Table 1 Laboratory data on admission

Hematology	
WBC	5300/μl
RBC	477 × 10^4/μl
Hb	14.7 g/dl
Ht	44.4%
PLT	24.6 × 10^4/μl
Biochemistry	
T-Bil	0.7 mg/dl
AST	84 IU/l
ALT	123 IU/l
Alb	3.4 g/dl
LDH	225 IU/l
GGT	27 IU/l
ALP	2910 IU/l
ALP isozyme	
1	0
2	16%
3	84%
4	0
5	0
BUN	15 mg/dl
Cr	0.6 mg/dl
Na	139 mEq/l
K	4.0 mEq/l
Cl	103 mEq/1
CRP	0.2 mg/dl
Coagulation	
PT	100%
Virus marker	
HBs-Ag	(−)
HCV-Ab	(+)
HCV genotype	2a
Quantitative HCV RNA	2900 KIU/ml

		Normal range
Bone and mineral metabolism		
Ca	78.8 mg/dl	8.7–10.3
IP	4.1 mg/dl	2.5–4.5
Bone-specific ALP	653 U/l	13.0–33.9
Osteocalcin	90 ng/ml	2.5–13.0
Vitamin D3	113 kIU	20.0–60.0
TSH	1.052 μl U/ml	0.350–4.94
F-T4	1.08 ng/dl	0.70–1.48
Intact PTH	224 pg/ml	10–65
Urine		
DPD	582.0 nmol/nmol Cr	2.8–7.6
NTX	7540 nmolBCE/l	9.3–54.3

PTH parathyoid hormone, *DPD* deoxypridinolin, *NTX* crosslinked N-telopeptide of type I collagen

considered that these findings pointed to secondary hyperparathyroidism apparently resulting from skeletal hyperaccretion of calcium.

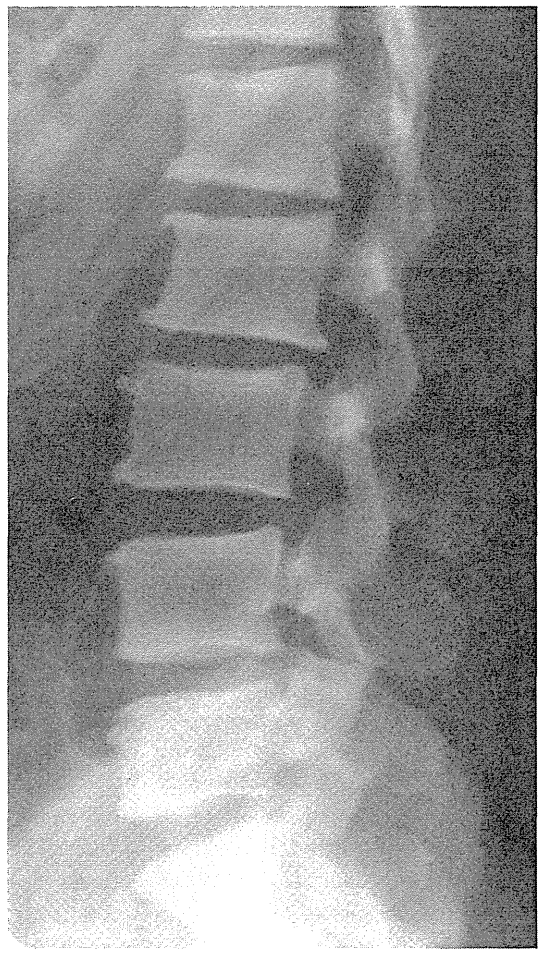

Fig. 1 Lateral view of lumbar spine shows generalized osteosclerosis of cancellous bone

Treatment of HCAO with vitamin D, calcitonin, pamidronate and etidronate that inhibits osteoclast function may decrease serum ALP activity (Table 3). However, subjective response to pharmacological treatment appears to vary. In our case, the administration of peginterferon alfa-2b and ribavirin obtained a sustained virological response and the severe bone pain improved. Approximately 55% of patients with chronic hepatitis C obtain a sustained virological response after treatment with peginterferon alfa and ribavirin [18, 19]. However, those with genotype 1 infection have a sustained virological response rate of 40–55 versus 70–90% among those with genotype 2 or 3 infection. Because our patient was infected with HCV genotype 2a, 24-week treatment of peginterferon alfa-2b and ribavirin induced a sustained viral clearance in our patient. Both interferon alfa and beta suppress bone resorption in osteoclasts and increase bone mineral density [20, 21]. Patients with HCAO who are treated with interferon experience greater bone formation. In our patient, it was thought that the function of osteoclasts was suppressed by the interferon treatment, resulting in increased bone

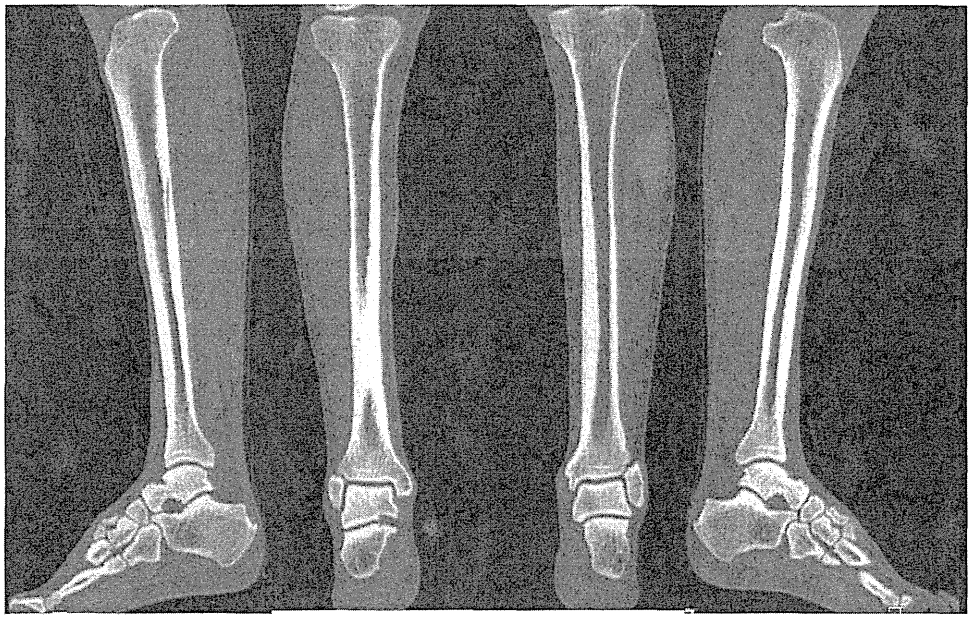

Fig. 2 Helical CT imaging shows osteosclerosis with marked cortical thickening along the diaphyses of the bilateral shank

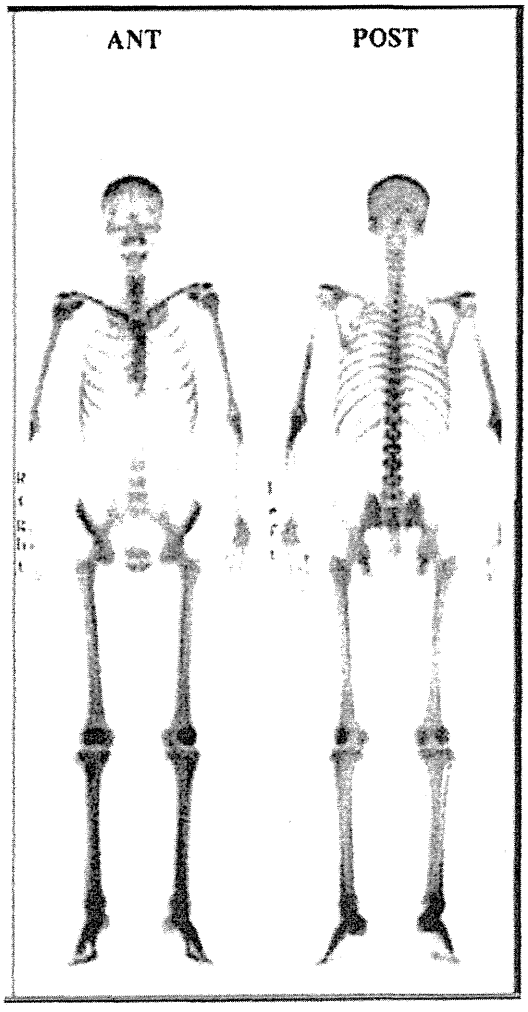

Fig. 3 A 99mTc-methylene diphosphonate bone scan demonstrates diffusely increased radionuclide activity in the spine and long trunk bones. This is consistent with markedly increased bone turnover

formation and worsening hypocalcemia. The patient was given calcium orally for hypocalcemia. However, the severity of the bone pain was non-progressive during interferon treatment. After the end of treatment with peginterferon alfa-2b and ribavirin, hypocalcemia was improved and bone pain was further improved. Therefore, when physicians treat HCAO patient with interferon, they must monitor the level of serum calcium and administer the calcium to correct hypocalcemia. However, this case report may suggest that interferon treatment is safe for the patient with HCAO because there was no osteocopic exacerbation.

