) were more frequent in non-SVR (n = 2, 8.0%) than rapid response (n = 0, 0%) patients (p = 0.082). There were no significant differences in the other substitution sites, including aa 70, aa 91, and aa 110 of the previous report [11], concerning the treatment efficacy of rapid response and non-SVR (table 3).

Predictive Factors Associated with Rapid Response in Multivariate Analysis

We then evaluated the data of all 86 patients who could be analyzed by the nucleotide sequences of core region to determine those factors that could predict rapid re-

Table 5. Factors associated with rapid response to combination therapy with IFN + ribavirin for 24 weeks in 86 patients infected with HCV genotype 2a, identified by multivariate analysis

Factor	Category	Odds ratio (95% CI)	p
Age, years	1: ≥50	1	
	2: <50	7.46 (1.79–31.3)	0.006
Level of viremia kIU/ml	1: ≥1,000	1	
	2: <1,000	4.33 (1.36–13.9)	0.013
Substitution of aa 4	1: asparagine	1	
	2: non-asparagine	9.97 (1.12–89.0)	0.039

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

sponse. Univariate analysis identified 5 parameters associated with rapid response that achieved statistical significance or marginal significance. As potential predictors of rapid response, table 4 indicates age (<50 years; p = 0.006), serum albumin (≥3.9 g/dl; p = 0.005), and level of viremia (<1,000 kIU/ml; p = 0.053). Furthermore, table 3 shows aa substitution of the core region in the pretreatment sample (substitution of aa 4 [non-asparagine], p = 0.032, and aa 23 [lysine], p = 0.082).

Multivariate analysis identified 3 parameters that independently influenced rapid response, including age (<50 years; p = 0.006), level of viremia (<1,000 kIU/ml; p = 0.013), and substitution of aa 4 (non-asparagine; p = 0.039) (table 5).

Discussion

Previous reports indicated that viral factors (e.g., viral load, aa substitutions in the NS5A region, early viral kinetics, and periods from the start of treatment to initial point of undetectable HCV-RNA) and host factors (e.g., body mass index, fibrosis stage, and level of soluble interleukin-2 receptor) might be important predictors of treatment response to 24-week IFN + ribavirin combination therapy in patients infected with HCV genotype 2a, in addition to treatment-related factors (e.g., treatment duration and ribavirin dose) [6–9, 23–28]. Using multivariate analysis, the present study identified viral- (viral load and substitution of aa 4) and host-related factors (age and serum albumin levels as surrogate markers of liver fibrosis [3, 11]) that influenced the virological response to 24-week combination therapy in patients with genotype 2a infection and a high viral load. IFN + ribavirin combination therapy carries potential serious side effects and is costly, especially when used long enough to achieve a high SVR. For these reasons, especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (≤16 weeks) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. Identification of these viral and host factors before the start of combination therapy should help design better therapeutic regimens.

Amino acid substitutions at position 70 and/or 91 in the core region of patients with genotype 1b infection and a high viral load are predictors of poor virological response to 48- and 72-week PEG-IFN + ribavirin combination therapy [11–15] and also affect clinical outcomes, including insulin resistance and hepatocarcinogenesis [16–18]. This study, based on 24-week combination therapy in patients with genotype 2a infection and a high viral load, identified substitution of aa 4 in the core region as the significant determinant of treatment efficacy, but did not identify substitutions of aa 70, aa 91, and aa 110. These discrepant findings might be due to the difference of genotype and treatment duration. Other mechanisms could be also explained by the small number of NVR patients with genotype 2a (only 3%), compared with about 25% of patients infected with genotype 1b [11]. Previous studies reported that the core region might be associated with resistance to IFN monotherapy involving the Jak-STAT signaling cascade [29–32]. The present result could also be interpreted to mean that aa substitutions in the core region are associated with those proteins involved in resistance to IFN monotherapy, such as SOCS proteins, which are known to inhibit IFN-α-induced activation of the Jak-STAT pathway and expression of the antiviral proteins 2′,5′-OAS and MxA [33]. Furthermore, this result also indicates that aa substitutions in the core region might serve as surrogate markers for other proteins associated with resistance to the antiviral actions of IFN. One limitation of this study based on the small number of patients was that only the nucleotide sequences of rapid response within SVR patients were analyzed, although all of SVR patients should have been investigated (e.g., possible type II error). Further large-scale studies that examine the structural and functional impacts of aa substitutions during combination therapy should be conducted to confirm the above findings.

