Table 1
Demographic and clinical characteristics of 126 patients with PBC

Characteristics	
Age, years[a]	57 (30–83)
Female/male	111/15
Biopsy at diagnosis, n (%)	65 (51.6)
Stage 1/2/3/4	49/8/4/4
Liver transplant, n (%)	4 (3.2)
AMA-positive, n (%)	106 (84.1)
ANA-positive, n (%)	100 (79.4)
Median values (normal values)[a]	
AST (12–37 IU/l)	35 (12–687)
ALT (7–45 IU/l)	32 (8–885)
ALP (124–367 mg/dl)	403 (131–2306)
γ-GTP (8–50 mg/dl)	104 (10–1029)
Total bilirubin (0.3–1.2 mg/dl)	0.73 (0.30–6.04)
IgM (35–220 mg dl)	232 (56–1390)
IgG (870–1700 mg dl)	1530 (764–3410)

Abbreviations: PBC, primary biliary cirrhosis; AMA, antimitochondrial antibody specific for the pyruvate dehydrogenase complex-E_2 component; ANA, anti-nuclear antibody; AST, aspartate amino transferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γ-GTP, γ-glutamyltransferase.
[a]Median (range).

age 51 years) were enrolled in this study. All subjects who visited the outpatient clinic at Shinshu University Hospital or affiliated hospitals were residents of Nagano Prefecture, Japan, and their ethnic background was Japanese. All control subjects had indicated the absence of major illness on a standard questionnaire, and were volunteers from hospital staff whose ethnic backgrounds were also all Japanese.

The diagnosis of PBC could be established when two of the following three criteria were met: biochemical evidence of cholestasis with elevation of alkaline phosphatase activity; presence of antimitochondrial antibody (AMA) specific for the pyruvate dehydrogenase complex-E_2 component antigen; and histopathologic evidence of nonsuppurative cholangitis and destruction of small or medium-sized bile ducts if a biopsy was performed [15]. Serum AMA was measured by enzyme-linked immunosorbent assay (ELISA). An index value of greater than 7 was considered a positive result. Liver samples were obtained from 65 patients (51.6%) and assessed using the Scheuer's classification [16,17] by investigators blinded to the results of other experiments. The numbers of histologically diagnosed PBC patients as Scheuer stage I, II, III, and IV were 49, 8, 4, and 4, respectively. These 65 patients were classified as having either early stage (Scheuer stage I or II) or late stage (Scheuer stage III or IV) PBC. Of all patients, 4 cases underwent orthotopic liver transplantation (OLT). We excluded patients with overlap syndrome from our cohort [18]. Clinical characteristics of the patients in this study are shown in Table 1.

Informed consent was obtained from all study participants. Our registry and present study conform to the ethical guidelines of the 1975 Declaration of Helsinki and have been approved by the Ethics Committee of Shinshu University School of Medicine.

2.2. Preparation of genomic DNA

Genomic DNA from patients and controls was isolated by phenolic extraction of sodium dodecyl sulfate–lysed and proteinase K-treated cells, as described previously [19–23].

2.3. Microsatellite typing

A total genome scan was carried out using 400 microsatellite markers (ABI Linkage Mapping Set v 2.5 – MD10; Applied Biosystems, Foster City, CA) with an average heterozygosity of 79% and an average intermarker distance of 9.4 ± 2.9 cM (mean ± SD). The entire marker set consisted of 28 panels, each containing markers pooled according to size and fluorescent tag (6-FAM, VIC, NED). The markers were amplified by polymerase chain reaction (PCR) in 10-μl reactions containing 40 ng of genomic DNA, according to the manufacturer's protocol. After PCR, pooled panels were analyzed using an ABI 3130 DNA Analyzer. Semi-automated genotyping was performed using GeneMapper v 3.5 software (Applied Biosystems).

2.4. SNP genotyping

One locus located on chromosome 16p13.3 (microsatellite marker D16S423) was identified as particularly associated with PBC. A protein-coding gene, ataxin 2-binding protein 1 (A2BP1), was located 27 kb on the centromeric side of the nearest D16S423 microsatellite marker and therefore further investigated as a candidate susceptible gene for PBC.

Seven SNPs (rs17139207, rs12926282, rs17139244, rs6500742, rs4146812, rs4124065, and rs889699) distributed within the A2BP1 gene were selected from the National Center for Biotechnology Information dbSNP database (build 36) and the SNP database from Applied Biosystems. All seven polymorphisms were typed by TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) using the Applied Biosystems 7500 Real-time PCR system, following the manufacturer's instructions.

2.5. Statistical analysis

The phenotypic frequency at polymorphic sites among the 400 microsatellite markers was estimated by direct counting. The significance of differences in the distribution of alleles between patients with PBC and healthy control subjects was determined by the χ^2 test. Fisher's exact probability test was used for comparisons with fewer than five samples. A p value of less than 0.05 was considered statistically significant. p Values were corrected by multiplying by the number of different alleles observed in each locus (p_c). The strength of association with PBC was estimated by calculating the odds ratios (OR) and 95% confidence intervals (CI). The Hardy–Weinberg proportion (HWP) for multiple alleles was calculated using the Markov chain method within the GENEPOP software package (http://genepop.curtin.edu.au/) [24,25].

3. Results

To identify the genetic intervals that might contain susceptible or protective loci for PBC, 126 Japanese patients with PBC and 95 healthy Japanese controls were enrolled for an association anal-

Table 2
Statistically significant alleles associated with susceptibility to PBC

Chromosome	Marker	Significant allele	PBC % (n = 126)	Control % (n = 95)	OR	χ^2	p	PC
1p31	D1S207	156	16.7	4.2	4.55	8.38	0.00380	0.04181
12q24.2	D12S86	145	85.7	68.4	2.77	9.53	0.00203	0.00283
16p13.3	D16S423	135	59.5	33.7	2.90	14.48	0.00014	0.00170
21q21	D21S1256	102	78.6	56.8	2.78	12.01	0.00053	0.00637
16p13.3	D16S0602i	210	36.8	22.9	1.96	4.91	0.02665	NC

Abbreviations: OR, odds ratio; Pc, corrected P:NC, not calculated.

Table 3
Statistically significant alleles associated with protection against PBC

Chromosome	Marker	Significant allele	PBC % ($n = 126$)	Control % ($n = 95$)	OR	χ^2	p	p_c
1p13	D1S2726	292	13.5	28.4	0.39	7.57	0.00593	0.04747
3p14.2	D3S1300	232	19.1	39.0	0.37	10.73	0.00105	0.01263
5p13.3	D5S426	285	36.5	57.9	0.42	9.98	0.00158	0.01738
10p15	D10S249	119	15.9	36.8	0.32	12.74	0.00036	0.00394
14q12	D14S275	149	4.8	15.8	0.27	7.66	0.00565	0.03953
20p12	D20S186	137	3.2	15.8	0.17	10.97	0.00093	0.01205
Xp22.3	DXS1060	246	15.1	19.0	0.30	10.43	0.00124	0.01236

Abbreviations: OR, odds ratio; p_c, corrected p value.

ysis using 400 microsatellite markers dispersed throughout the human genome. As shown in Table 2, the alleles in four microsatellites (*D1S207*, *D12S86*, *D16S423*, and *D21S1256*) were positively associated with PBC susceptibility. Conversely, the alleles in seven microsatellite markers were negatively associated with disease (Table 3).

3.1. Candidate genes associated with D16S423 on chromosome 16p13.3

Among the susceptible and protective markers, allele 135 of the D16S423 marker on chromosome 16p13.3 showed the strongest association with PBC (59.5% vs 33.7%, OR $=2.90$, $p_c = 0.0017$) (Table 2). We therefore chose D16S423 as a marker of interest for further analysis. To validate its association with PBC, we investigated the association with PBC of an additional marker, D16S0602i, which is closely situated to D16S423. D16S0602i was extracted from the Gene Diversity DataBase System, Japan Biological Informatics Consortium (http://jbirc.jbic.or.jp/gdbs/top.jsp). The allele 210 of D16S0602i also showed a significant association with PBC (36.8% vs 22.9%, OR $=1.96$, $p = 0.026\,65$) (Table 2). We next used the National Center for Biotechnology Information Map Viewer from the National Institutes of Health (http://www.ncbi.nlm.nih.gov/mapview/) to predict novel susceptibility genes associated with both significant markers. This revealed the *A2BP1* gene, located within a 100-kb span of the two markers, to be a promising candidate gene for susceptibility to PBC.