The disorder is almost certainly related to HCV; however, the pathogenesis remains incompletely understood. Fiore et al. [13] documented an increase of circulating osteoprotegerin (OPG) in a patient with HCAO, and a concentration of circulating receptor activator for nuclear factor-kappa-B ligand (RANKL) below the lower limit of the reference range. OPG is an inhibitor of osteoclast differentiation and RANKL is an osteoclast differentiation factor [22, 23]. OPG is produced mostly by osteoblasts, so if there is an increased osteoblast function in HCAO, one would expect higher OPG levels in HCAO than in healthy individuals. OPG acts as a decoy receptor for RANKL and prevents its interaction with receptor activator for nuclear factor-kappa-B (RANK), a cell surface receptor on pre-osteoclasts and osteoclasts [24, 25]. Fiore et al. suggested that the abnormalities of the OPG/RANKL system might contribute to the maintenance of the positive balance of bone remodeling that characterizes patients with HCAO. Tanaka et al. [14] hypothesized three explanations for the pathogenesis of HCAO: HCV directly infects bone cells, another unknown-infective agent, and cytokines or growth factors that hepatocytes or other tissues may produce. Kaji

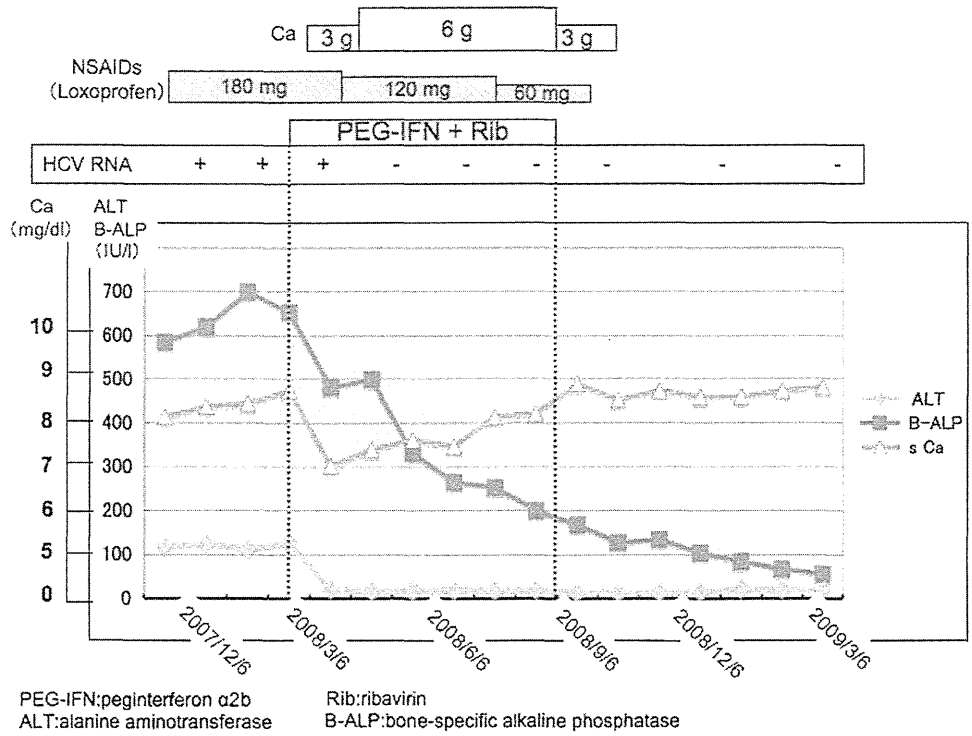

Fig. 4 Clinical course of the patient

Table 2 Change in Laboratory data

	Before	After	Normal range
Biochemistry			
AST (IU/l)	84	34	6–3
ALT (IU/l)	123	23	6–30
ALP (IU/l)	2,910	454	115–359
Ca (mg/dl)	7.8	9.1	8.7–10.3
IP (mg/dl)	4.1	4.5	2.5–4.5
Virus marker			
HCV RNA (KIU/ml)	2,900	Negative	
Bone and mineral metabolism			
Bone-specific ALP (U/l)	653	55.3	13.0–33.9
Osteocalcin (ng/ml)	90	20	2.5–13.0
Vitamin D3 (KIU)	113	62.4	20.0–60.0
Intact PTH (pg/ml)	224	119	10–65
Urine DPD (nmol/nmol Cr)	582.0	197.8	2.8–7.6

ALP alkaline phosphatase, *PTH* parathyroid hormone, *DPD* deoxypyridinolin

et al. [26] reported that serum soluble factors in patients with HCAO induce cell proliferation, ALP activity and transforming growth factor-beta signal in mouse osteoblastic cells. Khosla et al. [27] have also previously found that the insulin-like growth factor (IGF)-II E in HCAO patients circulates bound to insulin-like growth factor binding protein (IGFBP)-2. Moreover, they indicated that upon binding IGF- II, IGFBP-2 has greatly enhanced avidity for the osteoblast extracellular matrix. Thus, they have postulated that the IGF-II E/IGFBP-2 complex accumulates in bone in HCAO patients, and subsequently results in the stimulation of bone formation and osteosclerosis observed in these patients. Conover et al. [28] reported that in a rat model of osteoporosis, subcutaneous administration of IGF-II/IGFBP-2 complex stimulates bone formation and prevents loss of bone mineral density.

Because we did not investigate IGF-II, IGFBP-2, OPG and RANKL in our patient, we could not ascertain whether the levels of cytokines and growth factors were altered by the combination therapy with peginterferon alfa-2b and ribavirin. Previous reports have identified that these agents may play a role in the pathogenesis of HCAO. Also it was not clarified whether peginterferon alfa-2b and ribavirin affect cytokines and growth factors. However, we considered that cytokines and/or growth factors produced by hepatocytes or other tissues induced HCAO, and that in the HCV clearance state induced by peginterferon alfa-2b and ribavirin, these agents improved to normal levels. Therapy with drugs that inhibit osteoclast function may decrease serum ALP activity, but subjective response varies. There have been reports of patients benefiting from calcitonin injections or pamidronate infusions, whereas other patients have not improved (Table 3). We considered that HCV clearance by treatment with peginterferon alfa-2b and ribavirin was the best therapy for patients with HCAO. Because the HCV genotype in our patient was 2a, we treated our patient with peginterferon alfa-2b and ribavirin. HCV genotype has been shown to be the best parameter on which to base the individualization of therapy. In patients

Table 3 Review of previous case reports

Case	Age	Gender	ALP	OC	s-Ca	IPTH	Vit.D	HCAO therapy	Efficacy	HCV genotype	HCV therapy	References
1	28	F	↑			↑	↑	Non therapy	Improve			[3]
2	27	F	↑	↑	↓	→	↑	Calcitonin	Improve			[4]
3	38	M	↑		↓	→	↑	Calcitonin	Improve			[4]
4	38	M										[5]
5	52	M										[6]
6	37	M	↑	↑	↓	↑	↑	Pamidronate	Improve			[7]
7	37	M	↑	↑	→	→	→	Vit. D, pamidronatee, calcitonin	Progress			[8]
8	73	M	↑	↑	→	↑	→	Pamidronate, etidronate	Improve			[9]
9	38	M	↑	↑	→	→	→	Pamidronate	Improve			[9]
10	69	F	↑	↑	→	↑	↑					[10]
11	45	F	↑	→	→	→				2a		[11]
12	74	F										[12]
13	65	F	↑	↑	→	↑				1b		[13]
14	72	F	↑	↑	→	↑	↑	Vit. D, Ca	Improve	2		[14]
15	28	M	↑		→	→		Pamidronate	Progress			[15]
16	42	M	↑	↑	↓	↑	↑	PEG-IFN + RBV	Improve	2a	PEG-IFN + RBV	※

Ca calcium, *OC* osteocalcine, *IPTH* intact PTH, *Vit.D* 1,25(OH)2Vit.D3, *PEG-IFN* peginterferon alfa 2b, *RBV* ribavirin, ※ present case

with genotype 2, sustained virological response rates of 70–90% were obtained with 24 weeks of treatment [18, 19]. Unfortunately, interferon treatment is often associated with adverse effects such as depression and hematological abnormalities. Recent reports indicate one host-related factor could be genetic variations near the IL28B gene (rs8099917, rs12979860) on chromosome 19, which encodes interferon-λ-3; these variations are pretreatment predictors of virological response to therapy with peginterferon and ribavirin [29–31]. Physicians should consider HCV-genotype, viral load, age, complications and IL28B SNPs before starting treatment, and identify the patients with HCAO who are unlikely to benefit from this treatment.

It is possible that the actual prevalence of HCAO among HCV patients may be underestimated. We propose that physicians caring for patients with HCV who have invariably high levels of ALP activity should check the bone mineral density of their patients.

In conclusion, HCAO is improved by the combination therapy of peginterferon alfa-2b and ribavirin when the patients achieved sustained virological response. It was confirmed that HCAO was one of the extrahepatic manifestations of HCV.

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Research Article

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Indications and limitations for aged patients with chronic hepatitis C in pegylated interferon alfa-2b plus ribavirin combination therapy

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Background & Aims: This study investigated the efficacy and adverse effects of pegylated interferon (Peg-IFN) plus ribavirin therapy in aged patients with chronic hepatitis C (CH-C).

Methods: A total of 1040 naïve patients with CH-C (genotype 1, $n = 759$; genotype 2, $n = 281$), of whom 240 (23%) over 65 years old (y.o.), were treated with Peg-IFN alfa-2b plus ribavirin and assessed after being classified into five categories, according to age.

Results: The discontinuance rate was higher for patients over 70 y.o. (36%), the most common reason being anemia. In the presence of genotype 1, the SVR rate was similar (42–46%) among patients under 65 y.o. and declined (26–29%) among patients over 65 y.o. For patients over 65 y.o., being male (Odds ratio, OR, 3.5, $p = 0.035$) and EVR (OR, 83.3, $p < 0.001$) were significant factors for SVR, in multivariate analysis. The Peg-IFN dose was related to EVR, and when EVR was attained, 76–86% of patients over 65 y.o. achieved SVR. SVR was not achieved (0/35, 0/38, respectively) if a 1-log decrease and a 2-log decrease were not attained at week 4 and week 8, respectively. In the presence of genotype 2, the SVR rate was similar (70–71%) among patients under 70 y.o. and declined among patients over 70 y.o. (43%).

Conclusions: Aged patients up to 65 y.o. with genotype 1 and 70 y.o. with genotype 2 can be candidates for Peg-IFN plus ribavirin therapy. The response-guided therapy can be applied for aged patients with genotype 1.

Keywords: Pegylated interferon plus ribavirin therapy; Chronic hepatitis C; Aged patients.

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Abbreviations: HCV, hepatitis C virus; CH-C, chronic hepatitis C; HCC, hepatocellular carcinoma; Peg-IFN, pegylated interferon; SVR, sustained virologic response; RVR, rapid virologic response; EVR, early virologic response; LVR, late virologic response; NR, non-response; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Plt, platelet; G-CSF, granulocyte–macrophage colony stimulating factor.

Introduction

Pegylated interferon (Peg-IFN) plus ribavirin combination therapy has led to a marked progress in the treatment of chronic hepatitis C (CH-C) [1–4]. However, in aged patients, problems remain with respect to its anti-viral effect and tolerability [5–9]. Recently, the addition of a protease inhibitor to Peg-IFN plus ribavirin combination therapy has been reported, on the one hand, to improve the anti-viral effect, and, on the other hand, to increase side effects, especially severe anemia [10–11].