In conclusion, our results suggest that the aa substitution pattern in the core region in patients with a high viral load of HCV genotype 2a may partly affect the viro-

logical response to combination therapy. The limitations of this study were that it did not investigate other viral factors, such as the number of substitutions in aa 2193–2228 (the region corresponding to the IFN sensitivity-determining region [ISDR] of genotype 1b [34–36]) or aa 2163–2228 of NS5A in genotype 2a [10, 26, 37], the geographic diversities of the genotype 2a core region (distribution of consensus sequence), and other races apart from Asians in Japan. Further prospective studies, matched for aa substitutions of the core region and large groups of patients of different races, are required to determine the virological response to 24-week IFN + ribavirin combination therapy in patients with a high viral load of HCV genotype 2a.

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References

1 Simmonds P: Clinical relevance of hepatitis C virus genotypes. Gut 1997;40:291–293.
2 Haydon GH, Jarvis LM, Blair CS, Simmonds P, Harrison DJ, Simpson KJ, Hayes PC: Clinical significance of intrahepatic hepatitis C virus levels in patients with chronic HCV infection. Gut 1998;42:570–575.
3 Akuta N, Suzuki F, Tsubota A, Suzuki Y, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: Efficacy of interferon monotherapy to 394 consecutive naive cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartite interaction of viral, host and interferon treatment-related factors. J Hepatol 2002;37:831–836.
4 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling MH, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001;358:958–965.
5 Fried MW, Shiffman ML, Reddy R, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.
6 Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A: Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005; 352:2609–2617.
7 Mangia A, Minerva N, Bacca D, Cozzolongo R, Agostinacchio E, Sogari F, Scotto G, Vinelli F, Ricci GL, Romano M, Carretta V, Petruzzellis D, Andriulli A: Determinants of relapse after a short (12 weeks) course of antiviral therapy and re-treatment efficacy of a prolonged course in patients with chronic hepatitis C virus genotype 2 or 3 infection. Hepatology 2009;49:358–363.
8 Von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, Bergk A, Bernsmeier C, Häussinger D, Herrmann E, Zeuzem S: Peginterferon-α-2a (40 KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129:522–527.
9 Fujiwara K, Yokosuka O, Komine F, Moriyama M, Kato N, Yoshida H, Tanaka N, Imazeki F, Shiratori Y, Arakawa Y, Omata M; Tokyo Hepatitis Network: Twenty-four weeks of interferon-α-2b in combination with ribavirin for Japanese hepatitis C patients: sufficient treatment period for patients with genotype 2 but not for patients with genotype 1. Liver Int 2006;26:520–528.
10 Akuta N, Suzuki F, Tsubota A, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in nonstructural protein 5A of hepatitis C virus genotype 2a low viral load and response to interferon monotherapy. J Med Virol 2003;69: 376–383.
11 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayshi M, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48:372–380.
12 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410.
13 Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. J Med Virol 2009;81:452–458.
14 Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE: Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. J Virol 2007; 81:8211–8224.
15 Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H: Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol 2009 (in press).
16 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 2007; 46:1357–1364.
17 Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: Amino acid substitutions in the hepatitis C virus core region of genotype 1b are the important predictor of severe insulin resistance in patients without cirrhosis and diabetes mellitus. J Med Virol 2009;81:1032–1039.
18 Fishman SL, Factor SH, Balestrieri C, Fan X, Dibisceglie AM, Desai SM, Benson G, Branch AD: Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. Clin Cancer Res 2009;15:3205–3213.