3.2. A2BP1 SNP analysis in patients with PBC and healthy subjects

In total, seven SNPs in the *A2BP1* gene were genotyped in 126 PBC patients and 95 healthy controls (Table 4). We observed that the genotype distribution of all SNPs exhibited Hardy–Weinberg equilibrium, and that the minor allele frequencies of all SNPs were greater than 5% in patients and controls (Table 4). The major A allele at rs17139244 was significantly increased in an additive model (A/A+A/G vs G/G) (89.7% vs 78.9%, OR 2.318, 95% CI 1.100–4.883, $p = 0.027$) in patients compared with controls (Table 5). However, the significance of this allele disappeared after correction for multiple testing. In contrast, the genotype frequency of the major C allele at rs6500742 was significantly increased (62.3% vs 51.6%,

OR 1.55, 95% CI 1.05–2.27, $p = 0.024$) in patients compared with controls as well as in an additive model (C/C+C/T vs T/T) (86.5% vs 71.6%, OR 2.546, 95% CI 1.306–4.961, $p = 0.006$, $p_c=0.042$) (Table 5).

Evaluation of the seven SNPs between early and late stage PBC subgroups revealed no significant allelic associations. Moreover, a comparison of four OLT PBC cases and 122 non-OLT cases revealed no significant differences in allele frequencies (data not shown).

4. Discussion

The present study is a case-control association analysis to identify candidate regions of PBC pathogenesis susceptibility in Japanese patients using a previously reported [22] method of genome-wide microsatellite analysis. Our results showed that at least four susceptible and 7 protective markers were significantly associated with PBC patients. The four susceptible regions for PBC differed from those for autoimmune hepatitis in an earlier report (D11S902, D18S464) [22], suggesting a separate genetic etiology between the two. A recent report by Hirschfield et al. [7] showed that HLA class II, IL12A, and IL12RB2 loci had a strong association with PBC susceptibility, and that STAT4 and CTLA4 loci had a modest association with PBC in a genome-wide association analysis using 373,400 SNPs. A possible reason for the differing findings found here may be that the dense SNP marker sets offered more precise information than the lower resolution microsatellite scan [26]. Here, a total of 400 microsatellite markers were used throughout the whole human genome. With an intermarker distance of 9.4 cM, the number of microsatellite markers in this study was not sufficient to supersede information provided by 373,400 SNPs, considering that approximately 300,000 microsatellite markers are needed for whole genome association studies of complex diseases if charting at 100-kb intervals [27]. Thus, many regions associated with the pathogenesis of PBC might have remained undetected in our study. Nonetheless, four statistically significant susceptible regions and seven protective regions were identified, which imply that new or previously unidentified genes responsible for the pathogenesis of PBC exist within the proximity of the corresponding microsatellite markers because of the average length of linkage disequilibrium between the markers [27–29]. Several candidate genes were considered within a 100-kb perimeter of each marker for further analysis. Two candidate PBC susceptibility protein coding genes (*latrophilin 2* and *A2BP1*) and eight protective genes (*KCNA2*, *KCNA3*, *FHIT*, similar to *hCG1745223*, *RAI14*, *TTC23L*, *ZMYND11*, and *DIP2C*) were located within this 100-kb range. However, no previously reported linked genes, including *STAT4* [7], *MDR3* [11], *CTLA4* [8], *vitamin D receptor* [14], and *TNF-α* [10], were found to be related to PBC susceptibility.

Based on our findings, we sought to determine whether the *ataxin-2 binding protein 1 (A2BP1)* gene had a significant association with susceptibility to PBC by SNP analysis. The *A2BP1* gene was 27 kb on the centromeric side of the D16S423 microsatellite marker, which had shown the strongest significance to PBC. This gene has been described as a protein-coding gene of 1,691,715 bps in size

Table 4
Allele frequencies of 6 SNPs on the A2BP1 gene in PBC patients and controls

DbSNP	Alleles Major/minor	Position (Bp)	Patients ($n = 126$) MAF (%)	Patients ($n = 126$) HWE p value	Controls ($n = 95$) MAF (%)	Controls ($n = 95$) HWE p value
rs17139207	A/G	6,026,273	9.9	0.200	7.4	1.000
rs12926282	C/A	6,034,558	23.4	0.718	18.4	0.686
rs17139244	A/G	6,045,214	29.8	0.532	40.0	0.058
rs6500742	C/T	6,049,222	37.7	0.916	48.4	0.073
rs4146812	G/A	6,055,827	27.0	0.806	20.0	1.000
rs4124065	T/G	6,068,401	25.4	1.000	25.3	1.000
rs889699	A/G	6,074,528	34.9	0.494	35.8	0.489

Abbreviations: MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium.

Table 5
A2BP1 polymorphisms in patients with PBC and healthy subjects

DbSNP	Alleles	PBC (n = 126)			Controls (n = 95)			p^a	Additive model	
	(1/2)	1/1	1/2	2/2	1/1	1/2	2/2		p	OR (95% CI)
rs17139207	A/G	104	19	3	81	14	0	0.644	NC	NC
rs12926282	C/A	75	43	8	62	31	2	0.466	0.240	0.317 (0.071–1.423)
rs17139244	A/G	64	49	13	39	36	20	0.071	0.027	2.318 (1.100–4.883)
rs6500742	C/T	48	61	17	30	38	27	0.024	0.006	2.546 (1.306–4.961)
rs4146812	G/A	66	52	8	61	30	4	0.303	0.069	0.648 (0.190–2.208)
rs4124065	T/G	70	8	48	53	6	36	0.961	0.992	0.994 (0.332–2.976)
rs889699	A/G	51	62	13	37	48	10	0.974	0.960	1.023 (0.427–2.448)

Abbreviations: A2BP1, ataxin 2-binding protein 1; PBC, primary biliary cirrhosis; OR, odds ratio; 95% CI, 95% confidence interval; NC, not calculated.
[a]p Value in genotype frequency caluculated by χ^2 test of a 2×3 contingency table (df =2). p Value in additive model, which was compared 1/1+ 1/2 vs 2/2, was calculated by χ^2 test of a 2×2 contingency table (df = 1). If there were fewer than five data samples, Fisher's exact probability test was used.

that consists of 16 exons and is located on chromosome 16p13.3. The *A2BP1* gene encodes a ribonucleoprotein motif that is highly conserved among RNA-binding proteins [30,31]. This protein binds to the C-terminus of ataxin-2, which in turn signals encoding of many transcripts by alternative splicing [32]. A2BP1 is predominantly expressed in muscle and the brain [30]. *A2BP1* gene polymorphisms have been reported to be associated with autism in a subset of patients [33], as well as with smoking cessation [34], early-stage non–small-cell lung cancer [35], and osteoarthritis of the hand [36]. However, there have been no reports of any association between the *A2BP1* gene and PBC to date. The *A2BP1* gene was also reported to be a novel transcriptional regulator that mediates the neuron-specific splicing pattern of calcitonin-calcitonin gene-related peptide (CGRP) pre-mRNA [37]. CGRP is a neuropeptide produced in the neural body of dorsal root ganglion cells that is released from sensory nerve endings. One of the isoforms of CGRP, αCGRP, is produced mainly in the nervous system by the tissue-specific alternative splicing of the primary RNA transcript of the calcitonin-CGRP gene [38]. αCGRP was also reported to suppress the production of TNF-α and IL-12 in bone marrow–derived dendritic cells stimulated with lipopolysaccharide in *an vivo* study [39]. Therefore, we cannot exclude the possible involvement of *A2BP1* SNPs in the pathogenesis of PBC, as previous reports have implicated TNF-α and IL-12 SNPs with PBC as well [7,10].

Overall, our work with genome-wide microsatellite analysis yielded a combination of 10 candidate genes potentially associated with PBC by analysis of linkage disequilibrium (LD). However, our study was preliminary in nature because the numbers of cases and controls were limited, as well as the number of microsatellite markers. To overcome type I error, further studies are needed to analyze the relationship between gene polymorphisms and the expression and functions of these gene products in a second cohort or larger test group.

