cytokine response. *J Immunol* 1998; 160: 3487.
2) Cramp, M.E. *et al.*: Hepatitis C virus-specific T-cell reactivity during interferon and rebavirin treatment in chronic hepatitis C. *Gastroenterology* 2000; 118: 346.
3) Tam, R.C. *et al.*: Contact hypersensitivity response following ribavirin treatment *in vivo* are influenced by type 1 cytokine polarization, regulation of IL-10 expression, and costimulatory signaling. *J Immunol* 1999; 163: 3709.
4) Yasuki, K. *et al.*: Dynamics of hepatitis C viremia following interferon-α administration. *J Infect Dis* 1998; 177: 1475.
5) Nuemann, A.U. *et al.*: Hepatitis viral dynamics *in vivo* and the antiviral efficacy of interferon-α therapy. *Science* 1998; 282: 103.
6) Bekkering, F.C. *et al.*: Estimation of early hepatitis C viral clearance in patients receiving daily interferon and ribavirin therapy using a mathematical model. *Hepatology* 2001; 33: 419.
7) Buti, M. *et al.*: Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. *Hepatology* 2002; 35: 930.
8) Okanoue, T. *et al.*: Transient biochemical response in interferon therapy decreases the development of hepatocellular carcinoma for five years and improves the long-term survival of chronic hepatitis C patients. *Hepatol Res* 2002; 23: 62.
9) Poynard, T. *et al.*: Randomized trial of interferon alfa 2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alfa 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* 1998; 352: 1426.
10) Lindsay, K.L. *et al.*: A randomized, double-blind trial comparing peglated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; 34: 395.
11) Manns, M.P. *et al.*: Peginterferon alfa-2b in combination with ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: results of a randomized trial. *Lancet* 2001; 358: 958.

Intervirology

Intervirology 2003;46:394–399
DOI: 10.1159/000074998

Lamivudine Therapy for Japanese Patients with Cirrhosis B

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Key Words
Chronic hepatitis B · Cirrhosis · Lamivudine · Peptide nucleic acid · YMDD mutants

Abstract
Objective: The aim of this study was to investigate the emergence of YMDD mutants in patients with chronic hepatitis B during lamivudine therapy and to compare the emergence patterns of YMDD mutants in cirrhotic and noncirrhotic patients. ***Methods:*** Eighteen cirrhotic and 37 noncirrhotic patients with chronic hepatitis B were studied. The emergence of YMDD mutants was determined before, as well as at 1, 3, 6, 9 and 12 months after treatment using a highly sensitive method based on polymerase chain reaction. ***Results:*** Although YMDD mutants were elicited early, the emergence of YMDD mutants was not always associated with breakthrough hepatitis. YMDD mutants appeared in cirrhotic and noncirrhotic patients: in 22 and 8% at 1 month, 13 and 21% at 3 months, 46 and 19% at 6 months, 30 and 19% at 9 months, and 83 and 27% at 12 months, respectively. ***Conclusion:*** YMDD mutants emerge more frequently in cirrhotic than noncirrhotic patients during the early period on lamivudine treatment. The highly sensitive method may be useful for monitoring the development of YMDD mutants in patients with chronic hepatitis B during lamivudine therapy.

Introduction

Hepatitis B virus (HBV) infection is a major cause of progressive liver disease, including hepatic failure and hepatocellular carcinoma, and is associated with significant mortality rates [1]. Lamivudine is a potent inhibitor of RNA-dependent DNA polymerase of HBV and suppresses HBV replication [2, 3]. Treatment with lamivudine in patients with chronic hepatitis B is effective for the inhibition of HBV replication and decreases serum levels of alanine aminotransferase (ALT) and improves histopathology in the liver [4–9]. Prolonged administration of lamivudine, however, leads to the emergence of lamivudine-resistant HBV mutants, typically those with mutations in the YMDD motif for YIDD and YVDD accompanied by breakthrough hepatitis [10–15].

The occurrence of severe breakthrough hepatitis often results in hepatic failure or even fatality and is very harmful in the patient whose liver function is restricted, especially in those with cirrhosis. Thus, early detection of YMDD mutants is very important and beneficial, because it helps predict breakthrough hepatitis and can influence the planning of appropriate therapeutic strategies. A highly sensitive method has been developed with the use of peptide nucleic acid (PNA) and restriction fragment length polymorphism (RFLP) for the detection of YMDD mutants [16]. This PNA-mediated PCR clamping and RFLP enabled the detection of as little as 0.01–0.001% of mutants from the wild-type HBV, with the sen-

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Table 1. Baseline characteristics of patients with or without cirrhosis

Features	Cirrhotics (n = 18)	Noncirrhotics (n = 37)	Differences[a]
Age, years	50.1 ± 9.5	47.8 ± 12.3	NS
Men[b]	14 (78)	31 (84)	NS
ALT, IU/l	80 ± 53	360 ± 494	$p < 0.001$
Albumin, g/dl	3.5 ± 0.6	4.1 ± 0.4	$p < 0.001$
Total bilirubin, mg/dl	1.9 ± 1.1	1.1 ± 0.9	$p < 0.001$
Platelets, $\times 10^4/mm^3$	7.6 ± 3.1	17.3 ± 6.0	$p < 0.001$
HBeAg[b]	10 (56)	25 (68)	NS
HBV genotypes (A/B/C)	0/0/18	1/1/35	NS
HBV DNA, LGE/ml	6.6 ± 1.3	7.2 ± 1.1	NS
Follow-up period, months	7.4 ± 4.2	9.5 ± 3.8	NS

NS = Not significant.
[a] Evaluated by the χ^2 test, Fisher's exact test or Student's t test where appropriate.
[b] Figures in parentheses represent percentage.

sitivity being 100- to 1,000-fold higher than that of RFLP reported by Chayama et al. [17]. With the advent of this sensitive method, YMDD mutants were detected retrospectively in serial sera from a cirrhotic patient as well as in patients with chronic hepatitis B who had not been treated with lamivudine [16].

There have been several studies that sequentially examined the emergence of YMDD mutants from the start of lamivudine with RFLP [18, 19]. As far as we are aware, however, there have been no reports that investigate the emergence of YMDD mutants in sizable numbers of patients with chronic hepatitis B using this highly sensitive assay. In the present study, the problems associated with the emergence of YMDD mutants were investigated in 55 consecutive patients with chronic hepatitis B treated with lamivudine with the use of this highly sensitive method. Furthermore, cirrhotic patients were compared with noncirrhotic patients to sort out features responsible for the emergence of YMDD mutants in cirrhotic patients who tend to lapse into hepatic failure after developing breakthrough hepatitis.

Patients and Methods

Patients

During March 1999 and December 2002, 55 consecutive adult patients with chronic hepatitis B received lamivudine therapy (Glaxo Wellcome, Greenford, UK) at the Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine. They were all positive for hepatitis B surface antigen (HBsAg) and HBV DNA in serum, and had elevated levels of serum ALT for at least 6 months before the administration of lamivudine. Of these patients, 35 were positive for hepatitis B e antigen (HBeAg) and the remaining 20 were negative for HBeAg at the start of lamivudine therapy. Furthermore, 18 were cirrhotic and 37 were noncirrhotic (table 1). To confirm chronic hepatitis and cirrhosis, liver biopsies were done before lamivudine therapy except in 8 patients with decompensated cirrhosis (Child-Pugh class B or C). None of these patients were positive for antibodies to hepatitis C virus or immunodeficiency virus type 1 or had a past history of other liver diseases unrelated to HBV, such as autoimmune hepatitis and alcoholic liver disease. None of them had received any antiviral drug, such as interferon, at least 6 months before lamivudine therapy. Patients received oral lamivudine 100 mg daily for an average period of 9.1 months (range: 1–12 months).