19 Chayama K, Tsubota A, Arase Y, Saitoh S, Koida I, Ikeda K, Matsumoto T, Kobayashi M, Iwasaki S, Koyama S, Morinaga T, Kumada H: Genotypic subtyping of hepatitis C virus. J Gastroenterol Hepatol 1993;8:150–156.
20 Desmet VJ, Gerber M, Hoofnagle JH, Manna M, Scheuer PJ: Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–1520.
21 Okamoto H, Okada S, Sugiyama Y, Kurai K, Iizuka H, Machida A, Miyakawa Y, Mayumi M: Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: comparison with reported isolates for conserved and divergent regions. J Gen Virol 1991;72:2697–2704.
22 Kwok S, Higuchi R: Avoiding false positives with PCR. Nature 1989;339:237–238.
23 Nousbaum JB, Cadranel JF, Savary O, Legrand MC, Dumouchel P, Gouérou H: Sustained virological response after a short course of treatment with interferon and ribavirin in two chronic hepatitis C patients. J Hepatol 2003;39:655–656.
24 Dalgard O, Bjøro K, Hellum KB, Myrvang B, Ritland S, Skaug K, Raknerud N, Bell H: Treatment with pegylated interferon and ribavarin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology 2004;40:1260–1265.
25 Abe S, Narita R, Matsuhashi T, Oto T, Tabaru A, Otsuki M: Increased soluble IL-2 receptor levels during interferon and ribavirin treatment are associated with a good response in genotype 2a/2b patients with chronic hepatitis C. Eur J Gastroenterol Hepatol 2008;20:373–378.
26 Nagase Y, Yotsuyanagi H, Okuse C, Yasuda K, Kato T, Koike K, Suzuki M, Nishioka K, Iino S, Itoh F: Effect of treatment with interferon-α-2b and ribavirin in patients infected with genotype 2 hepatitis C virus. Hepatol Res 2008;38:252–258.
27 Nomura H, Miyagi Y, Tanimoto H, Ishibashi H: Impact of early viral kinetics on pegylated interferon-α-2b plus ribavirin therapy in Japanese patients with genotype 2 chronic hepatitis C. J Viral Hepat 2009;16:346–351.
28 Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kanamori A, Atsumi H, Nakano S, Arakawa T: Eight-week regimen of antiviral combination therapy with peginterferon and ribavirin for patients with chronic hepatitis C with hepatitis C virus genotype 2 and a rapid virological response. Liver Int 2009;29:120–125.
29 Blindenbacher A, Duong FH, Hunziker L, Stutvoet ST, Wang X, Terracciano L, Moradpour D, Blum HE, Alonzi T, Tripodi M, La Monica N, Heim MH: Expression of hepatitis C virus proteins inhibits interferon α signaling in the liver of transgenic mice. Gastroenterology 2003;124:1465–1475.
30 Bode JG, Ludwig S, Ehrhardt C, Albrecht U, Erhardt A, Schaper F, Heinrich PC, Häussinger D: IFN-α antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J 2003;17:488–490.
31 Melén K, Fagerlund R, Nyqvist M, Keskinen P, Julkunen I: Expression of hepatitis C virus core protein inhibits interferon-induced nuclear import of STATs. J Med Virol 2004;73:536–547.
32 De Lucas S, Bartolome J, Carreno V: Hepatitis C virus core protein downregulates transcription of interferon-induced antiviral genes. J Infect Dis 2005;191:93–99.
33 Vlotides G, Sörensen AS, Kopp F, Zitzmann K, Cengic N, Brand S, Zachoval R, Auernhammer CJ: SOCS-1 and SCOS-3 inhibit IFN-α-induced expression of the antiviral 2,5-OAS and MxA. Biochem Biophys Res Commun 2004;320:1007–1014.
34 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C: Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96:224–230.
35 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.
36 Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, Ichijo T, Yoshizawa K, Kiyosawa K, Tanaka E: Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology 2008;48:1753–1760.
37 Murakami T, Enomoto N, Kurosaki M, Izumi N, Marumo F, Sato C: Mutations in nonstructural protein 5A gene and response to interferon in hepatitis C virus genotype 2 infection. Hepatology 1999;30:1045–1053.

INTERNAL MEDICINE

ORIGINAL ARTICLE

The Efficacy of Interferon-beta Monotherapy for Elderly Patients with Type C Hepatitis of Genotype 2

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Abstract

Objective The aim of this study was to elucidate the efficacy of interferon (IFN)-beta monotherapy for elderly patients of ≥70 years with type C hepatitis (HCV) of genotype 2.

Methods The present study was a retrospective cohort study. Inclusion criteria were type C hepatitis patients with HCV genotype 2a or 2b, ≥70 years, and IFN-beta monotherapy of within 24weeks. Thirty-one consecutive patients who satisfied the above criteria were enrolled in the present study. Independent factors that might have influenced the sustained virological response (SVR) were studied using logistic regression analysis.

Results Background of clinical profiles was as follows: median (range) age =71 (70-76) years, male/female =13/18, and median (range) HCV-RNA=260 (<5-3,800) KIU/mL. Out of 31, 16 patients (51.6%) had SVR by the intention-to-treat analysis. The SVR was significantly associated with the serum HCV RNA level. Logistic analysis showed that SVR occurred when HCV RNA level was <100 KIU/mL (p=0.020). Based on the difference of the serum HCV RNA level, the SVR rate was 81.8% (9/11) in patients with a serum HCV RNA level of <100 KIU/mL and 35.0% (7/20) in patients with a serum HCV RNA level of ≥100 KIU/mL.

Conclusion IFN-beta monotherapy of ≤24 week is a possible therapy selection for elderly patients of ≥70 years with type C hepatitis of genotype 2.