In conclusion, we identified four candidate PBC susceptibility and seven candidate PBC resistance regions by genome-wide microsatellite analysis, which include two candidate susceptible genes and eight resistant genes to PBC by statistical LD analysis. Among these, *A2BP1* gene polymorphisms might be associated with the onset of PBC. As the direct functional effects of the *A2BP1* gene are still uncertain, further investigation is needed to clarify the interaction of this gene with PBC pathogenesis.

Acknowledgments

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INTERNAL MEDICINE

A Patient with Advanced Hepatocellular Carcinoma Treated with Sorafenib Tosylate Showed Massive Tumor Lysis with Avoidance of Tumor Lysis syndrome

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Abstract

A 33-year-old man presented with pain and palsy of the leg in 2008 for treatment of hepatocellular carcinoma with huge distant metastases. The patient's tumors had slowly enlarged despite several treatments. Oral administration of sorafenib at 800 mg/day with careful observation was commenced in 2009. Laboratory investigations on day 7 showed massive tumor lysis. An abdominal CT showed multiple low density areas and tumor markers decreased, indicating extended tumor necrosis. In conclusion, clinicians should bear in mind not only the published adverse effects, but also massive tumor lysis, when treating patients with large tumor burden by sorafenib.

Key words: hepatocellular carcinoma, sorafenib, tumor lysis, tumor lysis syndrome

Introduction

Sorafenib tosylate was approved by the Food and Drug Administration (FDA) in 2007 to treat hepatocellular carcinoma (HCC) and could be prescribed in Japan from 2009 to treat unresectable HCC. It is an oral multitargeted kinase inhibitor that inhibits tumor cell proliferation and angiogenesis (1, 2). Sorafenib is believed to mark the beginning of a new era in advanced HCC therapy since it is the first approved molecular targeted agent for HCC and can be prescribed in outpatient clinics. However, the most frequently reported adverse events of sorafenib have been observed in 80% of patients (1). We report a case of HCC who avoided tumor lysis syndrome (TLS) following massive tumor lysis from treatment with sorafenib.

Case Report

A 33-year-old man presented with pain and palsy of the left leg at our hospital in 2008 for further examination and treatment of huge masses in the pelvic cavity (70 mm in diameter) and liver (110 mm in diameter) that had been earlier detected with ultrasonography, computed tomography (CT) and magnetic resonance imagining (MRI). A specimen taken from a direct tumor biopsy from the sacral bone histologically showed well-differentiated hepatocellular carcinoma (HCC) with monotonous tumor cell proliferation (Fig. 1a) and immunohistologically stained for hepatocyte paraffin 1 (Fig. 1b). Laboratory tests were normal except for levels of serum HCC tumor makers, such as alpha-fetoprotein (AFP) at 2,580 ng/ml, AFP like the Lens culinaris agglutinin-reactive fraction (AFP-L3) at less than 0.5% of total AFP, and prothrombin induced by vitamin K absence or antago-
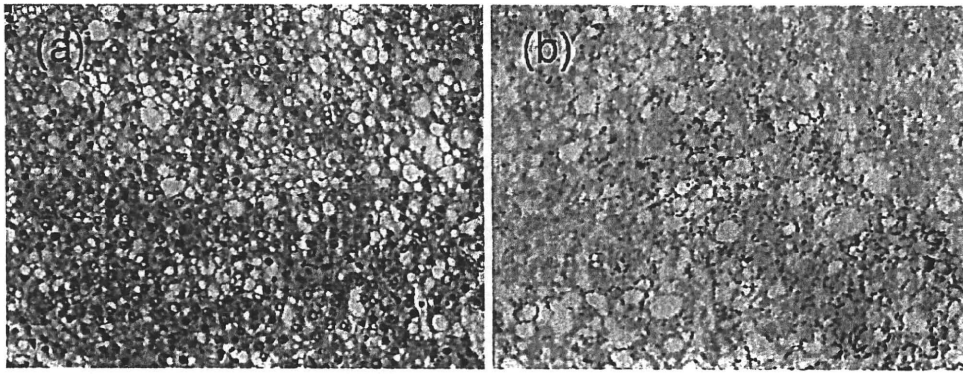
Figure 1. a) Microscopic examination revealed that the tumor was a well-differentiated hepato-cellular carcinoma with monotonous tumor cell proliferation. Hematoxylin and Eosin staining ×100. b) Cells were immunohistologically positive to antibodies against hepatocyte paraffin 1 (Hep Par 1) ×100.
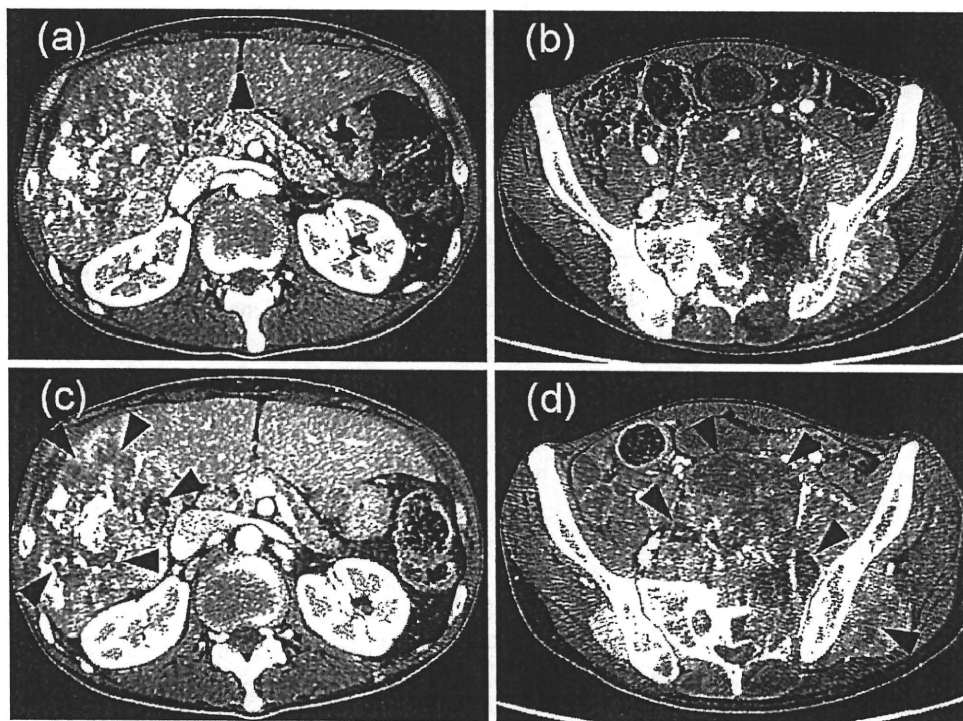

Figure 2. a) Contrast-enhanced computed tomography (CT) right before treatment with soraf-enib depicted a maximum 78×63 mm-wide tumor on the right robe in the liver with several small intrahepatic metastases. b) It also showed a maximum 102×110 mm-wide tumor in the pelvic cav-ity directly infiltrating out of the sacral bone. c) Contrast-enhanced CT on day 14 depicted multi-ple low density areas in the liver (black arrowheads). d) It also showed multiple low density areas in the pelvic cavity (black arrowheads), indicating extended tumor necrosis by sorafenib.

nist II (PIVKA II) at 781 mAU/mL. The patient's tumors had slowly become enlarged over 14 months despite several treatments with transarterial chemoembolization and transar-terial infusion chemotherapy for both hepatic and pelvic masses. He also suffered from complications of severe can-cer pain that were managed by local radiation therapies and several analgesic agents, including morphine.