The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and approved by the Ethics Committee of Kyoto Prefectural University of Medicine. Informed consent was obtained from every patient.

Blood Testing

Routine biochemical tests were performed with standard procedures before therapy and at least once a month during treatment. Serial blood samples taken before and during therapy were stored at −80° until molecular biological analysis. HBsAg, HBeAg and antibody to HBeAg (anti-HBe) were determined by enzyme immunoassay (Dainabot, Tokyo, Japan). Serum HBV DNA was measured by transcription-mediated amplification and hybridization protection (TMA) assay (Chugai Diagnostics, Tokyo, Japan), and the results were expressed as log genome equivalents per milliliter (LGE/ml). The lower limit of the assay was 3.7 LGE/ml, which was equivalent to approximately 5,000 copies/ml.

Highly Sensitive Detection of YMDD Mutants by the PNA-Mediated PCR Clamping with RFLP Assay

Nucleic acids were extracted from serum (200 µl) by phenol/chloroform extraction as described previously [16]. They were purified by chloroform, precipitated by ethanol and dissolved in 10 µl of distilled water. A 1-µl portion of the extracted nucleic acids was subjected to amplification by PCR.

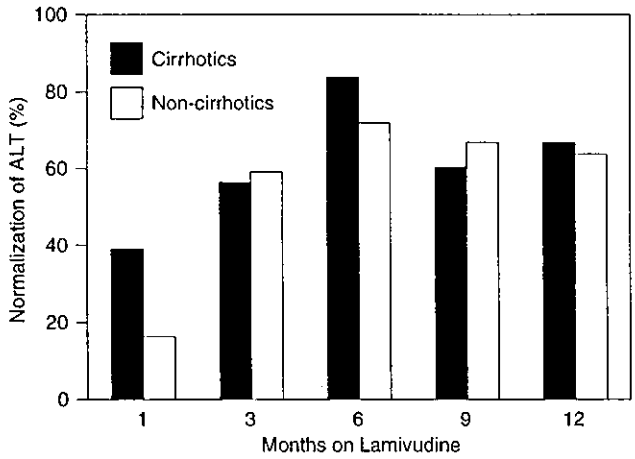

Fig. 1. Normalization of serum ALT in cirrhotic and noncirrhotic patients.

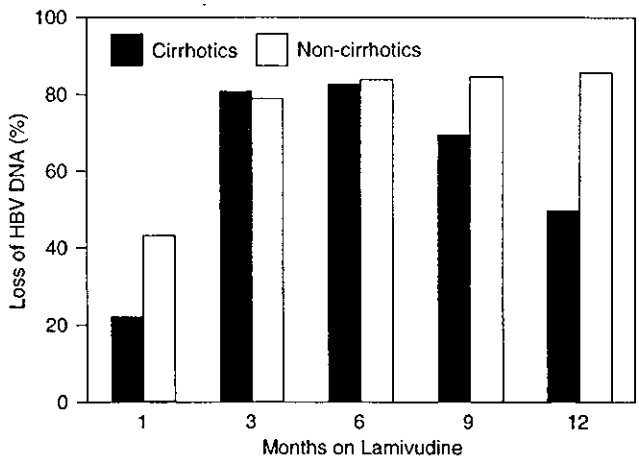

Fig. 2. Loss of HBV DNA detectable by TMA assay in serum of cirrhotic and noncirrhotic patients.

To detect mutations in the HBV DNA polymerase gene involving the YMDD motif, a highly sensitive PNA-mediated PCR clamping with RFLP assay was used [16]. Briefly, the first-round PCR was performed with primers F1 (sense: 5′-CAC TGT TTG GCT TTC AGT CAT-3′), which introduces restriction sites for *Nde*I and *Nla*III, in PCR products amplified on the wild-type and YVDD mutants, respectively, and R1 (antisense: 5′-CAG CAA AGC CCA AAA GAC CAC-3′) for 10 cycles (94°, 30 s; 60°, 30 s; 72°, 30 s) under standard PCR conditions with the fast-start Taq DNA polymerase (Roche Diagnostics, Tokyo, Japan). A 5-μl portion of the product of the first-round PCR was digested with 4 units of *Nde*I. Then PNA-mediated PCR clamping was performed with F2 (sense: 5′-CTG TTT GGC TTT CAG TCA T-3′) and R1 as well as PNA (H_2N-GCT TTC AGT CAT ATG-CON_2H), which exactly matches the wild-type YMDD sequence for allowing only YMDD mutants to serve as templates, for 25 cycles (94°, 60 s; 76°, 60 s; 60°, 60 s; 72°, 60 s) under the same conditions as the first-round PCR except for the addition of PNA. The third-round PCR was performed with primers F2 and R2 (antisense: 5′-AAG GGA GTA GCC CCA ACG TT-3′) for the detection of the YVDD mutant and F3 (sense: 5′-GTT TGG CTT TCA GTA ATA T-3′), which introduces the restriction site for *Ssp*I into only PCR products amplified on YIDD mutants and R2 for detection of YIDD mutants, respectively, for 35 cycles (94°, 30 s; 58°, 30 s; 72°, 30 s) under the same conditions. A negative control was amplified in parallel under the same conditions for every 10 samples, and no false-positive results were observed throughout this study. A 5-μl portion of the amplification products of the third-round PCR was digested with 10 units of *Nla*III (New England Biolabs, Mass., USA) for YVDD mutants or 4 units of *Ssp*I (Takara Shuzo, Osaka, Japan) for YIDD mutant at 37° for 1 h and then subjected to electrophoresis in a 3% (wt/vol) agarose gel.

This highly sensitive assay was carried out on serial sera from patients obtained before therapy as well as at 1, 3, 6, 9 and 12 months during therapy for detecting YMDD mutants.

Results

Baseline Characteristics

Table 1 compares the baseline characteristics between patients with cirrhotic and noncirrhotic hepatitis B before they received lamivudine therapy. As expected, levels of serum ALT and albumin as well as platelet counts were significantly lower, and levels of total serum bilirubin were higher in cirrhotic than noncirrhotic patients. There were no other significant differences in the baseline characteristics between these two groups of patients.

Biochemical and Virological Responses to Lamivudine

The normalization of serum ALT to 6–40 IU/l was defined as the initial biochemical response and HBV-DNA undetectable by TMA as the initial virological response. The rates of normalization of serum ALT in cirrhotic and noncirrhotic patients were 39 and 16% at 1 month, 56 and 59% at 3 months, 83 and 72% at 6 months, 60 and 67% at 9 months, and 67 and 64% at 12 months, respectively (fig. 1). They were not significantly different between the two groups of patients except for the determination at 1 month. The rate of normalization of serum ALT was higher in cirrhotics than noncirrhotics at 1 month. This may be caused by levels of serum ALT which are lower in the patients with than those without cirrhosis at the baseline. In either group, the rate of normalization of serum ALT tended to increase gradually for 6 months and then ceased to increase.

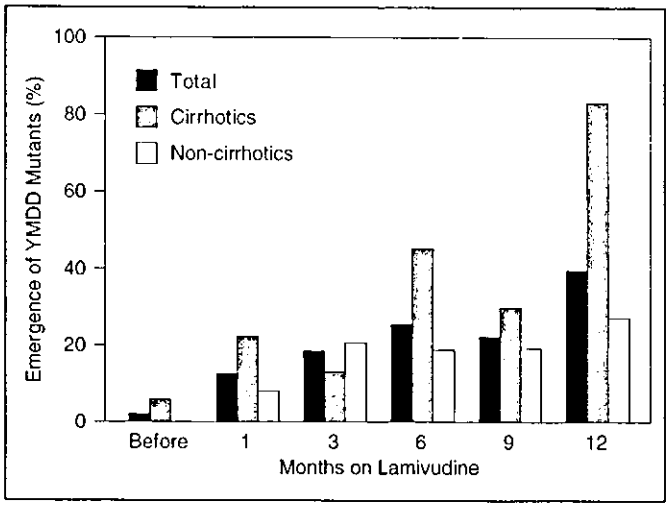

Fig. 3. Emergence of YMDD mutants at each time point in cirrhotic and noncirrhotic patients.