Therefore, this new therapy does not solve the problems encountered when treating aged patients.

With aging, the progression of liver fibrosis and the occurrence of hepatocellular carcinoma (HCC) have been shown to be accelerated, especially in patients over 60 y.o. [12–14]. In general, the anti-viral therapy can lead to an improvement in liver fibrosis and thus diminish the risk of HCC and ameliorate the prognosis in patients with CH-C [15–21]. Among aged patients, those results are mainly achievable upon eradication of the hepatitis C virus (HCV) [18,21]. Accordingly, the first goal of treatment of aged patients with a high-risk of HCC should be HCV elimination.

Thus, a treatment strategy, aiming at the improvement of the anti-viral efficacy in aged patients, should be established based on detailed large-scale studies.

Some points need to be further elucidated when using the Peg-IFN plus ribavirin combination therapy for the treatment of aged patients with CH-C: (i) the characteristics before treatment

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that would lead to the successful elimination of HCV, (ii) the prediction factors of treatment efficacy after the initiation of the therapy, and (iii) the utility of a response-guided therapy established in the treatment.

In the present study, using a large cohort, we aimed at clarifying these points taking into account the patients' age.

Patients and methods

Patients

This study was a retrospective, multicenter trial conducted by the Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 1040 naïve patients with CH-C were enrolled between December 2004 and June 2007. All patients were Japanese, infected with a viral load of more than 10^5 IU/ml, and treated with a combination of Peg-IFN alfa-2b plus ribavirin. Patients were excluded from the study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis), co-infection with hepatitis B or anti-human immunodeficiency virus. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

Treatment

All patients received Peg-IFN alfa-2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL; Schering-Plough). Treatment duration was 48 weeks for patients with genotype 1 and 24 weeks for those with genotype 2. As a starting dose, Peg-IFN alfa-2b was given once weekly, at a dosage of 1.5 μg/kg, and ribavirin was given at a total dose of 600–1000 mg/day based on body weight (body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg), according to a standard treatment protocol for Japanese patients.

Dose reduction and discontinuance

Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematologic adverse effects. The Peg-IFN alfa-2b dose was reduced to 50% of the assigned dose when the white blood cell (WBC) count was below 1500/mm^3, the neutrophil count below 750/mm^3 or the platelet (Plt) count below 8×10^4/mm^3, and was discontinued when the WBC count was below 1000/mm^3, the neutrophil count below 500/mm^3 or the Plt count below 5×10^4/mm^3. Ribavirin was also reduced from 1000 to 600 mg, 800 to 600 mg, or 600 to 400 mg when the hemoglobin (Hb) was below 10 g/dl, and was discontinued when the Hb was below 8.5 g/dl. Peg-IFN alfa-2b and ribavirin had to be both discontinued if there was a need to discontinue either of them. No ferric medicine or hematopoietic growth factors, such as epoetin alpha, or granulocyte–macrophage colony stimulating factor (G-CSF), were administered.

Virologic assessment and definition of virologic response

Serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 KIU/ml; Roche Diagnostics, Branchburg, NJ) and qualitatively analyzed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/ml; Roche Diagnostics). The rapid virologic response (RVR) was defined as undetectable serum HCV RNA at week 4; the early virologic response (EVR) as undetectable serum HCV RNA at week 12; and the late virologic response (LVR) as detectable serum HCV RNA at week 12 and undetectable serum HCV RNA at week 24. Moreover, the sustained virologic response (SVR) was defined as undetectable serum HCV RNA, 24 weeks after treatment.

According to the protocol, genotype 1 patients, with less than a 2-log decrease in HCV RNA level at week 12 compared to the baseline, or with detectable serum HCV RNA at week 24, had to stop the treatment and were regarded as non-response (NR). Treatment discontinuance was evaluated except for those patients who had discontinued the treatment at up to 24 weeks, due to absence of response. Anti-viral efficacy was evaluated, for all study patients, using the intention-to-treat analysis (ITT analysis) and the per protocol analysis (PP analysis) for patients without treatment discontinuation due to side effects, and was assessed considering the definition of EVR or LVR for genotype 1, and RVR or non-RVR for genotype 2, as previously reported [1].

Assessment of drug exposure

The amounts of Peg-IFN alfa-2b and ribavirin, taken by each patient during the full treatment period, were evaluated by reviewing the medical records. The mean doses of Peg-IFN alfa-2b and ribavirin were calculated individually as averages, on the basis of the body weight at baseline: Peg-IFN alfa-2b expressed as μg/kg/week, ribavirin expressed as mg/kg/day.

Statistical analysis

Patients' baseline data are expressed as means ± SD or median values. To analyze the difference between baseline data, ANOVA or Mantel–Haenszel Chi-square test were performed. Factors associated with the viral response were assessed by univariate analysis using the Mann–Whitney *U* test or Chi-square test and multivariate analysis using logistic regression analysis. A two-tailed p value <0.05 was considered significant. The analysis was conducted with SPSS version 15.0J (SPSS Inc., Chicago, IL).

Results

Patient's profile

Baseline characteristics of the patients categorized by age are shown in Table 1.

Genotype 1 patients ($n = 759$) were distributed into five categories: 266 patients were under 55 y.o. (group 1A), 159 were 55–59 y.o. (group 1B), 149 were 60–64 y.o. (group 1C), 134 were 65–69 y.o. (group 1D), and 51 were 70 y.o. or older (group 1E). With advancing age, the male-to-female ratio and peripheral blood cell count (WBC, neutrophil count, Red blood cell (RBC), Hb, Plt) decreased significantly. Patients with a progression of liver fibrosis (METAVIR fibrosis score 3 or 4) significantly increased with age (Table 1A).

Genotype 2 patients ($n = 281$) were also distributed into five categories: 145 patients were under 55 y.o. (group 2A), 43 were 55–59 y.o. (group 2B), 38 were 60–64 y.o. (group 2C), 41 were 65–69 y.o. (group 2D), and 14 were 70 y.o. or older (group 2E). As observed in genotype 1 patients, the peripheral blood cell count decreased and the ratio of advanced fibrosis (score 3–4) increased significantly with age (Table 1B). For both genotypes, the initial doses of Peg-IFN in patients over 70 y.o. were lower than in those under 70 y.o., this was not the case for the ribavirin doses.

Dose reduction and discontinuance for adverse event

The overall discontinuance rate of treatment was 15% (140/919); 18% (112/639) for genotype 1 and 10% (28/280) for genotype 2, respectively. Table 2 shows the reason for and the rate of treatment discontinuance according to age. The discontinuance rate increased with age, being 10% (36/363) for patients under 55 y.o., 15% (27/182) for patients with 55–59 y.o., 17% (28/169) for patients with 60–64 y.o., 19% (28/147) for patients with 65–70 y.o., and significantly higher, 36%, (21/58) for patients over 70 y.o. The discontinuance of treatment due to hemolytic anemia was significantly higher for patients over 70 y.o. as compared to those under 70 y.o. (<70 y.o., 1% (9/861) vs. ⩾70 y.o., 16% (9/58), p <0.0001).

The rate without dose reduction of both drugs decreased with age (<55 y.o., 41% (171/411); 55–59 y.o., 20% (40/202); 60–64 y.o., 26% (48/187); 65–69 y.o., 23% (41/175); ⩾70 y.o., 18% (12/65)). In the presence of genotype 1, the mean dose of Peg-IFN

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Table 1. Baseline characteristics of patients.

Patients with genotype 1							
Factor		<55 y.o.	55 - 59 y.o.	60 - 64 y.o.	65 - 69 y.o.	≥70 y.o.	*p* value
Number		266	159	149	134	51	
Age (y.o.)		44.4 ± 8.1	56.9 ± 1.4	62.0 ± 1.4	66.8 ± 1.4	71.4 ± 1.7	<0.001
Sex: male / female		160 / 106	64 / 95	57 / 92	54 / 80	23 / 28	<0.001
Body weight (kg)		64.6 ± 11.7	58.3 ± 9.4	58.1 ± 9.6	56.3 ± 9.3	56.3 ± 9.2	<0.001
White blood cells (/mm³)		5608 ± 1668	4901 ± 1664	4888 ± 1488	5113 ± 1426	4883 ± 1511	<0.001
Neutrophils (/mm³)		2923 ± 1214	2425 ± 1031	2559 ± 1155	2535 ± 1017	2599 ± 1149	<0.001
Red blood cells (×10⁴/mm³)		454 ± 47	432 ± 38	427 ± 40	424 ± 37	424 ± 46	<0.001
Hemoglobin (g/dl)		14.4 ± 1.5	13.8 ± 1.2	13.7 ± 1.3	13.6 ± 1.2	13.7 ± 1.4	<0.001
Platelets (×10⁴/mm³)		18.6 ± 6.2	16.3 ± 5.7	15.4 ± 5.3	15.1 ± 5.0	14.4 ± 4.2	<0.001
AST (IU/L)		62 ± 50	62 ± 45	64 ± 46	72 ± 45	64 ± 40	0.295
ALT (IU/L)		79 ± 68	76 ± 64	73 ± 63	77 ± 58	65 ± 41	0.657
Serum HCV RNA (KIU/ml)*		1800	1600	1700	1700	1700	0.691
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	177 / 19	99 / 20	90 / 19	76 / 28	21 / 9	0.001
	Activity, 0 - 1 / 2 - 3	117 / 79	63 / 56	59 / 50	47 / 57	13 / 16	0.146
Peg-IFN dose (μg/kg/week)¶		1.47 ± 0.14	1.47 ± 0.16	1.46 ± 0.18	1.44 ± 0.18	1.36 ± 0.24	<0.001
Ribavirin dose (mg/kg/day)¶		11.5 ± 1.1	11.5 ± 1.4	11.5 ± 1.4	11.5 ± 1.7	11.2 ± 2.2	0.65