Oral administration of sorafenib at 800 mg/day with care-ful observation was commenced in 2009 at an outpatient clinic visit after sorafenib became approved for unresectable

HCC in Japan. An abdominal CT right before treatment showed a maximum 78×63 mm-wide tumor (Fig. 2a) with several small intrahepatic metastases and a huge maximum 102×110 mm-wide tumor directly infiltrating out of the sac-ral bone (Fig. 2b). Pretreatment laboratory tests were as fol-lows: aspartate aminotransferase (AST), 102 IU/L; alanine aminotransferase (ALT), 28 IU/L; lactate dehydrogenase (LDH), 377 IU/L; uric acid (UA), 5.8 mg/dL; phosphorus (P), 3.9 mg/dL; creatinine (Cre), 0.39 mg/dL; potassium (K), 4.3 mmol/L; calcium (Ca), 10.0 mg/dL; AFP, 91,260
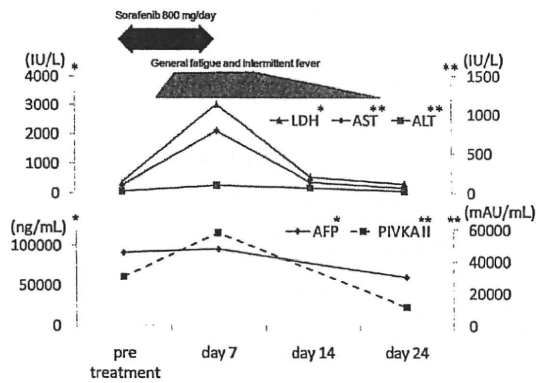
Figure 3.   Clinical course from the beginning of treatment with sorafenib. On day 4 of taking sorafenib, the patient started complaining of general fatigue and showed intermittent fever. Sorafenib treatment was discontinued because levels of lactate dehydrogenase (LDH, red line) and aspartate aminotransferase (AST, blue line) were elevated on day 7, accompanied by a discrepancy in the level of alanine aminotransferase (ALT, green line), which was indicative of massive tumor lysis. On day 24, levels of both alpha-fetoprotein (AFP, black line) and prothrombin induced by vitamin K absence or antagonist II (PIVKA II, broken black line) were decreased, indicating extended tumor necrosis by sorafenib. The patient's clinical symptoms gradually improved and the elevated levels of AST and LDH returned to pretreatment levels.

ng/mL (AFP-L3 less than 0.5%); and PIVKA II, 30,533 mAU/mL. On day 4 after taking sorafenib, the patient started complaining of general fatigue and showed intermittent fever. Laboratory investigations on day 7 were extremely exacerbated and were as follows: AST, 788 IU/L; ALT, 105 IU/L; LDH, 3,016 IU/L; UA, 4.4 mg/dL; P, 3.9 mg/dL; Cre, 0.38 mg/dL; K, 4.9 mmol/L; and Ca, 8.9 mg/dL. Sorafenib treatment was discontinued due to concerns of tumor lysis syndrome (TLS) development despite laboratory findings not entirely meeting the diagnostic criteria of TLS (3, 4). An abdominal CT on day 14 showed no interval changes in the size of the tumors, but multiple low density areas were evident (Fig. 2c in the liver; Fig. 2d in the pelvic cavity. Black arrowheads indicate multiple low density areas.). Furthermore, on day 24, levels of AFP and PIVKA II were decreased to 60,810 ng/mL (AFP-L3 less than 0.5%) and 11,701 mAU/mL, respectively, indicating extended tumor necrosis by sorafenib. The patient's clinical symptoms gradually improved and the elevated levels of AST and LDH returned to pretreatment levels after a 17-day interruption of sorafenib treatment (Fig. 3). He was then started on oral administration of sorafenib at a reduced dose of 400 mg/day on day 30 and showed no tumor lysis in blood examination, and has since received extended does of 400 mg/ 800 mg sorafenib on alternate days from day 38 and has been alive to date (day 171) despite showing tumor progression.

Discussion

Sorafenib increases the rate of tumor apoptosis by inhibition of the serine-threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors (VEGFRs)-1 (flt-1), -2 (KDR/flk-1), and -3 (flt-4) and platelet-derived growth factor receptor (PDGFR)-β (2). Cellular signaling mediated by Raf-1 and vascular endothelial growth factors (VEGFs) has been implicated in the molecular pathogenesis of HCC (5). One isoform of VEGF, VEGF-A, was reported to be expressed and related to angiogenesis along with VEGFR-1 and -2 in relatively well-differentiated HCC (6). Another VEGF isoform, VEGF-C, was found to be related to HCC disease progression together with VEGFR-2 and -3 (7). Clinically, the multicenter European randomized SHARP trial demonstrated a statistically significant survival benefit and disease-control rate according to the Response Evaluation Criteria in Solid Tumors (RECIST) for sorafenib in patients with advanced HCC in 2008 (1). In the present case of histologically well-differentiated HCC with distant metastases, the antitumor effect of sorafenib was clearly observed and resulted in massive tumor lysis.

The most frequently reported adverse events of sorafenib observed in 80% of patients include dermatologic events, such as hand-foot skin reactions, constitutional symptoms, such as weight loss, and gastrointestinal events like diarrhea (1). The present case showed none of these side effects although he only received sorafenib treatment for 7 days. TLS is an oncologic emergency that is caused by the release of intracellular tumor components due to massive tumor lysis, especially in cases that show a large tumor burden, a high proliferative tumor rate, or high sensitivity to cytotoxic therapy (3, 4). In general, TLS is less likely to occur in patients with solid tumors than in those with hematological malignancies (8) because the former tend to be more resistant to cytotoxic therapy. However, with the advent of multitargeted kinase inhibitors that show effectiveness against solid tumors, an increasing number of reports on TLS in these cases are surfacing (9-11). Recently, a 55-year-old man with HCC who had shown a good initial response to sorafenib later experienced TLS and died after 30 days of continuous treatment (12). Careful observation, especially in the initial period after sorafenib commencement, and earlier discontinuation of the drug may have prevented TLS development. Administration with sorafenib was halted at 7 days in the present case but it still yielded positive results without the onset TLS, indicating that further studies are required to determine the optimal dosage and treatment course with sorafenib in unresectable HCC patients such as the current case.

In conclusion, clinicians should bear in mind not only the published major adverse effects (1), but also the possibility of TLS brought on by massive tumor lysis, when starting treatment of advanced HCC patients with large tumor bur-

den using oral administration of sorafenib.

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Authors' disclosures of potential conflicts of interest

The authors indicated no potential conflicts of interest.

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Review Article

Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan

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In the 2008 guidelines for the treatment of patients with cirrhosis, who are infected with hepatitis B virus (HBV), the main goal is to normalize levels of alanine and aspartate aminotransferases by eliminating HBV or reducing viral loads. In patients with compensated cirrhosis, the clearance of HBV from serum is aimed for by entecavir, as the main resort, for histological improvement toward the prevention of hepatocellular carcinoma (HCC). In patients with decompensated cirrhosis, by contrast, meticulous therapeutic strategies are adopted for the reversal to compensation, toward the eventual goal of decreasing the risk of HCC. For maintaining liver function and preventing HCC, branched chain amino acids and nutrient supplements are applied, in addition to conventional liver supportive therapies. For patients with chronic hepatitis B, separate guidelines are applied to those younger than 35 years and those aged 35 years or older. Even for patients with chronic hepatitis who are negative for hepatitis e antigen (HBeAg), but who harbor HBV DNA in titers of 7 log copies/mL or more, a "drug-free state" is aimed for by sequential treatment with interferon (IFN) plus entecavir as the first line. For patients with chronic hepatitis B aged 35 years or older, who are HBeAg-negative and carry HBV DNA in titers of less than 7 log copies/mL, long-term IFN for 24–48 weeks is adopted anew. To HBeAg-negative patients who have either or both platelet counts of less than $150 \times 10^3/mm^3$ and less than 7 log copies of HBV DNA, also, long-term IFN for 24–48 weeks is indicated.

Key words: chronic hepatitis, cirrhosis, hepatitis B virus, hepatocellular carcinoma, interferon, liver supportive therapies, nucleos(t)ide analogs

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INTRODUCTION

SINCE THE FISCAL year 2002, guidelines for the treatment of patients with viral hepatitis have been compiled annually by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, under the auspice of the Ministry of Health, Labor and Welfare of Japan, supported by enduring efforts of many specialists recruited from all over the nation. Guidelines have been improved every year with many supplementary issues, which had surfaced as our understanding of many facets of viral hepatitis deepened and treatment options widened increasingly with time. For the fiscal year 2008, guidelines have been worked out for a comprehensive standardization of the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in Japan. These guidelines have been observed by more than 70% of practicing hepatologists treating patients with viral liver disease in Japan. It is hoped that these guidelines will continue being widely accepted and implemented to help as many patients as possible who are suffering from sequelae of persistent hepatitis virus infections.