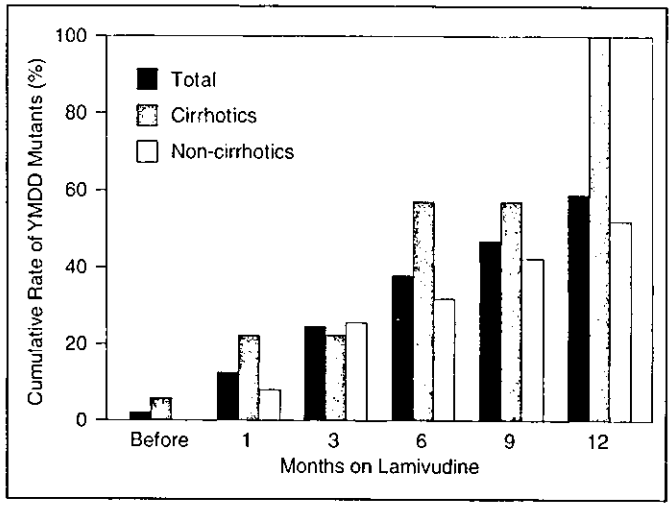

Fig. 4. Cumulative rate of YMDD mutants in cirrhotic and noncirrhotic patients.

Loss of detectable HBV DNA from serum occurred in 22 and 43% at 1 month in cirrhotic and noncirrhotic patients, respectively, 81 and 79% at 3 months, 83 and 84% at 6 months, 70 and 85% at 9 months, and 50 and 86% at 12 months (fig. 2). In either group, the loss of serum HBV DNA tended to increase for 6 months up to 84%. Thereafter, the rates of HBV DNA loss in cirrhotic patients started to decrease and reached 50% at 12 months. In contrast, the loss of HBV DNA in noncirrhotic patients was constantly higher than 80% throughout the 12 months.

Emergence of YMDD Mutants

YMDD mutants (YVDD) were detected in pretreatment serum samples in only 1 cirrhotic patient (2% of all patients), while no mutants were detected before lamivudine administration in the remaining 54 patients even with the highly sensitive method [16].

The emergence of YMDD mutants at each time point in cirrhotic and noncirrhotic patients was 6 and 0% (amounting to 2% of the total) before treatment, 22 and 8% (13%) at 1 month, 13 and 21% (18%) at 3 months, 46 and 19% (26%) at 6 months, 30 and 19% (22%) at 9 months, and 83 and 27% (39%) at 12 months, respectively (fig. 3). YMDD mutants emerged more frequently in cirrhotic than noncirrhotic patients at each point, except for 3 months after the start of lamivudine. Remarkably, a few YMDD mutants were detected as early as 1 and 3 months, with appearance rates of 13 and 18%, respectively. The appearance rate of YMDD mutants did not always increase in proportion to the duration of lamivudine. For example, the appearance rate of YMDD mutants in cirrhotics at 6 months was higher than that at 9 months. This would be caused by the reversion from positive to negative YMDD mutation in some patients.

The cumulative appearance rates of YMDD mutants in cirrhotic and noncirrhotic patients were 6 and 0% (2% of the total) before treatment, 22 and 8% (13%) at 1 month, 22 and 25% (24%) at 3 months, 57 and 32% (38%) at 6 months, 57 and 42% (47%) at 9 months, and 100 and 52% (59%) at 12 months, respectively (fig. 4). The highly sensitive method was capable of demonstrating that the rate of patients having YMDD mutants, either obvious or latent, was surprisingly high and reached approximately 60% at 12 months. The cumulative appearance rate of YMDD mutants in cirrhotic patients was also higher than that of noncirrhotic patients, except for 3 months after the commencement of lamivudine. It was remarkable that the cumulative appearance rates of YMDD mutant in cirrhosis were 2-fold higher than those of noncirrhotic patients and reached 100% at 12 months, although only 6 cirrhotic patients were continued on lamivudine at this time point.

Discussion

Lamivudine-resistant HBV mutants have been reported in patients with chronic hepatitis B treated with lamivudine for more than 6 months, and the reported rate of emergence during the therapy ranges from 14 to 43% during the first year [4–6, 8, 9]. Several studies attest to the emergence rates of YMDD mutants increasing gradually with the duration of lamivudine from 14% at 1 year to 38% at 2 years, and further to 53% at 3 years [9, 20]. There have been few reports, however, in which the rate of early emergence of YMDD mutants was investigated in sequential sera from patients within 6 months of lamivudine therapy. In most studies, YMDD mutants were detected in patients who had already developed virological breakthroughs with the emergence of YMDD mutants after long-term lamivudine [11, 12, 15]. This is attributed to direct sequencing or even the RFLP assay not being sensitive enough for detecting YMDD mutants coexisting with a huge excess of the wild-type HBV during an early stage of lamivudine treatment.

We have previously reported a highly sensitive method utilizing PNA and RFLP for the detection of YMDD mutant [16]. This PNA-mediated PCR clamping with RFLP assay made it possible to detect YMDD mutants coexisting with the wild-type HBV in as little as 0.01–0.001% of total HBV DNA with a sensitivity 100- to 1,000-fold higher than that of the conventional RFLP assay. By means of this sensitive method, YMDD mutants are found even in chronic hepatitis B patients who have not received lamivudine [16]. Paik et al. [19] previously reported early detection of YMDD mutants at 2 weeks (14.3%), as well as a high rate of their appearance at 12 weeks (60.7%) [19] with an RFLP assay. In the present study, a more sensitive assay than they used was applied for the detection of YMDD mutants in an early phase of lamivudine treatment. Furthermore, the difference of the effect of lamivudine therapy was evaluated and the features of the emergence of YMDD mutants were compared between cirrhotic and noncirrhotic patients.

The results obtained clearly demonstrated that YMDD mutants had been detected since a very early stage such as 1 month (13%) and 3 months (18%), which is attributed to a high sensitivity of the detection system employed [16]. YMDD mutants (YVDD) were also detected in pretreatment serum from a cirrhotic patient, in confirmation of preexisting mutants in patients with chronic hepatitis B who had not received lamivudine [16] and the detection of YMDD mutants in some lamivudine-naive asymptomatic HBV carriers [21].

The appearance rate of YMDD mutant was 39% at 1 year and not markedly different from those of previous reports. The cumulative appearance rate of YMDD mutants at 12 months, however, was surprisingly high and reached 59%. The appearance rate of YMDD mutants at 3 months was 18.4% and lower than the 60.7% previously reported [19]. It is hard to explain the discrepancy, because the method used for detecting YMDD mutants in the present study is more sensitive than the one they used. Although the reason is unclear, differences in HBV genotypes, geographic factors either endemic or nonendemic, and ethnic backgrounds of the patients studied may have led to inconsistent findings.

The cumulative appearance rate was considerably higher than the emergence rate of YMDD mutants at each point. It is remarkable that this was caused by YMDD mutants that would have appeared temporarily and disappeared later. Taken altogether, the emergence of YMDD mutants would not always be related to breakthrough hepatitis. There is a certain possibility that YMDD mutants had been got rid of by the host immunity that is known to clear them after acute exacerbation [18], or that these YMDD mutants are minor strains of replication-incompetent HBV variants. To comprehend whether detected mutants can induce breakthrough hepatitis, other potential factors such as HBV DNA levels of mutants and host immune responses to HBV should be analyzed.