Patients with genotype 2							
Factor		<55 y.o.	55 - 59 y.o.	60 - 64 y.o.	65 - 69 y.o.	≥70 y.o.	*p* value
Number		145	43	38	41	14	
Age (y.o.)		40.9 ± 8.9	56.7 ± 1.3	62.3 ± 1.4	66.7 ± 1.5	71.8 ± 1.8	<0.001
Sex: male / female		78 / 67	17 / 26	17 / 21	18 / 23	6 / 8	0.441
Body weight (kg)		63.4 ± 12.0	59.5 ± 11.5	58.6 ± 11.7	58.5 ± 9.8	55.9 ± 6.8	0.783
White blood cells (/mm³)		6011 ± 1965	4874 ± 1346	4982 ± 1210	5079 ± 1877	4414 ± 871	<0.001
Neutrophils (/mm³)		3214 ± 1511	2468 ± 971	2576 ± 950	2492 ± 1119	2521 ± 683	0.001
Red blood cells (×10⁴/mm³)		454 ± 48	430 ± 42	432 ± 50	430 ± 43	408 ± 48	<0.001
Hemoglobin (g/dl)		14.3 ± 1.6	13.5 ± 1.3	13.9 ± 1.4	13.9 ± 1.3	13.3 ± 1.2	0.001
Platelets (×10⁴/mm³)		21.3 ± 5.4	18.3 ± 6.1	17.0 ± 5.2	15.8 ± 5.4	13.9 ± 4.7	<0.001
AST (IU/L)		53 ± 59	57 ± 45	55 ± 38	83 ± 48	68 ± 29	0.029
ALT (IU/L)		65 ± 59	73 ± 70	68 ± 62	105 ± 62	78 ± 43	0.008
Serum HCV RNA (KIU/ml)*		1700	1100	900	1100	500	0.008
Histology (METAVIR)‡	Fibrosis, 0 - 2 / 3 - 4	102 / 0	25 / 3	29 / 2	21 / 9	7 / 1	<0.001
	Activity, 0 - 1 / 2 - 3	68 / 34	18 / 10	18 / 13	9 / 21	5 / 3	0.01
Peg-IFN dose (μg/kg/week)¶		1.48 ± 0.16	1.48 ± 0.14	1.45 ± 0.18	1.46 ± 0.15	1.28 ± 0.26	0.001
Ribavirin dose (mg/kg/day)¶		11.5 ± 1.1	11.4 ± 1.2	11.5 ± 1.4	11.3 ± 1.6	11.0 ± 1.4	0.55

*, Data shown are median values.
†, 201 Missing.
‡, 82 Missing.
¶, Initial doses.

during the whole treatment period was lower (1.1 ± 0.3 μg/kg/week) for patients over 70 y.o. than for those under 70 y.o. (1.3 ± 0.3 μg/kg/week) and that of ribavirin decreased with age (<55 y.o., 10.3 ± 1.9 mg/kg/day; 55–59 y.o., 9.8 ± 1.9 mg/kg/day; 60–64 y.o., 9.3 ± 2.3 mg/kg/day; 65–69 y.o., 9.2 ± 2.3 mg/kg/day; ⩾70 y.o., 8.5 ± 2.5 mg/kg/day). The same tendency was observed with genotype 2.

Sustained virologic response

In genotype 1 patients, the overall SVR rate was 40% (305/759), being 46% (123/266) for group 1A, 44% (70/159) for group 1B, 42% (62/149) for group 1C, 26% (35/134) for group 1D, and 29% (15/51) for group 1E, following ITT analysis. The same tendency was observed using the PP analysis ($n = 647$). The SVR rates for patients over 65 y.o. were significantly lower than those for patients under 65 y.o. (ITT analysis: ⩾65 y.o., 27% vs. <65 y.o., 44%, $p < 0.0001$; PP analysis: ⩾65 y.o., 31% vs. <65 y.o., 50%, $p < 0.0001$) (Fig. 1A). Among genotype 1 patients over 65 y.o., the SVR rate was significantly lower for female patients than for male patients (ITT analysis: male, 40% (31/77) vs. female, 18% (19/108), $p < 0.001$; PP analysis: male, 49% (27/55) vs. female, 20% (18/90), $p < 0.001$).

Moreover, for genotype 2 patients, the overall SVR rate was 78% (220/281), being 88% (128/145) for group 2A, 70% (30/43) for group 2B, 71% (27/38) for group 2C, 71% (29/41) for group 2D, and 43% (6/14) for group 2E, following ITT analysis. The same tendency was observed with the PP analysis ($n = 253$). The SVR rates for patients over 70 y.o. were significantly lower than those for patients under 70 y.o. (ITT analysis: ⩾70 y.o., 43% vs. <70 y.o., 80%, $p < 0.0001$; PP analysis: ⩾70 y.o., 56% vs. <70 y.o., 85%, $p < 0.05$) (Fig. 1B). Among patients over 70 y.o. with genotype 2, the difference according to gender was not clear because of the small sample.

Table 2. Reasons for treatment discontinuation.

Factor	<55 y.o. (n = 363)	55 - 59 y.o. (n = 182)	60 - 64 y.o. (n = 169)	65 - 69 y.o. (n = 147)	≥70 y.o. (n = 58)	Total (n = 919)
Neutropenia	2	3	0	0	0	5
Thrombopenia	1	0	1	1	0	3
Anemia	0	4	3	2	9	18
Fatigue	1	1	3	3	1	9
Gastrointestinal disorder	2	1	0	0	1	4
Cough, Dyspnea	1	0	3	0	0	4
Vertigo	1	0	0	0	3	4
Psychosis (depression)	7 (3)	7 (3)	4 (4)	3 (3)	2 (2)	23
Rash	5	2	5	7	1	20
Thyroid dysfunction	2	0	2	0	0	4
Fundal hemorrhage	0	2	0	2	0	4
Drug-induced hepatitis	3	1	0	0	0	4
Interstitial pneumonia	0	1	0	1	1	3
Cerebral hemorrhage, infarction	2	0	0	1	0	3
Others	9	5	7	8	3	32
Total	36 (10%)	27 (15%)	28 (17%)	28 (19%)	21 (36%)	140 (15%)

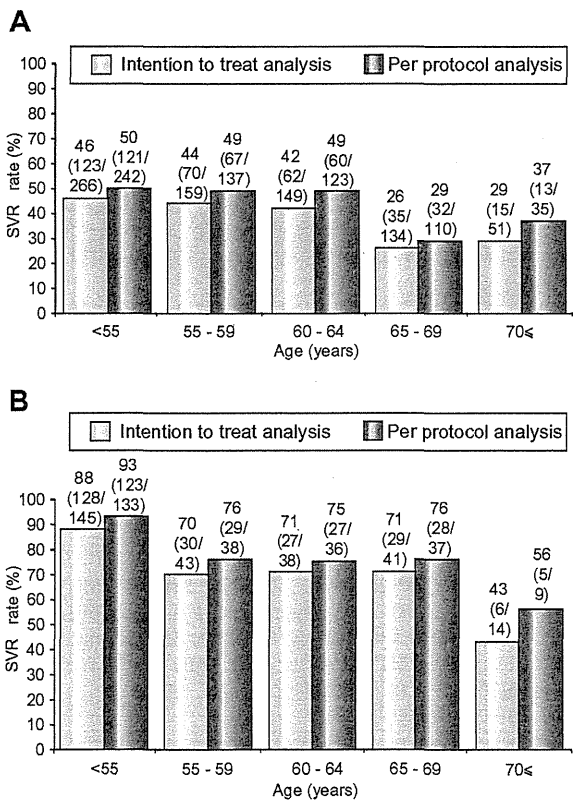

Fig. 1. SVR rate according to age. (A) Genotype 1. (B) Genotype 2.

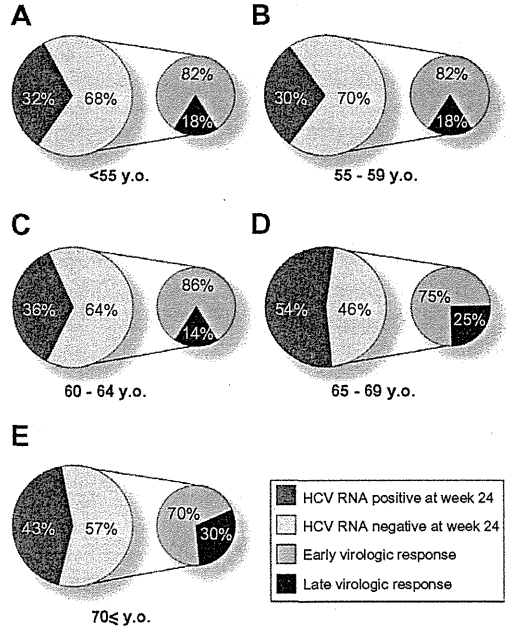

Fig. 2. Antiviral effect during treatment according to age. (A) <55 y.o. (B) 55–59 y.o. (C) 60–64 y.o. (D) 65–69 y.o. (E) ≥70 y.o.

Timing of HCV RNA negativation for genotype 1, according to age

Treatment responses distributing EVR, LVR, and NR according to age are shown in Fig. 2. The rates of NR were similar in patient groups under 65 y.o. (30–36%), but increased in almost half of the patients over 65 y.o. (p <0.0001). Moreover, among the virologic responders, the proportion of LVR tended to increase in patients over 65 y.o. (25–30%) compared to patients under 65 y.o. (14–18%) (p = 0.06).

SVR rate according to the timing of HCV RNA negativation

SVR rates according to EVR or LVR in genotype 1, and RVR or non-RVR in genotype 2 are summarized in Table 3. Genotype 1 patients with EVR achieved high SVR rates regardless of age; in particular, if EVR had been attained, 76% of patients with 65–69

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Table 3. SVR rate according to genotype and viral response in patients responding to PEG-IFN plus ribavirin combination therapy.

Factor	<55 y.o.	55 - 59 y.o.	60 - 64 y.o.	65 - 69 y.o.	≥70 y.o.
Genotype 1					
with EVR, % (n)	85 (114/134)	79 (62/79)	81 (55/68)	76 (29/38)	86 (12/14)
with LVR, % (n)	23 (7/30)	29 (5/17)	46 (5/11)	23 (3/13)	17 (1/6)
Genotype 2					
with RVR, % (n)	93 (57/61)	82 (14/17)	85 (17/20)	92 (11/12)	100 (4/4)
without RVR*, % (n)	96 (22/23)	60 (6/10)	57 (4/7)	50 (4/8)	0 (0/3)

RVR, rapid virologic response.
EVR, early virologic response.
LVR, late virologic response.
*, Serum HCV RNA was detectable at week 4, but undetectable at week 24.