Here, we relate excerpts of the 2008 guidelines for the treatment of patients with liver disease due to HBV, covering a wide range from those with chronic hepatitis to those with decompensated cirrhosis. The 2008 guidelines for the treatment of liver disease due to HCV are reported in an accompanying paper.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS B

PATIENTS WITH CHRONIC hepatitis B can stabilize the activity of liver disease in their natural course, after they have seroconverted from hepatitis B e antigen (HBeAg) to the corresponding antibody (anti-HBe), accompanied by decrease in HBV DNA titers. For that reason, treatment guidelines were constructed separately for the patients younger than 35 years and those aged 35 years or older.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS B YOUNGER THAN 35 YEARS

PATIENTS WITH CHRONIC hepatitis B younger than 35 years are treated in accordance with the guidelines summarized in Table 1. Criteria for the treatment eligibility are: (i) serum levels of alanine aminotransferase (ALT) of 31 IU/L or more; and (ii) HBV DNA titers of 5 log copies of more in HBeAg-positive patients and 4 log copies or more in HBeAg-negative patients. In the 2008 guidelines, the indication of treatment is extended to the patients with cirrhosis due to HBV who carry HBV DNA in titers of 3 log copies/mL or more.

In Japan, most HBeAg-positive patients with 7 log copies or more of HBV DNA have been infected with HBV of genotype C by perinatal infection at birth;

Table 1 Guidelines for the treatment of patients with chronic hepatitis B younger than 35 years

Eligibility criteria	ALT HBV DNA	≥31 IU/L HBeAg-positive patients: ≥5 log copies/mL HBeAg-negative patients: ≥4 log copies/mL Patients with cirrhosis: ≥3 log copies/mL
HBV DNA	≥7 log copies/mL	<7 log copies/mL
HBeAg-positive	(1) Long-term IFN for 24–48 weeks (2) Entecavir	(1) Long-term IFN for 24–48 weeks (2) Entecavir
HBeAg-negative	(1) Sequential treatment† (entecavir plus IFN) (2) Entecavir Start with entecavir in HBeAg-negative patients who have platelet counts <15 × 10³/mm³ and in those with advanced liver disease of stage F2 or higher.	(1) Regular follow up (2) Long-term IFN for 24 weeks

†Sequential treatment: patients who have lost hepatitis B virus (HBV) DNA after treatment with nucleos(t)ide analogs receive combined interferon (IFN) for 4 weeks, and then IFN monotherapy is continued for 20 weeks, and lifted thereafter. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen.

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Table 2 Guidelines for the treatment of patients with chronic hepatitis B aged 35 years or older

Eligibility criteria	ALT	≥31 IU/L
	HBV DNA	HBeAg-positive patients: ≥5 log copies/mL
		HBeAg-negative patients: ≥4 log copies/mL
		Patients with cirrhosis: ≥3 log copies/mL
HBV DNA	≥7 log copies/mL	<7 log copies/mL
HBeAg-positive	(1) Entecavir	(1) Entecavir
	(2) Sequential treatment† (entecavir plus IFN)	(2) Long-term IFN for 24–48 weeks
HBeAg-negative	Entecavir	(1) Entecavir
		(2) Long-term IFN for 24–48 weeks

†Sequential treatment: patients who have lost hepatitis B virus (HBV) DNA after treatment with nucleot(s)ide analog receive combined interferon (IFN) for 4 weeks, and then IFN monotherapy is continued for 20 weeks, and lifted thereafter. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen.

accordingly, they would be resistant to interferon (IFN) therapy. Should they receive nucleos(t)ide analogs, however, the duration would become inevitably longer, because they start the treatment when younger than 35 years old. Hence, IFN for 24–48 weeks is the first choice in their treatment. The standard treatment of 3 months is favored, which can be extended to the maximum of 6 months. Non-pegylated (standard) IFN-α is recommended to them, because self-injection at home is approved for preparations of IFN-α; it helps improve their quality of life (QOL). There are many patients who are refractory to IFN and in whom improvement of ALT levels and/or decrease in HBV DNA titers are hardly achievable. Therefore, as another option, monotherapy with entecavir can be applied for the purpose of clearing HBeAg from serum and lowering HBV DNA titers. For HBeAg-positive patients with lower HBV DNA titers (<7 log copies/mL), also, long-term IFN is endorsed as a rule.

There are HBeAg-negative patients in whom ALT levels increase to 31 IU/mL or more repeatedly. In the 2008 guidelines, sequential treatment with IFN and entecavir is introduced as a new arm of therapeutic options for such patients.[1]

For HBeAg-negative patients with less than 7 copies/mL of HBV DNA, in general, regular follow up without therapeutic intervention is deemed to suffice for the majority. For those of them in whom ALT levels flare to 31 IU/mL or more time after time, long-term IFN for 24 weeks is indicated. Because liver disease progresses in many HBeAg-negative patients, for those with platelet counts of less than $150 \times 10^3/mm^3$ or in fibrosis stage F2 or higher, treatment with entecavir is indicated.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS B AGED 35 YEARS OR OLDER

TABLE 2 SUMS up treatment modalities for patients with chronic hepatitis B who are aged 35 years or older. HBeAg-positive patients in this age range who carry HBV DNA in titers of 7 log copies/mL or more rarely, if ever, seroconvert to the loss of HBeAg by IFN-based therapies. Hence, entecavir is the first choice in their treatment.[2,3] Because HBV mutants resistant to entecavir can be elicited by it, sequential treatment with IFN plus entecavir is amended in the 2008 guidelines.[1] In view of low viral loads in patients who possess HBV DNA in titers of less than 7 log copies/mL, entecavir is selected as the first choice, followed by long-term IFN as the second choice of treatment in these patients. HBeAg-negative patients who have high viral loads (≥7 log copies/mL), on the other hand, can normalize ALT levels by monotherapy with entecavir. Therefore, entecavir becomes their first choice, and this is the case even in patients with HBV DNA titers less than 7 copies/mL.

GUIDELINES FOR THE TREATMENT WITH NUCLEOS(T)IDE ANALOGS OF PATIENTS WITH CHRONIC HEPATITIS B WHO ARE RECEIVING LAMIVUDINE

TABLE 3 DETAILS guidelines for the treatment with nucleos(t)ide analogs of patients with chronic hepatitis B who are receiving lamivudine. Because a number of drug-resistant HBV mutants emerge increasingly with time in patients on long-term treatment with lamivudine, the fundamental rule is to switch them to ente-

Table 3 Guidelines for the treatment with nucleos(t)ide analogs in patients with chronic hepatitis who are receiving lamivudine

Lamivudine	Less than 3 years		3 years or longer
HBV DNA			
<1.8 log copies/mL persistently	May be switched to entecavir 0.5 mg daily		Continued on lamivudine
≥1.8 log copies/mL	VBT (−)	May be switched to entecavir 0.5 mg daily	100 mg daily
	VBT (+)	Adefovir 10 mg daily add-on lamivudine	Adefovir 10 mg daily add-on lamivudine

HBV, hepatitis B virus; VBT, virological breakthrough.

cavir. For this reason, patients are stratified by the duration of lamivudine treatment, less than 3 years and 3 years or more, as well as HBV DNA titers persistently below 1.8 log copies/mL and 1.8 log copies/mL or more, and separate treatment strategies have been worked out for the patients in each category. Because by far the majority of patients with a duration of lamivudine treatment of less than 3 years and HBV DNA titers of less than 1.8 copies/mL possess drug-resistant mutants in low frequencies, they are recommended to switch to entecavir 0.5 mg daily as soon as possible. Likewise, patients who have received lamivudine for 3 years or longer, but in whom drug-resistant mutants have never developed, are recommended to switch to entecavir 0.5 mg daily. By contrast, for patients in whom drug-resistant mutants have emerged already and who have undergone virological breakthroughs,[4] adefovir 10 mg daily add-on lamivudine is started for the purpose of stabilizing liver function.[5] In regard of the patients who have received lamivudine for 3 years or longer, those without drug-resistant mutants can stay on lamivudine 100 mg daily.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS B (PART I)

FOR THE FISCAL year 2008, the following three items have been added to previous guidelines for the treatment of chronic hepatitis B (Table 4).
1 In the treatment of patients with chronic hepatitis B, IFN is the first resort for those younger than 35 years, toward the eventual goal of gaining a "drug-free state". For the patients aged 35 years or older, persistently negative HBV DNA is the aim of nucleos(t)ide analogs, with the first choice being entecavir in their primary treatment. On the other hand, for patients with HBV mutants resistant to lamivudine and/or entecavir, combined treatment with adefovir and lamivudine is the principal rule (Table 3).[6–8]

2 Therapeutic responses to antiviral treatment are much different in patients with chronic hepatitis B who are infected with HBV of distinct genotypes. It is recommended therefore to determine HBV genotypes before making a decision on the treatment choice. In particular, the patients infected with HBV of genotype A or B respond to IFN in high rates, even if they are aged 35 years or older. For these reasons, IFN becomes the first choice in their antiviral treatment.
3 The duration of IFN treatment is 24 weeks basically. In the patients in whom the efficacy of IFN has been achieved with decrease in HBV DNA titers and normalization of ALT, the treatment duration is better extended to 48 weeks.