The appearance rate of YMDD mutants was higher in cirrhotic than noncirrhotic patients. Especially, the appearance rate of YMDD mutants in cirrhotic patients at 12 months (83%) was much higher than that of noncirrhotic patients (27%); it was responsible for lower rates of HBV DNA loss in cirrhotic patients at 12 months. Previous studies have implicated HBV DNA levels and ALT levels, as well as HBeAg titers, in the appearance of YMDD mutants and DNA breakthrough [22, 23]. The results obtained suggest that the progression of liver disease would be related to the appearance of YMDD mutants.

In conclusion, the present study has demonstrated with the use of a highly sensitive method that YMDD mutants emerge more frequently in cirrhotic than noncirrhotic patients on lamivudine therapy. These mutants, however, were not always related to breakthrough hepatitis. Further and prolonged studies with larger numbers of patients are needed to explain this issue. It is to be hoped that the highly sensitive method is instrumental in monitoring YMDD mutants in patients during or before lamivudine treatment to obtain more insight into the therapeutic strategies to be adopted for treating chronic hepatitis B.

References

1 Hoofnagle JH: Type B hepatitis: Virology, serology and clinical course. Semin Liver Dis 1981;1:7–14.

2 Doong SL, Tsai CH, Schinazi RF, Liotta DC, Cheng YC: Inhibition of the replication of hepatitis B virus in vitro by 2′,3′-dideoxy-3′-thiacytidine and related analogues. Proc Natl Acad Sci USA 1991;88:8495–8499.

3 Chang CN, Skalski V, Zhou JH, Cheng YC: Biochemical pharmacology of (+)- and (–)-2′,3′-dideoxy-3′-thiacytidine as anti-hepatitis B virus agents. J Biol Chem 1992;267:22414–22420.

4 Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M: A preliminary trial of lamivudine for chronic hepatitis B infection. N Engl J Med 1995;333:1657–1661.

5 Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, Crowther L, Condreay LD, Woessner M, Rubin M, Brown NA: Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256–1263.

6 Dienstag JL, Schiff ER, Mitchell M, Casey DE Jr, Gitlin N, Lissoos T, Gelb LD, Condreay L, Crowther L, Rubin M, Brown N: Extended lamivudine retreatment for chronic hepatitis B: Maintenance of viral suppression after discontinuation of therapy. Hepatology 1999;30:1082–1087.

7 Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER: Histological outcome during long-term lamivudine therapy. Gastroenterology 2003;124:105–117.

8 Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF: A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med 1998;339:61–68.

9 Liaw YF, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Chien RN, Dent J, Roman L, Edmundson S, Lai CL: Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. Gastroenterology 2000;119:172–180.

10 Ling R, Mutimer D, Ahmed M, Boxall EH, Elias E, Dusheiko GM, Harrison TJ: Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology 1996;24:711–713.

11 Tipples GA, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DL: Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. Hepatology 1996;24:714–717.

12 Schalm SW: Clinical implications of lamivudine resistance by HBV. Lancet 1997;349:3–4.

13 Honkoop P, Niesters HG, de Man RA, Osterhaus AD, Schalm SW: Lamivudine resistance in immunocompetent chronic hepatitis B. Incidence and patterns. J Hepatol 1997;26:1393–1395.

14 Allen MI, Deslauriers M, Andrews CW, Tipples GA, Walters KA, Tyrrell DL, Brown N, Condreay LD: Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. Hepatology 1998;27:1670–1677.

15 Fischer KP, Gutfreund KS, Tyrrell DL: Lamivudine resistance in hepatitis B: Mechanisms and clinical implications. Drug Resist Updat 2001;4:118–128.

16 Kirishima T, Okanoue T, Daimon Y, Itoh Y, Nakamura H, Morita A, Toyama T, Minami M: Detection of YMDD mutant using a novel sensitive method in chronic liver disease type B patients before and during lamivudine treatment. J Hepatol 2002;37:259–265.

17 Chayama K, Suzuki Y, Kobayashi M, Tsubota A, Hashimoto M, Miyano Y, Koike H, Koida I, Arase Y, Saitoh S, Murashima N, Ikeda K, Kumada H: Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. Hepatology 1998;27:1711–1716.

18 Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM: Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 1999;30:567–572.

19 Paik YH, Chung HY, Ryu WS, Lee KS, Lee JS, Kim JH, Lee CK, Chon CY, Moon YM, Han KH: Emergence of YMDD motif mutant of hepatitis B virus during short-term lamivudine therapy in South Korea. J Hepatol 2001;35:92–98.

20 Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, Lim SG, Wu PC, Dent JC, Edmundson S, Condreay LD, Chien RN: Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: Results after 3 years of therapy. Hepatology 2001;33:1527–1532.

21 Kobayashi S, Ide T, Sata M: Detection of YMDD motif mutations in some lamivudine-untreated asymptomatic hepatitis B virus carriers. J Hepatol 2001;34:584–586.

22 Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL: Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. Hepatology 2001;34:785–791.

23 Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, Kleiner DE, Schmid P, Condreay LD, Gauthier J, Kuhns MC, Liang TJ, Hoofnagle JH: Long-term therapy of chronic hepatitis B with lamivudine. Hepatology 2000;32:828–834.

Journal of Viral Hepatitis, 2004, 11, 148–156

Interferon treatment improves survival in chronic hepatitis C patients showing biochemical as well as virological responses by preventing liver-related death

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Received June 2003; accepted for publication June 2003

SUMMARY. Interferon therapy for chronic hepatitis C reduces the risk of hepatocellular carcinoma, especially among virological and biochemical responders. However, little is known about the effect of interferon therapy on mortality. We studied the long-term effect of interferon therapy on mortality in patients with chronic hepatitis C. For this retrospective cohort study, 2954 patients with chronic hepatitis C were recruited, of whom 2698 received interferon therapy and 256 did not. The effect of interferon therapy on survival was assessed by standardized mortality ratio (SMR) based on published mortality data for the general Japanese population and by risk ratio calculated by proportional hazard regression. Over 6.0 ± 2.2 years follow-up, death from liver-related diseases was observed in 69 (68%) of 101 deaths among interferon-treated patients and in 42 (81%) of 52 deaths among untreated patients. Compared with the general population, overall mortality was high among untreated patients (SMR: 2.7; 95% CI: 2.0–3.6) but not among interferon-treated patients (SMR: 0.9; 95% CI: 0.7–1.1). Liver-related mortality was extremely high among untreated patients (SMR: 22.2; 95% CI: 16.0–30.0) and less among interferon-treated patients (SMR: 5.5; 95% CI: 4.3–6.9). The risk of death from all causes was lower for interferon-treated than untreated patients (risk ratio: 0.47; 95% CI: 0.261–0.836; $P = 0.01$). The risk of death from liver-related diseases was significantly lower for sustained virological responders (risk ratio: 0.04; 95% CI: 0.005–0.301; $P = 0.002$) compared with untreated patients, but not for nonsustained virological responders. Sustained biochemical responders (risk ratio: 0.03; 95% CI: 0.004–0.230; $P < 0.001$) and transient biochemical responders (risk ratio: 0.18; 95% CI: 0.063–0.532; $P = 0.002$) showed a significantly reduced risk of death from liver-related death, whereas biochemical nonresponders did not. Hence interferon treatment improved survival in chronic hepatitis C patients showing a biochemical as well as a virological response by preventing liver-related deaths.

Keywords: chronic hepatitis C, interferon, liver-related mortality, multivariate analysis, standardized mortality ratio.

Abbreviations: HCC, hepatocellular carcinoma; SMR, standardized mortality ratio.

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INTRODUCTION

Hepatitis C virus (HCV) infection rarely resolves spontaneously once it becomes chronic [1]. Consequently, most patients in Japan with chronic HCV infection are likely to progress steadily to liver cirrhosis and hepatocellular carcinoma (HCC), which develops approximately 30 years after blood transfusion [2–4]. HCC is one of the most common malignancies, especially in Southeast Asia, and a major cause of death for patients with chronic HCV infection. In the early 1990s, interferon was introduced worldwide as a therapy for patients with chronic hepatitis C and was effective in inducing normalization of serum alanine aminotransferase (ALT) [5,6], eliminating HCV RNA [7,8], and improving liver histological findings [9–11] in patients with chronic hepatitis C.