Table 4. Multivariate analysis for the factors associated with SVR among all patients.

Factor	Category	Odds ratio	95% CI	*p*
Age (y.o.)	<65 / ≥65	0.485	0.295 - 0.799	0.005
Sex	male / female	0.524	0.353 - 0.777	0.001
Platelets ($\times 10^4$/mm^3)	<12 / ≥12	1.780	1.039 - 3.049	0.040
Serum HCV RNA (KIU/ml)	<2000 / ≥2000	0.599	0.401 - 0.896	0.010
Histology (METAVIR): Fibrosis	0 - 2 / 3 - 4	0.599	0.333 - 1.076	0.090

y.o. and 86% of patients over 70 y.o. achieved SVR, and these SVR rates compared favorably with those of younger patients. On the other hand, the SVR rates for patients with LVR ranged from 17% to 46%, which were lower than those for EVR patients in each age group, and no significant differences of SVR rates were found among LVR patients by age.

With genotype 2, patients with RVR achieved high SVR rates ranging from 82% to 100% regardless of age. Even for patients without RVR, 96% of those under 55 y.o. attained SVR, a rate that was significantly higher than that for patients over 55 y.o. (50%, 14/28) (p <0.001).

Factors associated with SVR for genotype 1

The factors associated with SVR were assessed for the variables shown in Table 1. The factors selected as significant by the univariate analysis: age, gender, WBC, neutrophils, RBC, Hb, Plt, aspartate aminotransferase, serum HCV RNA level, the degree of liver fibrosis, and the initial dose of Peg-IFN, were evaluated by multivariate logistic regression analysis. The factor of age over 65 y.o. was the independent factor for SVR (p = 0.005), apart from the gender (p = 0.001), Plt value (p <0.05), and serum HCV RNA level (p = 0.01) (Table 4).

Factors associated with EVR and SVR for patients over 65 y.o. with genotype 1

The results of univariate analysis for EVR among patients over 65 y.o. are shown in Table 5A. Gender, Plt value, and mean dose of Peg-IFN during the first 12 weeks were factors significantly associated with EVR. In multivariate analysis, the mean dose of Peg-IFN during the first 12 weeks was the independent factor for EVR (p = 0.03), apart from gender (p = 0.002) (Table 5B). The EVR rates were 41% (41/101) in patients who received ⩾1.2 μg/kg/week on average during the first 12 weeks, and declined to 36% (8/22) in patients given 0.9–1.2 μg/kg/week of Peg-IFN, and to 14% (3/22) in patients administered with <0.9 μg/kg/week of Peg-IFN.

The baseline and on-treatment factors, which are correlated with the SVR among the patients over 65 y.o., were assessed by univariate and multivariate analyses. Univariate analysis showed that factors significantly associated with SVR were gender and virologic response (Table 6A), and they were also selected as significant independent factors in multivariate analysis (p = 0.035, p <0.001) (Table 6B).

Negative prediction of SVR for patients over 65 y.o. with genotype 1

We tried positive and negative predictions of SVR for aged patients, focusing on the decrease of HCV RNA at treatment week 4 and 8. The SVR rate was 47% (29/62) for patients with more than a 1-log decrease in HCV RNA level at week 4, while no patients with less than a 1-log decrease at week 4 attained SVR (0/35) (p <0.0001). Similarly, 55% (35/64) of patients with more than a 2-log decrease at week 8 attained SVR, whereas no patients with less than a 2-log decrease at week 8 attained SVR (0/38) (p <0.0001).

Discussion

Peg-IFN plus ribavirin combination therapy can improve anti-viral efficacy and is presently recommended as first-line therapy [1–4]. However, with respect to aged patients with CH-C, there have been only a few small-scale cohort studies which reported poor anti-viral effect and poor tolerability in comparison with non-aged patients [5–9]. The problem in the treatment of aged patients with CH-C is most serious in Japan, because HCV carriers in Japan are 10–20 years older than those in the United States and European countries [22]. Therefore, in the present study, we examined the efficacy and prevalence of side effects with a focus on patient's age using a large-scale cohort.

Table 5. Factors associated with EVR among patients over 65 y.o.

Univariate analysis				
Factor		EVR	Non-EVR	*p* value
Number		52	93	
Age (y.o.)		67.9 ± 2.3	67.8 ± 2.5	0.66
Sex: male / female		28 / 24	27 / 66	0.003
White blood cells (/mm^3)		5063 ± 1474	5001 ± 1422	0.76
Neutrophils (/mm^3)		2566 ±1110	2551 ± 1071	0.87
Red blood cells (×10^4/mm^3)		426 ± 36	421 ± 38	0.64
Hemoglobin (g/dl)		13.7 ± 1.2	13.5 ± 1.2	0.21
Platelets (×10^4/mm^3)		16.5 ± 5.5	14.0 ± 4.6	0.009
AST (IU/L)		70 ± 51	70 ± 40	0.49
ALT (IU/L)		76 ± 58	70 ± 41	0.80
Serum HCV RNA (KIU/ml)*		1700	1900	0.62
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	25 / 10	47 / 20	0.54
	Activity, 0 - 1 / 2 - 3	16 / 19	29 / 37	0.52
Peg-IFN dose (μg/kg/week)‡		1.35 ± 0.24	1.25 ± 0.31	0.03
Ribavirin dose (mg/kg/day)‡		10.0 ± 2.2	9.6 ± 2.3	0.40

Multivariate analysis				
Factor	Category	Odds ratio	95% CI	*p* value
Sex	male / female	0.309	0.149 - 0.644	0.002
Platelets (×10^4/mm^3)	<12 / ≥12	-	-	N.S
Peg-IFN dose (μg/kg/week)‡	<1.2 / ≥1.2	2.481	1.079 - 5.705	0.03

*, Data shown are median values.
†, 43 Missing.
‡, Mean doses during 0 to 12 weeks.
N.S., not statistically significant.

Table 6. Factors associated with SVR among patients over 65 y.o.

Univariate analysis				
Factor		SVR	Non-SVR	*p* value
Number		45	100	
Age (y.o.)		68.0 ± 2.4	67.7 ± 2.5	0.45
Sex: male / female		27 / 18	28 / 72	<0.001
White blood cells (/mm^3)		5006 ± 1516	5030 ± 1409	0.81
Neutrophils (/mm^3)		2575 ±1130	2548 ± 1063	0.96
Red blood cells (×10^4/mm^3)		427 ± 40	421 ± 36	0.53
Hemoglobin (g/dl)		13.8 ± 1.3	13.5 ± 1.2	0.14
Platelets (×10^4/mm^3)		16.1 ± 5.6	14.3 ± 4.7	0.09
AST (IU/L)		71 ± 54	69 ± 40	0.47
ALT (IU/L)		76 ± 56	70 ± 43	0.77
Serum HCV RNA (KIU/ml)*		1700	2000	0.51
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	21 / 8	51 / 22	1.00
	Activity, 0 - 1 / 2 - 3	14 / 15	31 / 41	0.66
Peg-IFN dose (μg/kg/week)‡		1.27 ± 0.28	1.23 ± 0.33	0.31
Ribavirin dose (mg/kg/day)‡		8.8 ± 2.1	9.1 ± 2.5	0.38
Virologic response: EVR / non-EVR		41 / 4	11 / 89	<0.001

Multivariate analysis				
Factor	Category	Odds ratio	95% CI	*p* value
Sex	male / female	0.283	0.088 - 0.914	0.035
Virologic response	EVR / non-EVR	0.012	0.004 - 0.043	<0.001

*, Data shown are median values.
†, 43 Missing.
‡, Mean doses during treatment.

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With respect to the side effects and discontinuance rate of treatment in aged patients with CH-C, treated with Peg-IFN plus ribavirin combination therapy, Reddy et al. reported that there was no difference related to the incidence and reason for side effects between non-aged and aged patients [6]. Another paper reported that the incidence of side effects was more frequent in aged patients [5]. In our study, not only the continuance rate without reduction of both drug decreased with age, but also the discontinuance rate of treatment increased with age, with a third of the patients over 70 y.o. discontinuing the treatment. The discrepancy, existing between our results and those reported in the former study cited above, is due to the difference in the number of aged patients enrolled; Reddy' s study analyzed a small cohort including only a few cases of patients over 65 y.o. and classified all those over 50 y.o. as aged patients.

Discontinuance of treatment due to progression of anemia was significantly higher in patients over 70 y.o., accounting for 43% (9/21) of the discontinuance in this group. Although the ratio of advanced fibrosis (score 3–4) increased with age, the high discontinuance rate due to anemia among patients over 70 y.o. was similar regardless of the progression of fibrosis (F0-2: <70 y.o., 1% (6/559) vs. ⩾70 y.o., 21% (6/28), p <0.0001; F3-4: <70 y.o., 0% (0/83) vs. ⩾70 y.o., 22% (2/9), p <0.0001). It is possible that poor hematopoietic function and renal function led to the progression of anemia in aged patients. For patients who develop severe anemia, using epoetin alpha or taribavirin, which are ribavirin prodrugs, has been shown to result in a lower incidence of anemia, although no significant increase of SVR has been reported so far, even with the addition of taribavirin to Peg-IFN [23–24].

With genotype 1 patients, the SVR rates were almost equal up to 65 y.o. (49–50%), but decreased to 31% (45/145) among the patients that were over 65 y.o., and even for those who completed the entire treatment schedule in this study. Since the degree of liver fibrosis and drug exposure have been shown to be associated with anti-viral efficacy, the progression of liver fibrosis or decrease of drug exposure with age could account for the reduction of SVR rate among the aged patients. However, the stratified analysis, according to the progression of liver fibrosis and drug exposure, revealed that older patients still yielded low a SVR rate (F0-2, Peg-IFN during the first 12 weeks ⩾1.2 μg/kg/week:<65 y.o., 55% (143/261) vs. ⩾65 y.o., 33% (15/46), p <0.0001; F0-2, Peg-IFN during the first 12 weeks <1.2 μg/kg/week:<65 y.o., 43% (26/60) vs. ⩾65 y.o., 23% (6/26), p = 0.07), which means that older patients would be difficult to treat. From our results showing a low SVR rate and a high discontinuance rate for patients over 65 y.o., the genotype 1 patients under 65 y.o were those who benefited the most from Peg-IFN plus ribavirin combination therapy. The high prevalence of treatment failure (non-SVR) among the aged patients seems to be due to the high populations of NR and LVR (Fig. 2). A high population of LVR is considered to lead to a higher transient response rate among aged patients, since those over 65 y.o. with LVR showed a much higher relapse rate (79%, 15/19) than those with EVR (21%, 11/52) (p <0.0001), as can be seen from Table 3.