Table 4 Supplements to guidelines for the treatment of patients with chronic hepatitis B (part I)

1 Treatment of patients with chronic hepatitis B aims at a "drug-free state" by IFN-based therapies in those younger than 35 years, and at persistently negative HBV DNA in those aged 35 years or older, with entecavir as the first choice in the primary therapy. Lamivudine plus adefovir forms the basis for the treatment of HBV mutants resistant to lamivudine or entecavir.
2 In view of antiviral response much different in patients infected with HBV of distinct genotypes, it is desired to make treatment choices based on genotypes. In particular, because genotypes A and B respond to IFN with high efficacy, even in patients aged 35 years or older, IFN is recommended as the first treatment choice in these patients.
3 The duration of IFN is for 24 weeks basically, but extension to 48 weeks is recommended in patients who respond to IFN with decrease in HBV DNA titers and normalization of ALT levels.

ALT, alanine aminotransferase; HBV, hepatitis B virus; IFN, interferon.

Table 5 Supplements to guidelines for the treatment of patients with chronic hepatitis B (part II)

- Self-injection of IFN at home is recommended to patients, who are eligible to do it, for improving their quality of life.
- Treatment with nucleos(t)ide analogs should be continued in patients in whom cirrhosis or HCC has been cured.
- Antiviral treatment is considered in patients with ALT levels of ≥31 IU/L. To patients aged 35 years or older in whom viral replication persists, even to those with normal ALT levels, antiviral treatments are indicated. It is possible, however, to follow for outcomes in patients who are elderly or HBeAg-negative and in whom antiviral treatments are difficult, while they receive liver supportive therapy (e.g. SNMC, UDCA).
- In patients co-infected with HBV and HIV, entecavir cannot be used due to the possibility for emergence of HIV variants resistant to antiretroviral therapies.
- Immunosuppressive and anticancer drugs should be used with utmost caution, even in patients with low HBV DNA titers and normal ALT levels, because they can induce severe liver damage along with elevation in HBV DNA titers.

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS B (PART II)

FURTHER, THE FOLLOWING five supplements have been added to the 2008 guidelines (Table 5).

To patients who are eligible, self-injection of IFN at home is recommended, taking into consideration their QOL. Because IFN-based therapies are not recommended for patients in whom HBV has been transmitted by perinatal infection, sequential treatment with IFN plus entecavir serves as another option in their antiviral treatment.

Treatment with nucleos(t)ide analogs should be extended to patients in whom cirrhosis or hepatocellular carcinoma (HCC) has been cured after successful therapies.

Antiviral treatment has to be considered in patients with ALT levels of 31 IU/L or more. Patients aged 35 years or older with normal ALT levels but in whom HBV replication persists, need to be considered for antiviral treatments. Elderly and HBeAg-negative patients, as well as those to whom the administration of antiviral drugs is difficult, can be followed regularly while they

receive liver supportive therapy (e.g. stronger neo-minophagen C,[9] ursodeoxycholic acid [UDCA][10]).

Patients co-infected with HBV and HIV type 1 cannot receive entecavir due to the possibility of emergence of HIV mutants resistant to antiretroviral drugs.

Even in patients with low HBV DNA titers and normal ALT levels, HBV DNA loads can increase massively to induce severe liver damages in them, while they receive immunosuppressive or anticancer drugs. Hence, utmost caution should be exercised if they are to undergo antiviral treatments.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CIRRHOSIS DUE TO HBV

TABLE 6 SUMMARIZES guidelines for the treatment of patients with type B cirrhosis. Patients with compensated or decompensated cirrhosis, who are infected with HBV, receive entecavir for persistent clearance of HBV DNA detectable by the real-time polymerase chain reaction and normalization of aspartate aminotransferase as well as ALT levels. Combined lamivudine plus adefovir therapy are indicated for patients in whom HBV mutants resistant to lamivudine or entecavir have developed. Guidelines for maintaining liver function, for preventing the development of HCC, include liver supportive therapy with glycyrrhizin and UDCA, either alone or in combination. For treatment toward sup-

Table 6 Guidelines for treatment of type B cirrhosis

Principles
  Compensated: termination of HBV infection by antiviral treatment with entecavir as the mainstay.
  Decompensated: reversal to compensation and prevention of HCC.
Methods
  (1) Eradication of HBV and normalization of ALT/AST (compensated and decompensated cirrhosis).
    a) Entecavir.
    b) Combined lamivudine and adefovir (for patients with HBV mutants resistant to lamivudine or entecavir).
  (2) Maintenance of liver function (improvement of ALT/ AST and albumin) for preventing HCC.
    a) Liver supportive therapy such as SNMC or UDCA.
    b) Branched chain amino acids (Livact).
  (3) Supplementation with nutrients (for stabilizing liver function in decompensated cirrhosis).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

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pressing the development of HCC, branched chain amino acids (BCAA)[11] are implemented. Also, nutrient supplements are utilized for stabilizing liver function.

DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HBV and HCV ranging from chronic hepatitis to cirrhosis of various severities annually, since the fiscal year 2002. The principal aim of these guidelines is to decrease the incidence of HCC due to hepatitis virus infections in Japan. In accordance with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic viral liver disease every fiscal year. This article summarizes guidelines for the treatment of liver disease due to HBV. Guidelines for the treatment of liver disease due to HCV for the fiscal year 2008 are reported in the accompanying paper. They are formulated on evidence-based data that have been accumulated by members and cooperators of the study group. It will be necessary to improve these guidelines in the next fiscal year and henceforth, in accordance with many pieces of new evidence that are expected to evolve through enduring efforts and keen insights of members and cooperators of the study group.

In the treatment of chronic hepatitis B, novel therapeutic strategies have continued to evolve in previous guidelines. In guidelines of the fiscal year 2008, diverse new treatment arms are introduced for gaining the eventual goal of the "drug-fee state".

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has been drafted and displayed on the web site (www.jsh.or.jp/medical/index.html [in Japanese]) as well, guidelines for the treatment of a spectrum of liver diseases due to HBV, ranging from chronic hepatitis to cirrhosis of various severities for the fiscal year 2008. In view of the eventual goal of decreasing the incidence of HCC due to HBV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been introduced to the treatment of cirrhosis due to HBV infection. As a general rule, antiviral treatments are the mainstay in guidelines for the treatment of chronic hepatitis B. In addition to them, it is necessary to always keep in mind the fundamental concepts of these guidelines. It is our sincere hope that, for the treatment of each patient, readers will conduct their

clinical practice on the basis of these concepts, and then refer to appropriate individual guidelines, when they make decisions regarding treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in making treatment choices.

We can foretell that there is no end to the treatment of patients with chronic hepatitis and cirrhosis due to HBV, as it will keep evolving and improving in future guidelines. The enduring efforts of doctors and scientists, in pursuit of this goal, will fill in wide social and economic gaps in medical practices being served to the nation, and produce substantial and efficient interest in the medical economy on a national basis. In conducting treatment of patients with liver disease due to HBV infection, according to these guidelines, many new and unforeseen facets may surface that will require further improvements. Hence, it will be necessary to evaluate the therapeutic efficacy of these guidelines, and revise or add necessary supplements to them as required in the future.