To evaluate the effect of interferon therapy on the incidence of HCC and the risk of mortality for chronic hepatitis C patients, a randomized controlled trial is needed. However, a prospective randomized trial with untreated control patients is ethically impossible, because interferon therapy has already been established as a standard treatment for patients with chronic hepatitis C. Therefore, almost all chronic hepatitis C patients, except for cases with medical conditions such as depression, autoimmune disease and severe diabetes mellitus, have been treated with interferon in Japan. Recently, several investigators have reported this therapy as being effective for reducing the incidence of HCC among patients who showed normalization of ALT during and after interferon therapy, as well as among those in whom HCV was eradicated [12–17]. However, a reduced risk of HCC does not necessarily lead to improvement in survival. Indeed, little is known about the effects of interferon therapy on the mortality of patients with chronic hepatitis C. Several investigators [14, 18–23] have tried to evaluate the impact of interferon therapy on mortality. Four of these studies indicated that interferon therapy significantly reduced the mortality of compensated HCV-related cirrhotic patients [18,20] or of chronic hepatitis C patients including patients with compensated cirrhosis [21,23]. However, lack of analysis on response to interferon [18,20–23] or lack of information on disease-specific mortality [20,21] has made it difficult to evaluate the benefits of interferon for survival. Recently, Yoshida *et al.* [24] demonstrated that interferon therapy improved survival by preventing liver-related deaths of chronic hepatitis C patients showing a sustained virological response. However, whether a biochemical response to interferon therapy results in a reduced risk of mortality has not been investigated.

We conducted a multi-centre, large-scale, retrospective cohort study of patients with chronic hepatitis C, who had been enrolled at the end of 1997 at participating hospitals in order to analyse the effect of interferon therapy on the incidence of HCC. The aim of the present study was to examine the effect of interferon therapy on the mortality and causes of death among chronic hepatitis C patients.

PATIENTS AND METHODS

Patients

We recruited chronic hepatitis C patients from four previous studies which were conducted to assess the effect of interferon therapy on the incidence of HCC [12,14,15,17]. All patients meeting the following criteria were included in this study: (i) histological diagnosis of chronic hepatitis or cirrhosis; (ii) no history of clinical signs at entry into the study of complications of cirrhosis, i.e. ascites, jaundice, encephalopathy, or variceal bleeding; (iii) no evidence of HCC at entry into the study as assessed by ultrasonography and/or computed tomography; (iv) absence of serum hepatitis B surface antigen; (v) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis; (vi) absence of excessive alcohol consumption (>80 g/day); and (vii) absence of human immunodeficiency virus antibodies, as described previously [12,14,15,17]. A total of 3025 patients who met these criteria and whose initial sera tested positive for anti-HCV as determined by either first- or second-generation ELISA (Ortho Diagnostics, Tokyo, Japan) and HCV RNA were included in the study. The sera of patients who had been diagnosed as non-A, non-B hepatitis before anti-HCV testing became available (i.e. before 1989) had been frozen at −80 °C and were retrospectively assayed.

Of the 3025 chronic hepatitis C patients, 2762 had received interferon after 1987, when interferon became available in Japan. Interferon-treated patients received a 4–12-month course of interferon therapy, which was initiated within 1 month of liver biopsy. The remaining 263 patients did not undergo interferon therapy or any other antiviral therapy, including almost all patients with biopsy-proven chronic hepatitis who had refused interferon treatment due to adverse effects, lack of time for therapy, or their inability to undergo treatment as a consequence of depression, severe diabetes mellitus or other medical conditions.

Criteria for biochemical and virological responses to interferon therapy

The biochemical response during the follow-up up to 6 months after the completion of interferon therapy was defined according to previously described criteria with minor modifications [8,9]. In the sustained response group, ALT levels decreased to the normal range during therapy and remained within that range up to 24 weeks after therapy without any abnormal elevation. In the transient response group, ALT levels decreased to the normal range by the end of therapy, remained normal during therapy but returned to abnormal levels during the 24 weeks following interferon therapy. In the no-response group, ALT levels did not decrease to the normal range, or fluctuated during therapy and the subsequent 24 weeks. Both biochemical transient

and nonresponders were designated as nonsustained biochemical responders.

A sustained virological response was defined as HCV RNA negativity at more than 6 months after the cessation of interferon therapy. Patients showing positive HCV RNA at the same time were designated as nonsustained virological responders.

Histological evaluation

Liver biopsy was carried out before interferon therapy in all cases. Specimens were fixed in formaldehyde and embedded in paraffin. The sections were stained with haematoxylin-eosin and Azan-Mallory and analysed by two pathologists without any knowledge of the clinical and laboratory data. Histological findings were scored according to the classification of Desmet *et al.* [25].

Follow-up

The starting date of the follow-up for both the interferon-treated and untreated groups was defined as the date of liver biopsy. Biochemical examinations including α-fetoprotein and abdominal ultrasonography were carried out before interferon therapy and every 3–6 months thereafter at the outpatient clinic of the respective hospitals. The end of the follow-up was the date of death or the latest confirmation of survival. Follow-up data on the patients were obtained from the participating hospitals. Follow-up data that were not available from the hospitals were collected from the resident registry of the local municipal office. Death from liver-related disease was defined as death from HCC, liver failure determined by the presence of one or more of ascites, jaundice and hepatic encephalopathy, or variceal bleeding diagnosed on the basis of endoscopic findings of patients presenting with upper gastrointestinal haemorrhage.

Five untreated patients were observed for over 162 months, which corresponded to the longest period of observation of those treated with interferon. In these subjects, only the follow-up data up to 162 months were considered. Seventy-one patients whose follow-up period was shorter than 12 months were excluded from the study. The final numbers of study subjects were 2698 for the interferon-treated group and 256 for the untreated group.

Informed consent was obtained from each patient included in the study. The study protocol was in accordance with the Helsinki Declaration of 1975 (revised in 1983) and approved by the Ethical Committee of the Osaka University Graduate School of Medicine.

Statistical analysis

The chi-square test was used to compare the frequency of gender between the interferon-treated and untreated groups. The difference in age at liver biopsy and ALT between the two groups, expressed as median, was assessed for significance with the Student's *t*-test. The Wilcoxon rank-sum test was used to compare the distribution of age at liver biopsy and histological staging. Cumulative survival curves were determined with the Kaplan–Meier method, and the log-rank test was used to compare the cumulative survival rates.

The observed number of deaths was compared with the expected number, which was calculated by applying sex, 5-year age, 5-year calendar time, and cause-specific mortality rates for the general population in Japan, as prepared by the Statistics and Information Department, Japan Ministry of Health and Welfare [26]. The standardized mortality ratio (SMR) was expressed by dividing the observed number of deaths by the expected number of deaths. The standard error and the 95% CI of SMR were estimated by assuming Poisson's distribution, and differences in mortality between the study cohort and the general population were considered to be significant if the CI did not include unity.

Survival was also analysed by using Cox proportional hazards regression controlling for age (continuous variable), gender, stages of liver fibrosis (stage: 0/1/2/3/4) and time at liver biopsy (1991/1992). Risk ratios attributable to biochemical sustained, transient and no responses and to virological sustained and nonsustained responses were calculated in comparison with no treatment by using dummy variables.

Data analysis was performed with the SAS/PC statistical package (SAS Institute, Cary, NC, USA). All reported *P*-values were two-sided and $P < 0.05$ was considered to be significant.