In this study, multivariate analysis for SVR, in patients over 65 y.o., showed that the factors associated with SVR were EVR and gender. This indicates that better SVR can be expected even with older patients if EVR is attained and response-guided therapy guidelines can be useful for aged patients. A low SVR rate among aged female patients was as previously reported [7], although the mechanism remains unclear. This finding suggests that female patients should be treated before 65 y.o.

The next question is how aged patients should be treated in order to attain EVR. We have examined the impact of drug exposure on treatment efficacy [25–26] and reported that Peg-IFN is dose-dependently correlated with EVR [25]. In this study, the dose-dependent efficacy of Peg-IFN for EVR was also revealed in aged patients over 65 y.o., with less than 0.9 μg/kg/week of Peg-IFN leading to a low EVR rate for aged patients. If patients are difficult to treat with more than 1.2 μg/kg/week of Peg-IFN, using as much Peg-IFN as possible is desirable, in order to attain higher EVR rates. Accordingly, a reduction of Peg-IFN to 80% may need to be considered, although the manufacturer's drug information recommends reducing the dose of Peg-IFN to 50% of the assigned one. Since reduction of Peg-IFN has been reported to not affect the SVR rate after HCV RNA disappearance [26], using G-CSF for aged patients who develop severe neutropenia can be beneficial, especially in the first 12 weeks.

We also examined the negative prediction of SVR, i.e. an HCV RNA decrease at an earlier point of treatment than the usual prediction at treatment week 12 of a 2-log decrease, among aged patients with CH-C treated by Peg-IFN plus ribavirin combination therapy. We found that none of the patients without a 1-log decrease at week 4 or a 2-log decrease at week 8 could attain SVR, even if the complete treatment duration was given, the negative predictive value (NPV) for SVR equaled 100%. This earlier prediction is applied just as well to aged patients as to non-aged patients in order to avoid additional adverse effects. Recently, a genetic polymorphism near the *IL28B* gene has been reported to be associated with non-response to Peg-IFN plus ribavirin combination therapy [27–29], which is beneficial to patients. Nevertheless, even in the presence of this genetic polymorphism, NPV for SVR remains at 57–87%; 100% accuracy is not guaranteed. Thus, in addition to the pretreatment prediction, an earlier negative prediction for SVR during treatment is also considered to be useful.

We have shown in this study that, in the presence of genotype 2, HCV was easily eliminated even among aged patients; the SVR rates were over 75% for patients who had completed the treatment, and these rates were similar up to 70 y.o. The SVR rate of genotype 2 patients over 70 y.o. was 43%, however, the age limitation of the treatment among patients over 70 y.o. remains unclear, because of the small number of patients enrolled in this study. We have reported that the reduction of treatment drugs had little effect on anti-viral efficacy for patients with genotype 2, meaning that SVR can be attained even with aged patients who are usually given lower drug doses than non-aged patients [30]. Patients under 70 y.o. with genotype 2 should, at least, benefit from this therapy. The SVR rate was maintained among genotype 2 patients being 65–69 y.o., compared to genotype 1 patients. The higher efficacy with shorter treatment duration in genotype 2 aged patients can account for it.

In conclusion, the strategy of a response-guided therapy and an earlier negative prediction for SVR may be beneficial for aged patients, especially those with genotype 1. At present, aged patients up to 65–70 y.o. with CH-C can be candidates for Peg-IFN plus ribavirin combination therapy, if its efficacy and adverse effects are fully taken into account. At the same time, there is an urgent need to establish new treatment procedures, such as combination therapy with protease inhibitor plus polymerase inhibitor without Peg-IFN or ribavirin, for non-responders or patients

with poor tolerability for Peg-IFN plus ribavirin combination therapy among aged patients.

Conflict of interest

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

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REVIEW

Changing etiologies and outcomes of acute liver failure: A perspective from Japan

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Key words
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Abstract

Acute liver failure in Japan usually consists of fulminant hepatitis (FH) due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury. The annual incidence of FH was estimated at 429 cases in 2004. FH is classified into acute or subacute type, and the prognosis of the latter is poor. Hepatitis B virus (HBV) is the most frequently identifiable agent that causes FH in Japan. Transient HBV infection is more prevalent in the acute than subacute type, whereas the frequency of HBV carriers is greater in the subacute type. FH due to HBV reactivation from resolved hepatitis B has been increasingly observed in patients with malignant lymphoma treated with rituximab and corticosteroid combination therapy. The prognosis is poor in HBV carriers with acute exacerbation, especially in patients with HBV reactivation from resolved hepatitis B. Despite careful investigation, the etiology is still unknown in 16% and 39% of the acute and subacute type of FH, respectively. Autoimmune hepatitis and drug-allergy-induced liver injury are found in 7% and 10%, respectively, and are more frequently observed in the subacute type of FH. Living donor liver transplantation is now the standard care for individuals with poor prognosis. Artificial liver support with plasmapheresis and hemodiafiltration plays a central role while waiting for a donor liver or for the native liver to regenerate. Further research is necessary to identify the causes of unknown origin. In addition, to improve the prognosis of FH, it is necessary to establish treatment modalities that are effective for liver regeneration.

Introduction

Acute liver failure is a clinical syndrome that is marked by the sudden loss of hepatic function in a person without chronic liver disease. The causes of acute hepatic failure are varied and differ geographically. In Japan, fulminant hepatitis (FH) is defined as having hepatitis, when grade II or worse hepatic encephalopathy develops within 8 weeks of the onset of the disease symptoms, with a prothrombin time of ≤40%. FH due to viral infection, autoimmune hepatitis and drug-allergy-induced liver injury is the main cause of acute liver failure in Japan. In contrast, other causes, including paracetamol overdose, other drug toxicity, metabolic liver disease, and acute fatty liver of pregnancy, are infrequent.

The Intractable Hepato-biliary Diseases Study Group of Japan annually performs a nationwide survey of patients with FH and late-onset hepatic failure (LOHF). This paper summarizes the results of the survey and addresses the characteristics and trends of acute liver failure in Japan.

Definition and methods

In 1969, Trey and Davidson defined acute liver failure as the occurrence of encephalopathy within 8 weeks of the onset of acute hepatic illness, and in the absence of pre-existing liver disease.[1] Thereafter, patients with hepatic encephalopathy that develops between 8 and 24 weeks after disease onset are defined as having LOHF.[2] Other definitions based on the duration of illness have subsequently been used to classify patients:[2–4] hyperacute, <7 days; acute, 7–28 days; and subacute, 28 days to 6 months. In Japan, patients with FH are classified into acute or subacute type, in which the encephalopathy occurs within 10 days, or later than 11 days, respectively, of the onset of disease symptoms.[5,6] Based on the previous survey, patients with FH who present within 10 days of symptom onset have significantly higher survival rates than similar patients who present with encephalopathy at 10 days after symptom onset.[7,8]

The survey was performed in hospital with active members of the Japan Society of Hepatology and the Japanese Society of Gastroenterology. Patients who meet the diagnostic criteria for FH and LOHF were entered into the survey (Table 1). Besides the diagnostic criteria, patients under 1 year of age and those with alcoholic hepatitis were excluded from the analysis.

The etiology of acute liver failure is classified into five categories: viral infection, autoimmune hepatitis, drug-allergy-induced liver injury, unknown, and indeterminate (Table 2). Patients with viral infection consist of those with hepatitis A virus (HAV),

Table 1 Diagnostic criteria for fulminant hepatitis in Japan according to the Intractable Liver Diseases Study Group of Japan, the Ministry of Health, Welfare and Labour (2003)

Fulminant hepatitis (FH) is defined as hepatitis in which hepatic encephalopathy of coma grade greater than II develops in the patients within 8 weeks after the onset of disease symptoms with highly deranged liver functions showing prothrombin time less than 40% of the standardized values.

FH is classified into two subtypes: the acute typeand subacute type in which the encephalopothy occurs within 10 days and later than 11 days, respecitvely.

Note 1: Patients with chronic liver diseases are excluded from FH, but asymptomatic HBV carriers who develop acute exacerbation are diagnosed with FH.

Note 2: Acute liver failure accompanying no liver inflammation, such as drug or chemical intoxication, microcirculatory disturbance, acute fatty liver of pregnancy, and Reye's syndrome are excluded from FH.

Note 3: The grading of hepatic encephalopathy is based on the criteria from the Inuyama Symposium in 1972.

Note 4: The etiology of FH is based on the criteria from the Intractable Liver Diseases Study Group of Japan in 2002 (Table 2).

Note 5: Patients with no hepatic encephalopathy or encephalopathy of coma grade I, even showing prothrombin time <40% of the standardized values, are diagnosed with severe acute hepatitis. Patients in whom encephalopathy develops between 8 and 24 weeks after disease onset, with prothrombin time <40% of the standardized values, are diagnosed with late onset hepatic failure (LOHF). Both are related to FH, but are regarded differently from FH.

hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV) and other viruses. Patients with HBV infection are further classified into transient infection and acute exacerbation of HBV carrier status. In 2002, the criteria were modified to define FH due to autoimmune hepatitis and HEV, and the etiology of patients between 1998 and 2001 was re-assessed according to these new criteria.

Demographic features

From 1998 to 2006, 934 patients were enrolled in the surveillance.[9] Among these patients, 856 (432, acute type and 424, subacute type) were classified as having FH and 78 as having LOHF (Table 3). Based on the nationwide epidemiology surveillance, the annual incidence of FH was estimated at 3700 cases in 1972, 1050 cases in 1995, and 429 cases in 2004.[10] About 30% of patients with severe acute hepatitis were presumed to develop hepatic encephalopathy of coma grade II or more.[11]

The male : female ratio was higher for the acute type than subacute type and LOHF. The age of the patients was significantly higher for the subacute type and LOHF than for the acute type. The frequency of HBV carriers was highest for the subacute type and lowest for LOHF. There were many patients with complications, such as metabolic syndrome, malignancy and psychiatric disorders, which preceded the onset of acute liver failure, and most of these patients had received daily medication. This tendency was more obvious in patients with the subacute type and LOHF.