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Review Article

Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan

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In the 2008 guidelines for the treatment of patients with chronic hepatitis C, pegylated interferon (Peg-IFN) combined with ribavirin for 48 weeks are indicated for treatment-naive patients infected with hepatitis C virus (HCV) of genotype 1. Treatment is continued for an additional 24 weeks (72 weeks total) in the patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA during 13–36 weeks on treatment. Re-treatment is aimed to either eradicate HCV or normalize transaminase levels for preventing the development of hepatocellular carcinoma (HCC). For patients with compensated cirrhosis, the clearance of HCV RNA is aimed toward improving histological damages and decreasing the development of HCC. The recommended therapeutic regimen is the initial daily dose of 6 million international units (MIU) IFN continued for 2–8 weeks that is extended to longer than 48 weeks, if possible. IFN dose is reduced to 3 MIU daily in patients who fail to clear HCV RNA by 12 weeks for preventing the development of HCC. Splenectomy or embolization of the splenic artery is recommended to patients with platelet counts of less than $50 \times 103/mm^3$ prior to the commencement of IFN treatment. When the prevention of HCC is at issue, not only IFN, but also liver supportive therapy such as stronger neo-minophagen C, ursodeoxycholic acid, phlebotomy, branched chain amino acids (BCAA), either alone or in combination, are given. In patients with decompensated cirrhosis, by contrast, reversal to compensation is attempted.

Key words: chronic hepatitis, cirrhosis, hepatocellular carcinoma, hepatitis C virus, interferon, liver supportive therapy, pegylated interferon, ribavirin

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INTRODUCTION

SINCE THE FISCAL year 2002, guidelines for the treatment of patients with viral hepatitis have been compiled annually by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, under the auspice of the Ministry of Health Labor and Welfare of Japan, recruiting many specialists from all over the nation. They have been improved every year with many supplementary issues that have evolved, as our understanding of various aspects of viral hepatitis deepens and treatment options widen with time. For the fiscal year 2008, guidelines have been worked out for a comprehensive standardization of the treatment of chronic hepatitis and cirrhosis due to infection with hepatitis C virus (HCV) in Japan. It is hoped that these guidelines will be accepted widely and implemented for helping as many patients as possible who suffer from sequelae of persistent HCV infection.

Here, we relate excerpts of the 2008 guidelines for the treatment of patients with HCV-induced liver disease covering a wide range from those with normal aminotransferase levels to those with decompensated cirrhosis.

GUIDELINES FOR THE PRIMARY TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

TABLE 1 SUMMARIZES the antiviral therapy of treatment-naive patients with chronic hepatitis C. In comparison with previous guidelines, the duration of combined treatment with pegylated interferon (Peg-IFN) and ribavirin is extended to 48–72 weeks for patients infected with HCV of genotype 1 in high viral loads (HVL: ≥5 log IU/mL by the Japanese criteria).[1,2] For patients infected with HCV of genotype 2 in HVL, Peg-IFN-α2b and ribavirin for 24 weeks are indicated.

To patients with HCV-1 in low viral loads (LVL: <5 log IU/mL), either the standard IFN (not conjugated with polyethylene glycol) for 24 weeks, or the weekly monotherapy with Peg-IFN-α2a for 24–48 weeks, is given.[3] Patients with HCV-2 in LVL receive either the standard IFN for 8–24 weeks, or the weekly monotherapy with Peg-IFN-α2a for 24–48 weeks.

GUIDELINES FOR THE RE-TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

FOR PATIENTS WHO receive re-treatment, first, it is imperatively prerequisite to: (i) identify factors for non-response to previous treatments; and (ii) decide whether to aim for clearance of HCV or to prevent the progression of hepatitis that can accelerate the development of hepatocellular carcinoma (HCC), and this can be monitored by alanine aminotransferase (ALT) and α-fetoprotein (AFP) levels toward normalizing or stabilizing their levels (Table 2).[4] Second, IFN combined with ribavirin is the mainstay of re-treatment of patients with chronic hepatitis C. Third, long-term IFN monotherapy is recommended to patients who are not indicated to IFN/ribavirin or who have failed to respond to the combination therapy. However, some patients do not tolerate IFN due to side-effects or their complicating morbidities. In addition, IFN monotherapy does not always improve ALT levels. Such patients need to receive liver supportive therapy including stronger neo-minophagen C (SNMC)[5] and ursodeoxycholic acid (UDCA),[6] as well as phlebotomy, either alone or in combination. Therapeutic target ALT levels are: (i) within ×1.5 the upper limit of normal (ULN) for patients in fibrosis stage 1 (F1); and (ii) less than 30 IU/L in those in fibrosis stages 2 or 3 (F2/F3), as far as possible.

Table 1 Guidelines for the primary treatment of patients with chronic hepatitis C

Genotypes	Genotype 1	Genotype 2
Viral loads		
High viral load ≥5.0 log IU/mL ≥300 fmol/L ≥1 Meq/mL	• Peg-IFN-α2b (Peg-Intron) + ribavirin (Rebetol) for 48–72 weeks • Peg-IFN-α2a (Pegasys) + ribavirin (Copegus) for 48–72 weeks	• Peg-IFN-α2b (Peg-Intron) + ribavirin (Rebetol) for 24 weeks
Low viral load <5.0 log IU/mL <300 fmol/L <1 Meq/mL	• Standard IFN for 24 weeks • Peg-IFN-α2a (Pegasys) for 24–48 weeks	• Standard IFN for 8–24 weeks • Peg-IFN-α2a (Pegasys) for 24–48 weeks

Peg-IFN, pegylated interferon.

Table 2 Guidelines for re-treatment of chronic hepatitis C

Principles

Selection has to be made between termination of HCV infection and normalization/stabilization of ALT as well as AFP levels (toward preventing aggravation of liver disease and development of HCC), after evaluating factors for non-response in the primary IFN treatment.

1 "IFN plus ribavirin" is the mainstay of re-treatment of patients who have failed to respond to the primary IFN therapy.

2 Long-term IFN is recommended to patients in whom ribavirin is not indicated or who have failed to respond to IFN/ribavirin; self-injection at home is approved for IFN-α (not for Peg-IFN).

3 Patients who are not indicated to IFN or have failed to improve ALT and AFP levels, in response to IFN, receive liver supportive therapy (SNMC, UDCA) and phlebotomy, either alone or in combination.

4 For preventing aggravation of liver disease (and development of HCC), ALT levels need to be controlled within $1.5 \times$ ULN in patients in stage 1 fibrosis (F1), and as far as possible, 30 IU/L or lower in those in fibrosis stages 2–3 (F2/F3).

5 In treatment combined with ribavirin, dose and mode need to be selected, taking into consideration factors contributing to the response, such as age, sex, progression of liver disease, mutations in the HCV genome (amino acid substitutions in the core protein [aa70/aa91] and ISDR) and HCV RNA titers determined by the real-time PCR.

AFP, α-fetoprotein; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS C

FOR THE FISCAL year 2008, the following items were supplemented to the treatment of chronic hepatitis C (Table 3).

1 The treatment of patients infected with HCV-1 in HVL with Peg-IFN/ribavirin for 72 weeks is modified by the early virological response (EVR) within 12 weeks after the start. Patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.[1,2]

2 Patients with HCV-1 in HVL who fail to clear HCV RNA detectable by real-time PCR but in whom

ALT levels normalize are continued on Peg-IFN/ribavirin until 48 weeks, so that normalized ALT levels endure longer after the completion of therapy.[7]

3 Patients who are not indicated to Peg-IFN/ribavirin, or who have failed to respond to previous treatments, receive long-term IFN monotherapy. During the first 2 weeks, IFN in the conventional dose is given daily or three times a week. Patients who do not clear HCV RNA during the maximal treatment period of 8 weeks receive half the conventional dose of IFN indefinitely.[8]

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C IN NORMAL ALT LEVELS

AS IN PREVIOUS guidelines, patients with chronic hepatitis C having normal ALT levels are stratified into four groups by ALT levels and platelet counts (Table 4). Patients with chronic hepatitis C who have normal ALT levels are reported to gain the sustained virological response (SVR) to antiviral treatments comparably frequently as those having elevated ALT levels. Taking this into consideration, patients with ALT levels of 30 IU/L or less and platelet counts of $150 \times 10^3/\text{mm}^3$ or more are followed for ALT every

Table 3 Supplements to guidelines for chronic hepatitis C

1 Criteria for extending the duration of Peg-IFN/ribavirin (to 72 weeks) in patients infected with HCV-1b in HVL: patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.[1,2]

2 Patients with HCV-1b in HVL who fail to lose HCV RNA detectable by real-time PCR, but in whom ALT levels normalize by 36 weeks, Peg-IFN/ribavirin is given till 48 weeks for maintaining normalized ALT levels long after the completion of treatment.