RESULTS

Patient characteristics at entry

Of the 2698 patients treated with interferon, 901 (33.3%) had a sustained biochemical response, 701 (26.0%) a transient biochemical response and the remaining 1096 patients (40.6%) were classified as biochemical nonresponders. Serum HCV RNA remained negative at more than 6 months after cessation of interferon therapy in 738 (81.9%) of the sustained biochemical responders, designated as sustained virological responders, whereas serum HCV RNA remained positive in 133 (14.8%). Serum HCV RNA was not examined after the termination of interferon therapy in 30 sustained biochemical responders, who were excluded from the analysis according to virological responses to interferon. Positive HCV RNA after interferon therapy was detected in all of the biochemical transient and nonresponders.

The demographic and clinical features of interferon-treated patients according to virological and biochemical responses to interferon and of untreated patients at the time of enrolment are summarized in Table 1. Untreated patients were significantly older than interferon-treated patients ($P = 0.04$), but frequency distribution of age at liver biopsy

Table 1 Characteristics of interferon-treated patients according to virological and biochemical responses to interferon and of untreated patients

	Interferon-treated							
	Virological response		Biochemical response					
	SVR (n = 738)	non-SVR (n = 1930)	SBR (n = 901)	TBR (n = 701)	BNR (n = 1096)	Total (n = 2698)	Untreated (n = 256)	P-value
Median age (range)	51 (20–72)	54 (20–76)	52 (20–73)	53 (20–75)	54 (20–76)	53 (20–76)	54 (21–72)	0.04
Age at biopsy (%)								
≤49	337 (45.7)	687 (35.6)	392 (43.5)	277 (39.5)	369 (33.7)	1038 (38.5)	75 (29.3)	0.12
50–59	240 (32.5)	759 (39.3)	303 (33.6)	280 (39.9)	428 (39.1)	1011 (37.5)	123 (48.9)	
≥60	161 (21.8)	484 (25.1)	206 (22.9)	144 (20.5)	299 (27.3)	649 (24.1)	58 (22.7)	
Sex (M/F)	507/231	1210/720	595/306	440/261	703/393	1738/960	157/99	0.32
Median ALT (U/L), SD	91	92	87	79	103	92	98	0.57
(range)	(7–1110)	(11–1195)	(7–1110)	(13–1195)	(13–828)	(7–1195)	(9–563)	
Stage of fibrosis (%)								
0	5 (0.7)	11 (0.6)	7 (0.8)	4 (0.6)	5 (0.9)	16 (0.6)	9 (3.5)	0.34
1	259 (35.1)	476 (24.7)	337 (37.4)	228 (32.5)	190 (17.3)	755 (28.0)	84 (32.8)	
2	263 (35.6)	614 (31.8)	297 (33.0)	238 (34.0)	349 (31.8)	884 (32.8)	40 (15.6)	
3	189 (25.6)	725 (37.6)	235 (26.1)	209 (29.8)	471 (43.0)	915 (33.9)	93 (36.3)	
4	22 (3.0)	104 (5.4)	25 (2.8)	22 (3.1)	81 (7.4)	128 (4.7)	30 (11.7)	

SVR, sustained virological responders; SBR, sustained biochemical responders; TBR, transient biochemical responders; BNR, biochemical nonresponders; ALT, alanine aminotransferase.

and the stages of liver fibrosis, gender and ALT did not differ significantly. In sustained biochemical responders, the ratio of male patients and median ALT levels were significantly higher for patients with HCV eradication than for those without it ($P < 0.001$, each), whereas median age and the frequency distribution of the stages of liver fibrosis were not significantly different between the two groups.

Follow-up data

The mean period of observation (total cases: 6.0 ± 2.2 years) of the interferon-treated and untreated patients was 5.8 and 8.0 years, respectively, with the former being significantly shorter than the latter ($P = 0.0001$) because interferon therapy was not introduced in Japan until 1987.

Table 2 Follow-up data for interferon-treated patients according to virological and biochemical responses to interferon and for untreated patients

	Interferon-treated						
	Virological response		Biochemical response				
	SVR (n = 738)	non-SVR (n = 1930)	SBR (n = 901)	TBR (n = 701)	BNR (n = 1096)	Total (n = 2698)	Untreated (n = 256)
Mean period of observation, year (SD)	5.7 (2.0)	5.8 (1.9)	5.6 (2.0)	5.7 (1.8)	5.9 (1.9)	5.8 (1.9)	8.0 (3.4)
No. of deaths	7	94	10	10	81	101	52
Liver-related deaths	1	68	1	5	63	69	42
Death from HCC	1	57	1	4	53	58	31
Death from other liver diseases	0	11	0	1	10	11	11
Liver-unrelated deaths	9	26	9	5	18	32	10

SVR, sustained virological responders; SBR, sustained biochemical responders; TBR, transient biochemical responders; BNR, biochemical nonresponders; HCC, hepatocellular carcinoma.

The sustained virological responders, nonsustained virological responders, sustained biochemical responders, transient biochemical responders and biochemical nonresponders were observed for a mean of 5.7, 5.8, 5.6, 5.7 and 5.9 years, respectively (Table 2).

We identified 153 deaths from all causes during the follow-up. The 153 patients who died consisted of 10 sustained biochemical responders (seven of whom were sustained virological responders and three of whom were sustained biochemical responders without HCV eradication), 10 transient biochemical responders, 81 biochemical nonresponders and 52 cases without interferon treatment. Death from all causes did not occur in 30 sustained biochemical responders whose serum HCV RNA was not examined after cessation of interferon therapy. Death from liver-related disease was identified in 111 (73%) of the 153 patients who died: only one death (10%) from liver-related disease (death from HCC) was found among sustained responders with HCV eradication, five (50%) among transient biochemical responders (death from HCC in four cases), 63 (78%) among biochemical nonresponders (death from HCC in 53 cases) and 42 (81%) among untreated patients (death from HCC in 31 cases) (Table 2).

Cumulative survival

The cumulative survival rates from all causes of death were found to be significantly higher for interferon-treated than for untreated patients ($P < 0.001$) (Fig. 1a) The respective 5-year survival rates of interferon-treated and untreated groups were 97.8 and 95.3%, and the 10-year survival rates 87.2 and 77.1%. The cumulative survival rates for sustained virological responders were significantly higher than for nonsustained virological responders ($P < 0.001$) (Fig. 1b), with 5-year survival rates of 99.5 and 97.1%, and 10-year survival rates of 97.8 and 81.9%, respectively. The cumulative survival rates for sustained biochemical responders were significantly higher than for nonsustained biochemical responders ($P < 0.001$). When nonsustained biochemical responders were divided into transient biochemical responders and biochemical nonresponders, the cumulative survival rates for the transient biochemical responders were significantly higher than for the biochemical nonresponders ($P < 0.001$) (Fig. 1c). The respective cumulative survival rates for sustained biochemical responders, transient biochemical responders and biochemical nonresponders were 99.2, 99.1 and 95.8% at the end of the fifth year and 97.8, 97.6 and 72.6% at the end of the 10th year. Among sustained biochemical responders, the cumulative survival rates for sustained virological responders and sustained biochemical responders without HCV eradication were 99.5 and 99.2% at the end of fifth year and 97.8 and 99.2% at the end of the 10th year, showing no statistical significance ($P = 0.18$).