The survival rates of non-liver-transplanted patients were 54% for acute and 24% for subacute type FH, and 15% for LOHF. The prognosis of patients with subacute type FH and LOHF was evidently poor. These annual rates have not improved between 1998 and 2006. When compared to a previous survey,[12] prognosis of FH in acute type patients improved until 1998, although the prognosis remained poor in the subacute type with no liver transplantation during that period (Fig. 1). This improvement was probably achieved by progress in artificial liver support.

Table 2 Criteria for etiology of fulminant hepatitis and late onset hepatic failure

I. Viral infection
  1. HAV: positive for serum IgM anti-HAV
  2. HBV: positive for either serum HBsAg, IgM anti-HBc or HBV DNA
    A. Transient infection: fulfilling either (a) or (b):
      (a) Negative for serum HBsAg before onset of acute liver injury.
      (b) Positive for serum IgM anti-HBc and negative for anti-HBc in serum diluted to 1:200.
    B. Acute exacerbation of carrier status: fulfilling either (a) or (b):
      (a) Positive for serum HBsAg before onset of acute liver injury
      (b) Negative for serum IgM anti-HBc and positive for anti-HBc in the serum diluted to 1:200.
    C. Undetermined: neither (a) nor (b)
  3. HCV: fulfilling either (a) or (b):
    (a) Negative for serum anti-HCV or HCV RNA before onset of acute liver injury.
    (b) Positive for serum HCV RNA and low titer positive for serum anti-HCV core protein.
  4. HEV: positive for serum HEV-RNA
  5. Other virus: e.g. EBV.

II. Autoimmune hepatitis: fulfilling either (a) (b) or (c):
  (a) Diagnosed as definite or probable according to the International Scoring System for autoimmune hepatitis.
  (b) Attenuation of liver injury after glucocorticosteroid administration and/or aggravation of liver injury following withdrawal of glucocorticoid.
  (c) Positive for serum antinuclear antigen and/or serum IgG levels >2 g/dL.

III. Drug-allergy-induced: drugs responsible for liver injury are determined by clinical course of liver injury and/or d-LST.

IV. Unknown: etiology is unknown despite sufficient examinations available.

V. Undetermined: etiology is undetermined because of insufficient examinations.

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; EBV, Epstein-Barr virus; d-LST, drug-induced lymphocyte stimulation test.

Causes of FH

Viral hepatitis

In Japan, the cause of FH has been identified as HAV, HBV or other viruses in about 50% of patients (Table 4). The causes of acute liver failure differed depending on the disease type. The frequencies of viral infection were 69% and 31% for patients with the acute and subacute types of FH, respectively, and 17% for LOHF patients.

Table 3 Demographic features of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Men/women	431/423	228/203	197/226	33/45
Age (years; mean ± SD)	48 ± 17	46 ± 16	49 ± 17**	53 ± 15**
HBV carrier rate (%)	14	12	16*	7***
Complications (%)	39	35	44*	49*
History of medication (%)	46	41	51**	54*
Survival rate (no LT) (%)	40	54	24**	15**
Survival rate (LT) (%)	77	73	79	81

*$P < 0.05$; **$P < 0.01$ versus acute type; ***$P < 0.05$ versus subacute type.
HBV, hepatitis B virus; LT, liver transplantation.

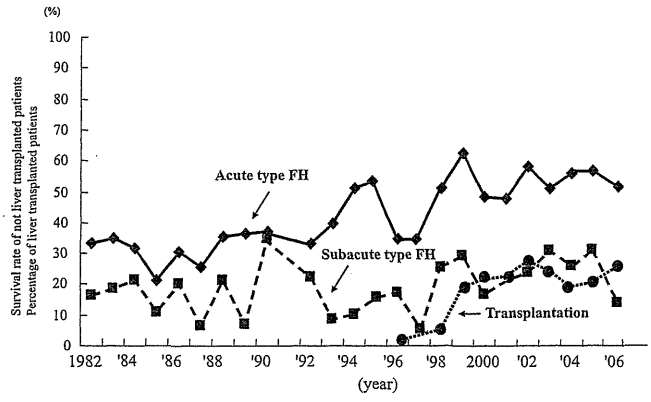

Figure 1 Survival rate of not liver transplanted patients with fulminant hepatitis(FH) and percentage of liver transplanted patients.

Infection with HAV was found in 6% of patients with FH and frequently observed in the acute type. As annual incidence of acute hepatitis A has declined over the past decade,[13] so too has the incidence of FH. However, as the overall immunity of the Japanese population to hepatitis A is only 12%[14] and is decreasing gradually as in other non-endemic areas, the increasing risk of future outbreaks of acute hepatitis A is probable. With regard to the severity of hepatitis A, age, sex, and drug toxicity have been identified as potential contributing factors.[15] HAV susceptibility and the risk of severity have likely increased recently.

In most of the patients, viral infections were due to HBV. HBV infection was found in 42% of patients with FH and 13% of those with LOHF. Among these, transient HBV infection was more frequent than acute exacerbation of HBV carrier status. Transient HBV infection was more frequent in the acute type (40%) than subacute type (9%) of FH, whereas the frequency of HBV carrier status was greater in the subacute type (16%) than in the acute type (11%). Annual incidence of FH due to HBV infection, both in transient HBV infection and acute exacerbation of HBV carrier status, has declined over the past decade. The routes of transmission of HBV indicate that, at present, sexual transmission from HBV carriers is a major route for FH. The preventive administration of HBV hyperimmune globulin and vaccination against HBV of neonates born to HBV-carrier mothers has been practiced nationwide since 1985 in Japan.[16] Therefore, the HBV carrier rate in the population has significantly decreased, and as a result, a marked decrease in the incidence of FH caused by HBV is expected.

Table 4 Percentage etiology of fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total ($n = 856$)	Acute type ($n = 432$)	Subacute type ($n = 424$)	($n = 78$)
Viral infection	51	69	31	17
HAV	6	11	1	1
HBV	42	56	27	13
(Transient infection)	(25)	(40)	(9)	(5)
(Carrier)	(13)	(11)	(16)	(4)
(Undetermined)	(4)	(6)	(2)	(4)
HCV	1	1	1	1
HEV	1	1	1	0
Other virus	1	1	1	1
Autoimmune hepatitis	7	2	12	18
Drug-allergy-induced	10	8	13	15
Unknown	30	18	42	47
Indeterminate	3	3	3	3

HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Reactivation of HBV is a well-recognized complication in patients with chronic HBV infection who are undergoing cytotoxic chemotherapy or immunosuppressive therapy. HBV reactivation can be clinically severe and result in death from acute liver failure. Among acute exacerbation of HBV carrier status in the survey, HBV reactivation has been increasingly observed in patients with hematological malignancies. Furthermore, among the 12 patients with HBV reactivation, six with serological evidence of resolved hepatitis B [without hepatitis B surface antigen (HBsAg), but with antibody to hepatitis B core antigen (anti-HBc) and/or antibody to HBsAg (anti-HBs) in serum] developed reactivation with reappearance of HBsAg in serum. Most of these patients had received rituximab and coriticosteroid. Recently, combination therapy with rituximab and corticosteroid has been identified as a risk factor for HBV reactivation in HBsAg-negative patients with malignant lymphoma.[17,18] A study in Japan has revealed that 22% of *de novo* hepatitis B and that caused by HBV reactivation from resolved

hepatitis developed into fulminant hepatic failure, and mortality was 100%.[19] This problem deserves careful attention, because HBsAg-negative, anti-HBc-and/or anti-HBs-positive patients, which account for 20–25% of hospitalized patients in Japan, represent a high-risk group.[20]

HCV infection is rare in the etiology of patients with FH and LOHF. HCV infection was found in 1% of patients with FH, independent of the disease type. Reactivation of HCV as a cause of acute liver failure following chemotherapy has been reported.[21] However, none of these patients were found in the survey.

HEV infection was found in 1% of FH patients. HEV is a common cause of acute hepatitis in endemic areas, such as South Asia, Africa and South America.[22] The virus is now also known to exist indigenously in Japan, and can contribute to acute liver disease.[23,24] In Japan, the zoonotic transmission from pigs, wild boar and deer, either food-borne or otherwise, is the cause of HEV infection in non-endemic areas.[24,25] As for the geographical distribution of clinical HEV infection in Japan, it has been reported that there was wide variation with a higher prevalence in the northern part of Japan (Hokkaido Island and the northern part of mainland Honshu).[26] In the survey, two-thirds of the patients were from this area. Moreover, most of the patients were elderly men and there were no pregnant women, who have the highest attack rate of the virus in endemic areas.

In the survey, Epstein–Barr virus, cytomegalovirus, herpes simplex virus, human herpesvirus type-6 and parvovirus were infrequent causes of other forms of viral hepatitis.

Autoimmune hepatitis

Although autoimmune hepatitis is a chronic disease, an acute presentation occurs in approximately 22% of patients, and an even smaller number present with acute liver failure.[27] In the survey, autoimmune hepatitis was found in 7% of patients with FH and 18% of those with LOHF, respectively. In 2001, FH due to autoimmune hepatitis was recognized in Japan, because there were patients with non-HAV/HBV FH in which IgG levels were >2 g/dL, with positive antinuclear antigen in the serum. Although the diagnosis generally relies on the presence of serum autoantibodies, higher IgG levels (>2 g/dL), liver histology (if available), and response to corticosteroid therapy, the diagnosis of acute-onset autoimmune hepatitis is often difficult. The serum gammaglobulin or IgG concentrations are often lower than those in patients with chronic hepatitis.[28]

Drug-allergy-induced liver injury

Formation of toxic reactive metabolites has been suggested as a potential mechanism for causing idiosyncratic drug-induced liver injury.[29] Drug-allergy-induced liver injury was seen in 13% of patients with subacute type FH and in 15% of those with LOHF. The diagnosis relied mostly on the clinical course or drug-induced lymphocyte stimulation test (D-LST). Numerous types and classes of drugs have been implicated. Anti-tuberculosis agents (isoniazid, rifampicin, ethambutol and pyrazinamide), nonsteroidal anti-inflammatory drugs (loxoprofen, lornoxicam and acetaminophen), anti-cancer agents (tegafur, UFT and flutamide), drugs for metabolic syndrome (allopurinol and acarbose), and various herbal and natural remedies were the probable causative agents in the survey.