Key words: elderly patients, hepatitis C virus, genotype 2a or 2b, interferon monotherapy, sustained virological response

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Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral (HCV) infection has been directed at viral clearance. Recent studies have reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-6). However, IFN is expensive and has a number of serious side effects. The adverse events have a tendency to occur in elderly patients (7, 8). Therefore, in the case of elderly patients, the physician in charge often avoids IFN therapy because of IFN side effects. However, recently, the lifespan has been long in Japan. Thus, in the near future, a large number of patients with HCV will be >60 years of age. Also, HCV-related hepatocellular carcinoma (HCC) patients have been shown to become old with a peak around age 70 (9-11). When such aged patients with chronic abnormal ALT levels consult a doctor, the decision of whether or not to use therapy for chronic hepatitis is problematic. Moreover, when the use of treatment for chronic hepatitis C is decided for such aged patients, whether or not IFN therapy should be second problem.

A few studies have targeted IFN therapy and prolonged prognosis in elderly patients with chronic hepatitis C. Our

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investigation showed that the clearance of hepatitis C virus reduces the onset of HCC in elderly patients with HCV (12). Imai et al reported that IFN therapy reduces liver-related mortality in aged patients with chronic hepatitis C, especially in those exhibiting a biochemical response as well as a sustained virological response (13). Thus, in Japan, elderly patients with HCV are often treated with IFN.

In IFN therapy for chronic hepatitis C, several predictive factors of sustained virological response (SVR) to IFN have been identified, and these include short duration of disease, young age, absence of liver cirrhosis, genotype 2, low HCV-RNA levels, HCV and mutant type of nonstructual5A region (14-18). Thus, HCV patients with genotype 2 or low HCV-RNA levels might have the possibility of eradication of HCV RNA with a small dose or a short period of IFN. Now, there is also controversy about the indication and administration method of the IFN therapy in elderly patients with HCV.

Thus, in this study, we evaluated the efficacy of interferon (IFN)-beta monotherapy for type C patients of ≥70 years with genotype 2.

Abbreviation: ALT: alanine aminotransferase, AST: aspartate aminotransferase, CH: chronic hepatitis, HCV: hepatitis C virus, IFN: interferon, LC: liver cirrhosis, MU: million unit, SVR: sustained virological response

Materials and Methods

Patients

A total of 31 consecutive cirrhotic type C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 2000 and 2007 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: ≥70 years; positive serum HCV RNA; genotype 2a or 2b; IFN-beta monotherapy; treatment period of ≤24 weeks. We excluded from the study all of the following patients: 1) those with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) leukocytes <3,000/mm^3, platelets <70,000/mm^3 and bilirubin >1.5 mg/mL before IFN therapy; 4) decompensated liver cirrhosis with ascites or encephalopathy.

IFN therapy

For the first IFN treatment regimen, the IFN treatment consisted of 3 to 6 million units (MU) of IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan). For the IFN treatment regimen, one group of 20 patients was given to receive IFN-beta intravenously every day for the first 2-8 weeks and then two or three times a week for the following 16-22 weeks (long-term group). Another group of 11 patients were treated with IFN by intravenous injection daily for 6-8 weeks (short-term group). The physician in charge primarily determined the method of IFN treatment and dose of IFN. We regarded sustained virological response (SVR) to therapy as clearance of HCV RNA by amplicor method (19) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained to each patient the purpose and method of the treatment as well as the potential adverse reactions, and informed consent for treatment was then obtained.

Blood testing

Blood samples were obtained just before IFN therapy and stored at -80℃. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, Branchburg, NJ, USA) (20).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay (19). The lower detection limit of the qualitative assay is 100 copies/mL. HCV genotype was examined by the PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (21).

Liver staging

Ideally, the severity of chronic liver disease should be determined histologically from the results of liver biopsy. Only 12 (38.7%) of 31 patients underwent peritoneoscopy or liver biopsy before the age of 70; the remaining 19 patients did not undergo histological assessment on the first visit owing to their advanced age. In these patients, liver staging was determined by calculation using the equation to discriminate chronic hepatitis (CH) and liver cirrhosis (LC) as described by Ikeda et al (22).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, body mass index, HCV RNA level, HCV genotype 2a or 2b, liver staging, biochemical factors (AST, ALT), platelet count, total IFN dose, and IFN regimen. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Abbreviation: AST: aspartate aminotransferase

Results

Patients' characteristics

Table 1 shows the characteristics of the 31 patients who had received IFN-beta monotherapy. Clinical profiles were as follows: median (range) age =71 (70-76) years, male/female =13/18, median (range) HCV-RNA=260 (<5-3,800) KIU/mL, and CH/LC =19/12. All LC patients were catego-