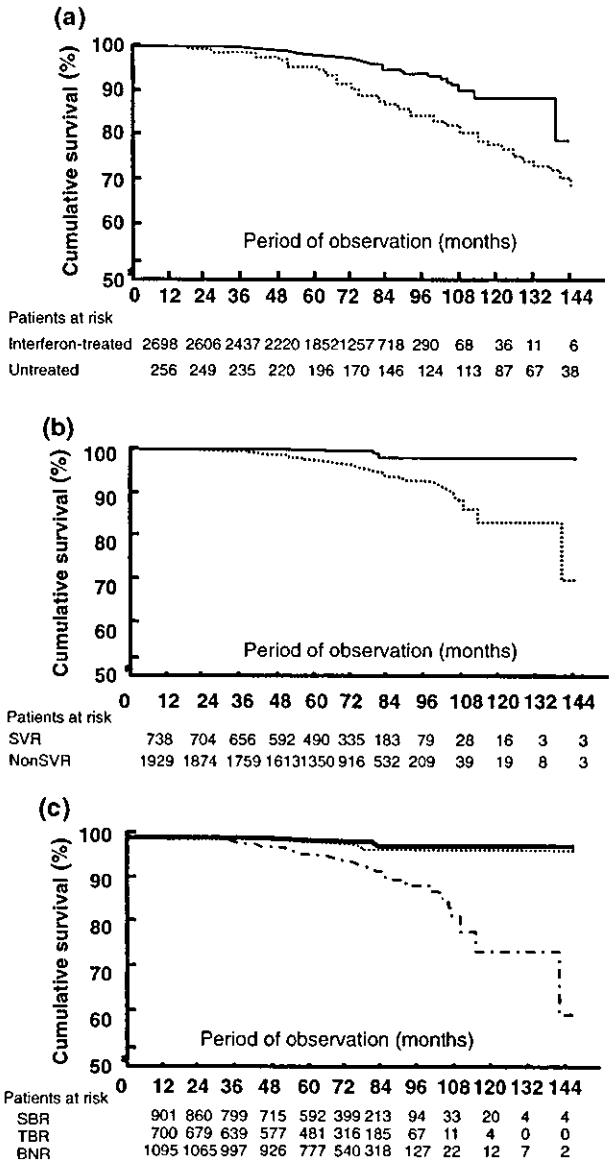

Fig. 1 Cumulative survival rates from all causes of death for patients with chronic hepatitis C. (a) For interferon-treated patients (solid line) and untreated patients (dotted line). (b) According to the virological response to interferon therapy; sustained virological responders (SVR) (solid line) and nonsustained virological responders (non-SVR) (dotted line). (c) In terms of the biochemical responses to interferon, sustained biochemical responders (SBR) (solid line), transient biochemical responders (TBR) (dotted line) and biochemical nonresponders (BNR) (dash-and-dot line).

Standardized mortality ratio

Differences in mortality among interferon-treated and untreated patients from the general population were further assessed by calculating SMR, the ratio of the observed number of deaths to the expected number. Overall mortality

Table 3 Standardized mortality ratios (SMR) in patients with chronic hepatitis C according to virological and biochemical responses to interferon

	Overall deaths			Liver-related deaths			Liver-unrelated deaths		
	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)
Untreated	52	19.2	2.7 (2.0–3.6)	42	1.9	22.2 (16.0–30.0)	10	17.3	0.6 (0.3–1.1)
Interferon-treated	101	112.7	0.9 (0.7–1.1)	69	12.6	5.5 (4.3–6.9)	32	100.0	0.3 (0.2–0.5)
Virological response									
Sustained (HCV RNA negative)	7	29.8	0.2 (0.1–0.5)	1	3.3	0.3 (0.0–1.7)	6	26.5	0.2 (0.1–0.5)
Nonsustained (HCV RNA positive)	94	82.2	1.1 (0.9–1.4)	68	9.2	7.4 (5.8–9.4)	26	73.0	0.4 (0.2–0.5)
Biochemical response									
Sustained response	10	36.5	0.3 (0.1–0.5)	1	4.0	0.3 (0.0–1.4)	9	32.5	0.3 (0.1–0.5)
Transient response	10	27.5	0.4 (0.2–0.7)	5	3.2	1.6 (0.5–3.7)	5	24.3	0.2 (0.1–0.5)
No response	81	48.8	1.7 (1.3–2.1)	63	5.4	11.6 (8.9–14.9)	18	43.3	0.4 (0.3–0.7)

Difference from the expected number of deaths was considered significant if 95% CI of SMR did not include unity.

for untreated patients (SMR: 2.7; 95% CI: 2.0–3.6) but not for the interferon-treated patients (SMR: 0.9; 95% CI: 0.7–1.1) was significantly higher than for the general population. Liver-related mortality was high for untreated patients (SMR: 22.2; 95% CI: 16.0–30.0) and also for interferon-treated patients, although to a lesser degree (SMR: 5.5; 95% CI: 4.3–6.9) (Table 3). For sustained virological responders overall mortality was low (SMR: 0.2; 95% CI: 0.1–0.5), and liver-related mortality (SMR: 0.3; 95% CI: 0.0–1.7) was equivalent to that for the general population. In contrast, liver-related mortality was high for nonsustained virological responders (SMR: 7.4; 95% CI: 5.8–9.4).

Sustained and transient biochemical responders showed a low overall mortality compared with that for the general population (SMR: 0.3; 95% CI: 0.1–0.5, and SMR: 0.4; 95% CI: 0.2–0.7, respectively), whereas overall mortality was high for biochemical nonresponders (SMR: 1.7; 95% CI: 1.3–2.1). Liver-related mortality was not high for sustained and transient biochemical responders (SMR: 0.3; 95% CI: 0.0–1.4, and SMR: 1.6; 95% CI: 0.5–3.7, respectively) compared with that for the general population, but it was high for biochemical nonresponders (SMR: 11.6; 95% CI: 8.9–14.9) (Table 3). Overall and liver-related mortality for sustained biochemical responders without HCV eradication was equivalent to that for the general population (SMR: 0.5; 95% CI: 0.1–1.5, and SMR: 0.0; 95% CI: 0.0–6.1, respectively).

Interferon-treated patients had a statistically lower risk of liver-unrelated death than the general population (SMR: 0.3; 95% CI: 0.2–0.5), whereas untreated patients did not (SMR: 0.6; 95% CI: 0.3–1.1).

Multivariate analysis

The effect of interferon on the risk of death was assessed by Cox proportional hazards regression controlling for age, gender, score of liver fibrosis and time at liver biopsy. Interferon therapy significantly reduced the risk of overall death to a ratio of only 0.47, in comparison with no treatment. When patients were classified according to virological responses to interferon, sustained virological responders showed reduced risks of overall death (risk ratio: 0.14; 95% CI: 0.056–0.352; $P < 0.001$) and liver-related death (risk ratio: 0.04; 95% CI: 0.005–0.301; $P = 0.002$) compared with untreated patients, whereas nonsustained virological responders did not. Similarly, sustained biochemical responders showed a lower risk of death from all causes (risk ratio: 0.16; 95% CI: 0.069–0.354; $P < 0.001$) and liver-related diseases (risk ratio: 0.03; 95% CI: 0.004–0.230; $P < 0.001$). Transient biochemical responders had a high, but still significantly reduced risk of overall death (risk ratio: 0.19; 95% CI: 0.083–0.445; $P < 0.001$) and liver-related death (risk ratio: 0.18; 95% CI: 0.063–0.532; $P = 0.002$), whereas the risk for nonresponders and untreated patients did not

Table 4 Risk of death in patients with chronic hepatitis C according to virological and biochemical responses to interferon

	All causes of deaths			Liver-related deaths		
	Risk ratio	95% CI	*P*-value	Risk ratio	95% CI	*P*-value
Untreated	1.00			1.00		
Interferon-treated	0.47	0.261–0.836	0.010	0.59	0.312–1.097	0.095
Virological response						
Sustained (HCV RNA negative)	0.14	0.056–0.352	<0.001	0.04	0.005–0.301	0.002
Nonsustained (HCV RNA positive)	0.59	0.327–1.057	0.08	0.76	0.402–1.417	0.380
Biochemical response						
Sustained response	0.16	0.069–0.354	<0.001	0.03	0.004–0.230	<0.001
Transient response	0.19	0.083–0.445	<0.001	0.18	0.063–0.532	0.002
No response	0.78	0.432–1.393	0.394	1.02	0.543–1.900	0.962

Adjusted for age, sex, score of liver fibrosis and period at liver biopsy.

change (Table 4). The risk of overall death for sustained biochemical responders without HCV eradication was lower than for untreated patients, although it did not reach a statistical significance (risk ratio: 0.31; 95% CI: 0.09–1.07; $P = 0.06$).