Table 5 Survival rates and etiology of patients with fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006)

		FH		LOHF
	Total	Acute type	Subacute type	
	(n = 678)	(n = 369)	(n = 309)	(n = 62)
Viral infection	45	55	23*	36*
HAV	74	77	40	100
HBV	39	50	18*	38
(Transient infection)	(51)	(56)	(32*)	(33)
(Carrier)	(22)	(35)	(13*)	(67)
(Undetermined)	(23)	(33)	(0)	(0)
HCV	67	75	60	0
HEV	60	100	33	—
Other virus	60	50	67	0
Autoimmune hepatitis	21	25	21	18
Drug allergy-induced	42	58	29*	0*
Unknown	36	54	26*	10*
Indeterminate	28	36	14	0

*P < 0.05 versus acute type.
HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

Unknown etiology

The etiology was unknown in 42% and 47% of patients with subacute type FH and LOHF, respectively. Although the roles of GB virus C (GBV-C)/hepatitis G virus (HGV) and transfusion transmitted virus (TTV) have been discussed, in this survey, neither GBV-C/HGV or TTV appeared to be a major cause of FH. It is possible that the patients with drug-allergy-induced liver injury were contaminated with those of unknown etiology, because the ratio of medication history was high in these patients. The relationship between daily dose of oral medication or medication with significant hepatic metabolism and idiosyncratic drug-induced liver injury has been reported.[30,31] The higher numbers of patients with complications and daily medication in the survey support this evidence. Furthermore, HEV infection needs further investigation, because serum HEV RNA and IgM antibody to HEV were measured less in the survey.

Prognosis

The prognosis of patients with FH and LOHF differed depending on the etiology (Table 5). It was excellent in patients with HAV infection: the survival rate was 77% and 40% in patients with acute and subacute types of FH, respectively, and 100% in those with LOHF. In contrast, the prognosis was especially poor in HBV carriers who showed acute exacerbation. The survival rates of acute and subacute types of FH were 35% and 13%, respectively. It is noteworthy that none of the patients with HBV reactivation from resolved hepatitis B after rituximab and corticosteroid combination therapy survived. In contrast, the survival rate was 56% in acute type FH and 32% in subacute type in patients with transient HBV infection. The prognosis was poor in autoimmune hepatitis independent of disease type. Prognosis was also poor in patients

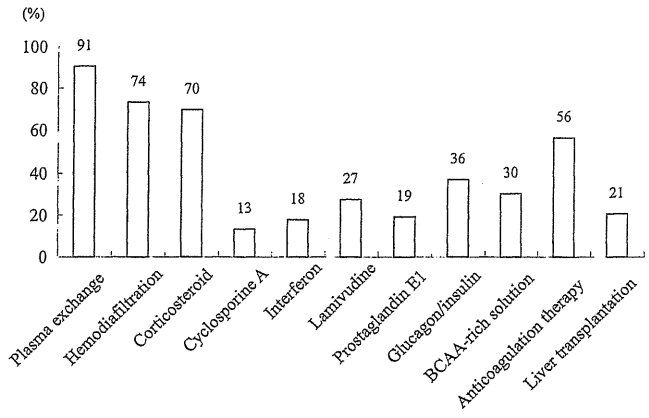

Figure 2 Percentage incidence of therapies performed for fulminant hepatitis (FH) and late onset hepatic failure (LOHF) in Japan (1998–2006). BCAA, branched-chain amino acid.

with subacute type FH and LOHF caused by drug-allergy-induced liver injury, and in those of the unknown etiology.

Complications

Complications that occurred during the course of acute liver failure also seemed to affect patient prognosis. Disseminated intravascular coagulation, renal failure and bacterial infection were found as complications in >30% of patients. Brain edema, gastrointestinal bleeding and congestive heart failure were seen in about 30%, 20% and 10%, respectively. Any of these complications significantly decreased survival rate. Furthermore, the number of these complications influenced prognosis.

Management

Specific therapies

The frequency of antiviral therapy with lamivudine has increased since 1998. As antiviral agents, lamivudine and interferon have been used in 27% and 18% of patients with FH and LOHF, respectively, between 1998 and 2006 (Fig. 2). Lamivudine has been used in 67% of patients with HBV-related FH or LOHF. Lamivudine has been reported to be efficacious for acute liver failure.[31,32] Recently, another guanosine nucleoside analog, entecavir, has been administered more frequently.[33] A preliminary study of entecavir for acute liver failure has revealed that the agent beneficially affects disease course. Lamivudine therapy is more efficacious when started early in acute liver failure. However, in the case of HBV reactivation from HBsAg-negative patients, it is difficult to prevent development of liver failure, even when lamivudine is administered after the onset of hepatitis. Two study groups in Japan have proposed guidelines for prevention of immunosuppressive-therapy- or chemotherapy-induced HBV reactivation. These guidelines recommend that patients with resolved infection should be routinely monitored for liver function and HBV DNA levels during and after chemotherapy, and antiviral therapy should be administered immediately when HBV DNA increases above the detection levels.

Corticosteroids were administered in 70% of patients with FH and LOHF. Steroid pulse therapy, methylprednisolone at a daily dose of 1 g injected intravenously, was administered to attenuate liver necrosis by suppressing excessive immune response. The efficacy of corticosteroids for improving the prognosis of acute liver failure is still obscure. Some randomized controlled trials have shown that corticosteroids provide no benefit overall in acute liver failure.[34] However, FH due to autoimmune hepatitis might be a candidate for therapy.[35] Anticoagulant therapy was performed in 56% of patients with FH and LOHF. Antithrombin III concentrate and protease inhibitor compounds such as gabexate mesylate and nafamostat mesylate were used as anticoagulants. They were effective for inhibition of disseminated intravascular coagulation and microcirculatory disturbance due to sinusoidal fibrin deposition. Glucagon/insulin, branched-chain amino acid-rich solution, cyclosporine A and prostaglandin E1 therapy was administered less frequently, and the frequency decreased compared to that in patients in the previous survey between 1995 to 1997.

Methods of liver support

In Japan, powerful artificial liver support with plasmapheresis and hemodiafiltration plays a central role in the treatment of acute liver failure. Plasmapheresis and hemodiafiltration were performed in 91% and 74% of patients with FH and LOHF, respectively (Fig. 2). In the late 1990s, hemodiafiltration therapy was developed and plasma exchange combined with hemodiafiltration therapy became popular. The increased frequency of this combination therapy in the 1990s could be implicated in the tendency for the survival rate to increase for acute type FH (Fig. 1). The effect of plasmapheresis on survival from acute liver failure has been difficult to determine. However, these support systems are efficacious for helping patients to remain in good condition until sufficient regeneration of the liver can be obtained, or liver transplantation can be performed. Recently, more powerful hemodiafiltration using large buffer volumes[36] or on-line hemodiafiltration[37] has been developed and has shown greater efficacy for improving hepatic coma.

Liver transplantation

Despite significant advances in critical care and an improved understanding of the pathophysiology of acute liver failure, the mortality rate remains high. Liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. In Japan, living donors have been used because of the insufficiency of organ donation since 1988. Living donor liver transplantation was performed in 17% of patients with FH and LOHF between 1998 and 2006, and the frequency in those patients was significantly greater in the subacute type (21%) than in the acute type (13%). Recently, these frequency ratios have been almost steady (Fig. 1). The survival rates were 77% and 81% in patients with FH and LOHF, respectively, and there was no difference in the rates among the disease types. Patient and graft survival rates were 94% and 87% at 1 year, and 91% and 81% at 5 years, respectively. There was no significant difference in patient and graft survival according to etiology.[38]

Appropriate judgment to move forward to liver transplantation is the most important step. The indications for liver transplantation

in cases of FH are determined according to the 1996 Guidelines of the Acute Liver Failure Study Group of Japan. Re-evaluation of the guidelines has revealed that the accuracy in patients not receiving liver transplantation was 68% and 78% in acute and subacute types of FH, respectively, and 84% among those with LOHF.[39] The sensitivity and specificity of the assessment in patients with acute and subacute types were very low. To improve this situation, new guidelines for using a scoring system have been proposed by the Intractable Hepato-biliary Disease Study Group of Japan.[40] By using these guidelines, the accuracy in patients not receiving liver transplantation was increased to 75% and 87% in acute and subacute types of FH, respectively.

Experimental methods of liver support

To improve the prognosis of acute liver failure, advances in the treatment for liver regeneration are urgently needed. Hepatocyte growth factor (HGF) acts as a stimulator of liver regeneration, as well as an anti-apoptotic factor. We have started a clinical trial to examine the effects of recombinant human HGF (rhHGF) in patients with FHor LOHF, and in the four patients with FH or LOHF enrolled in this study; repeated doses of rh-HGF did not produce any severe side effects. Although two patients were rescued in this study, evaluation of this therapeutic agent is still under investigation.[41]

Several clinical trials of bone marrow cell infusion in patients with liver cirrhosis have shown clinical improvement. A clinical trial of autologous bone marrow infusion for patients with advanced liver cirrhosis due to chronic HBV infection has shown clinical improvement with no serious adverse events.[42] The recent discovery of pluripotent stem cells has yielded a new cell type for potential application in regenerative medicine. Strategies to achieve high levels of hepatocyte survival and the development of methods to engineer a functional liver system *in vivo* are expected in the future.

Conclusion

In Japan, the incidence of FH has decreased gradually and the clinical characteristics of patients and the therapeutic approach have changed in the past decade. The prognosis differs in patients with FH and LOHF depending on the disease type and etiology. HBV is the major cause of FH in Japan. Recently, careful attention has been necessary because of an increase in HBV reactivation from resolved hepatitis B. Despite careful investigation, a significant group with FH of unknown origin remains and needs further investigation. Living donor liver transplantation is the only life-saving treatment available beyond the supportive care of a critical unit. Artificial liver support systems are efficacious while waiting until the native liver regenerates or a donor is found. New therapeutic modalities are required to regenerate the liver, in particular, for the subacute type of FH.

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