3 Long-term IFN monotherapy in patients who are not indicated to Peg-IFN/ribavirin, or have failed to respond to it: the usual dose of IFN daily or three times in week is given for the first 2 weeks, and when HCV RNA does not disappear within the maximal duration of 8 weeks, long-term treatment with half the usual dose of IFN is continued indefinitely.

ALT, alanine aminotransferase; HCV, hepatitis C virus; HVL, high viral loads; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon.

Table 4 Guidelines for the treatment of patients with normal ALT levels toward preventing the development of HCC

Platelets	$\geq 150 \times 10^3/mm^3$	$< 150 \times 10^3/mm^3$
ALT		
≤ 30 IU/L	• Follow for ALT every 2–4 months. • If ALT levels elevate, start antiviral treatments taking into consideration the possibility of SVR and risk for HCC.	• Liver biopsy, if possible, and consider antiviral treatments for patients in A2/F2. • Follow for ALT every 2–4 months, and consider antiviral treatments when ALT levels elevate, for patients without biopsy.
31–40 IU/L	• Consider antiviral treatments for patients younger than 65 years.	• Start treatments for chronic hepatitis C. • Select treatments according to genotypes, viral load, age of patients, etc.

ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

2–4 months. If ALT levels increase in them, antiviral treatments are considered based on the possibility of resolving HCV infection and the risk for developing HCC. In view of significant fibrosis present in patients with platelet counts of less than $150 \times 10^3/mm^3$, they are recommended to receive liver biopsy, if this is possible. Patients in fibrosis stage F2 or higher are evaluated for the indication to antiviral treatments. Patients with ALT levels between 31 and 40 IU/L are classified by platelet counts. Antiviral treatments are considered in those aged younger than 65 years who have platelet counts of $150 \times 10^3/mm^3$ or more, while guidelines for patients with chronic hepatitis are applied to those with platelet counts of less than $150 \times 10^3/mm$.[9,10]

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CIRRHOSIS DUE TO HCV

PATIENTS WITH COMPENSATED cirrhosis who are not infected with HCV-1 in HVL receive either IFN-β or IFN-α (Table 5). Since the fiscal year 2008, IFN-α has been approved for the treatment of patients infected with HCV-1 in HVL, with the aim of resolving infection and normalizing ALT as well as AFP levels by long-term therapy. Treatment duration was set at 1 year or longer, and because the longer the treatment duration the higher the SVR rate, 36 weeks has been recommended as the optimal treatment duration. Because the normalization of ALT/AST is important, even in patients who fail to clear HCV infection by these therapeutic regimens, treatment is better conducted for maintaining normal ALT/AST levels. Guidelines for maintaining liver function for preventing the development of HCC include liver supportive therapy with glycyrrhizin[5] and UDCA,[6] either alone or in combination. For treatment toward suppressing the

development of HCC, branched chain amino acids (BCAA)[11] or phlebotomy are adopted. Also, nutrient supplements are applied for stabilizing liver function.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CIRRHOSIS DUE TO HCV

THE FOLLOWING ITEMS have been appended to supplement guidelines for the treatment of type C cirrhosis (Table 6).

Table 5 Guidelines for treatment of type C cirrhosis

Principles
  Compensated: termination of HCV infection
  Decompensated: reversal to compensation and prevention of HCC
Methods
  (1) Eradication of HCV and normalization of ALT/AST (for patients with compensated cirrhosis).
    a) HCV-1b in HVL (≥ 5 log IU/mL)
      IFN-α (Sumiferon)
    b) Others
      IFN-α (Sumiferon)
      IFN-β (Feron)
  (2) Maintenance of liver function (improvement of ALT/AST and albumin) for preventing HCC.
    a) Liver supportive therapy
      Stronger neo-minophagen C (SNMC), ursodeoxycholic acid (UDCA), etc.
    b) Branched chain amino acids (BCAA [Livact])
    c) Phlebotomy
  (3) Supplementation with nutrients (for stabilizing liver function in decompensated cirrhosis).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HVL, high viral loads; IFN, interferon.

Table 6 Supplements to guidelines for type C cirrhosis

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1 To start with, IFN for compensated cirrhosis is desired at 6 MIU daily for 2–8 weeks, as far as possible, and to continue for 48 weeks or longer, as for chronic hepatitis C.

2 In patients with compensated cirrhosis who fail to clear HCV RNA within 12 weeks on IFN, long-term therapy at 3 MIU should be considered for preventing HCC.

3 In patients with platelet counts $<50 \times 10^3/mm^3$, splenectomy or embolization of splenic artery is recommended before re-treatment, and after thorough evaluation has been made on the response to IFN to be expected.

4 For the prevention of HCC, not only IFN, but also liver supportive therapy (SNMC, UDCA, etc.), phlebotomy and branched chain amino acids, either alone or in combination, are recommended for improving ALT/AST and AFP levels.

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MIU, million international units; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

1 For treatment of type C cirrhosis with IFN, the initial dose of 6 million international units (MIU) daily is continued as long as possible (2–8 weeks). Thereafter, long-term IFN for 48 weeks or longer is desired as in the treatment of chronic hepatitis C.

2 In the treatment of type C cirrhosis, patients who fail to achieve EVR with the clearance of HCV RNA from serum within 12 weeks should receive long-term IFN at a dose of 3 MIU.

3 For patients with type C cirrhosis who have platelet counts of less than $50 \times 10^3/mm^3$, splenectomy or embolization of the splenic artery is desirable before commencing IFN therapy, after the efficacy of IFN has been evaluated thoroughly.[12]

4 For preventing the development of HCC, improvement in ALT, AST and AFP levels are aimed. Toward this end, not only IFN, but also liver supportive therapy (SNMC and UDCA), phlebotomy and BCAA are used, either alone or in combination.

DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HCV ranging from chronic hepatitis to cirrhosis of various severities for the fiscal year 2008. The principal aim of these guidelines is to decrease the incidence of HCC due to HCV infection in Japan. In accord with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic hepatitis C. They are prepared on evidence-based data that have been accumulated by members and cooperators of the study group. It is necessary to improve these guidelines in the next fiscal year and thereafter, in accordance with many pieces of new evidence that are expected to emerge through enduring efforts of members and cooperators of the study group.

In the treatment of chronic hepatitis C, the duration of antiviral treatments is extended to 72 weeks, which has been approved as of the fiscal year 2008, and criteria for the eligibility of extended treatment duration are clearly defined. Long-term antiviral treatments, extended up to 72 weeks, are hoped to increase the SVR even further. In addition, comprehensive guidelines for the treatment of cirrhosis have been improved with substantial additions, and their criteria for the indication made explicit.

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has drafted, and also displayed online (www.jsh.or.jp/medical/index.html [in Japanese]), guidelines for a spectrum of liver diseases due to HCV, from chronic hepatitis to cirrhosis of various severities. In view of the eventual goal of decreasing the incidence of HCC due to HCV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been constructed for the treatment of cirrhosis due to HCV infection. As a general rule, antiviral treatments constitute the main body of guidelines for the treatment of chronic hepatitis C. Furthermore, the fundamental concept of these guidelines would need to be kept in mind always. It is our sincere hope that, for the treatment of each patient, readers will base their clinical practice on these guidelines, and refer to appropriate individual guidelines, when they make a decision on the treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in treatment choice.

It is our sincere desire that treatment of patients with chronic hepatitis and cirrhosis due to HCV will proceed following these guidelines. Efforts along these lines will rectify a wide gap in medical treatment served to the nation and raise substantial and efficient interest in the medical economy on the national basis. In practicing treatment according to these guidelines, it will be nec-