DISCUSSION

We previously demonstrated that interferon treatment could reduce the risk of HCC development in patients with chronic hepatitis C [12]. Following this, five retrospective studies [13–17] showed a similar effect of interferon on the risk of HCC, especially for virological and biochemical responders. These results suggest that interferon therapy for chronic hepatitis C can prevent the development of HCC, possibly leading to improvement in long-term survival. However, only a few previous studies have assessed the effects of interferon therapy on survival [18–24], and whether interferon therapy also reduces mortality from liver-related disease in patients with chronic HCV infection has not been thoroughly investigated. It is also still unclear what type of response to interferon results in the improvement of long-term survival.

To evaluate the effect of interferon therapy on the risk of mortality for chronic hepatitis C patients, a randomized controlled trial should be carried out. However, a prospective randomized trial with untreated control patients is ethically impossible, because interferon therapy has already been established as the standard modality for patients with chronic hepatitis C. Only two randomized controlled trials of a small number of HCV-related cirrhotic cases have evaluated the effect of interferon therapy on mortality [19,21], but with discrepant results. In contrast, large-scale prospective and retrospective cohort studies [23,24] indicate that interferon therapy for HCV-related cirrhosis or chronic hepatitis C improves long-term survival. In particular, Yoshida *et al.* [24] demonstrated in their recent retrospective cohort study that interferon therapy improved survival of chronic hepatitis C patients by preventing liver-related deaths. However, its beneficial effect was considered to be limited to patients with a sustained virological response.

As ours is a retrospective cohort study, it may be subject to several biases. The interferon-treated and untreated groups had different demographic characteristics, including age and gender. These factors were adjusted for multivariate regression analysis and considered when calculating SMR by applying the corresponding mortality for the general population. Severity of chronic liver disease was adjusted by using the stage of liver fibrosis for multivariate analysis. As the time of liver biopsy of untreated patients was earlier than for interferon-treated patients, mortality for untreated patients may be generally higher than for interferon-treated patients. To avoid this bias, we adjusted the time at liver biopsy for multivariate analysis, and 5-year time-specific mortality rates for the general population were prepared in the SMR analysis. Moreover, the number of untreated patients was small, because most Japanese chronic hepatitis C patients, except for cases with medical problems, have been treated with interferon. However, the relatively small number of untreated patients in comparison with the large number of interferon-treated patients is not likely to have resulted in a substantial overestimation of the effect of interferon therapy on survival as several of the biases already mentioned were controlled in the analyses.

When we compared the observed mortality with the expected mortality for the matched general population by calculating SMR, we were able to demonstrate that chronic hepatitis C patients had higher overall and liver-related mortality than the general population, and that the majority of deaths were liver-related. However, interferon-treated patients had a significantly lower risk of liver-unrelated mortality, whereas untreated patients did not. This may represent a selection bias in the use of interferon therapy, which included patients with no medical problems

except for having chronic liver diseases. However, our multivariate regression analysis clearly showed that interferon therapy reduced the risk of liver-related death in virological responders by 96% and in biochemical responders by 82–97%. These findings indicate that a significant reduction in the risk of death from all causes for patients treated with interferon, shown in the analysis of SMR, was not caused by a selection bias but is mainly attributable to the prevention of liver-related death by interferon therapy.

Our multivariate analysis made it clear that the risks of overall and liver-related deaths for chronic hepatitis C patients displaying a sustained virological response were 86 and 96% lower than for untreated patients. The risk reduction for sustained biochemical responders was almost equal to that for sustained virological responders. Similarly, the SMR analyses showed that liver-related mortality for these patients was equivalent to that for the general population. Thus, and as expected, when patients treated with interferon belong to the sustained virological or biochemical response group, they appear to have the highest long-term survival rate.

Of nonsustained virological responders, the risk of death from all causes and liver-related diseases for transient biochemical responders was significantly lower than for untreated patients, but higher than for sustained biochemical and virological responders. The same effects of interferon therapy on survival were observed in the SMR analyses. Although the follow-up period was not sufficiently long for a reliable and accurate examination of mortality, we would like to emphasize that the risk of death from all causes and liver-related diseases was significantly lower for chronic hepatitis C patients for whom interferon was effective in normalizing ALT than for patients who did not receive interferon, even when HCV was not eradicated. However, the risk of death from all causes and liver-related diseases was not reduced in biochemical nonresponders.

In conclusion, the findings reported here indicate that interferon therapy improves long-term survival in chronic hepatitis C patients showing a biochemical as well as a virological response by preventing liver-related deaths.

REFERENCES

1 Yokosuka O, Kato N, Hosoda K *et al.* Efficacy of long-term interferon treatment in chronic liver disease evaluated by sensitive polymerase chain reaction assay for hepatitis C virus RNA. *Gut* 1995; 37: 721–726.

2 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma. Analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; 12: 671–675.

3 Yano M, Kumada H, Kage M *et al.* The long-term pathological evolution of chronic hepatitis C. *Hepatology* 1996; 23: 1334–1340.

4 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; 28: 930–938.

5 Davis GL, Balart LA, Schiff ER *et al.* Treatment of chronic hepatitis C with recombinant interferon alpha. A multicenter randomized controlled trial. *N Engl J Med* 1989; 321: 1501–1506.

6 Di Bisceglie AM, Martin P, Kassianides C *et al.* Recombinant interferon alpha therapy for chronic hepatitis C. A randomized, double blind placebo-controlled trial. *N Engl J Med* 1989; 321: 1506–1510.

7 Hagiwara H, Hayashi N, Mita E *et al.* Detection of hepatitis C virus RNA in serum of patients with chronic hepatitis C treated with interferon-α. *Hepatology* 1992; 15: 37–41.

8 Hagiwara H, Hayashi N, Mita E *et al.* Quantitative analysis of hepatitis C virus RNA in serum during interferon alfa therapy. *Gastroenterology* 1993; 104: 877–883.

9 Kasahara A, Hayashi N, Hiramatsu N *et al.* Ability of prolonged interferon treatment to suppress relapse after cessation of therapy in patients with chronic hepatitis C: a multicenter randomized controlled trial. *Hepatology* 1995; 21: 291–297.

10 Shiratori Y, Imazeki F, Moriyama M *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have showed sustained response to interferon therapy. *Ann Intern Med* 2000; 132: 517–524.

11 Hiramatsu N, Hayashi N, Kasahara A *et al.* Improvement of liver fibrosis in chronic hepatitis C patients treated with natural interferon alpha. *J Hepatol* 1995; 22: 135–142.

12 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatology* 1998; 27: 1394–1402.

13 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. *Ann Intern Med* 1999; 131: 174–181.

14 Tanaka H, Tsukuma H, Kasahara A *et al.* Effect of interferon therapy on the incidence of hepatocellular carcinoma and mortality of patients with chronic hepatitis C: a retrospective cohort study of 738 patients. *Int J Cancer* 2000; 87: 741–749.

15 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. *Ann Intern Med* 1998; 129: 94–99.

16 Ikeda K, Satoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124–1130.

17 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage; a retrospective study of 1146 patients. *J Hepatol* 1999; 30: 653–659.

18 Benvegnu L, Chemello L, Noventa F, Fattovich G, Pontisso P, Alberti A. Retrospective analysis of the effect of interferon therapy on the clinical outcome of patients with viral cirrhosis. *Cancer* 1998; 83: 901–909.
19 Valla DC, Chevallier M, Marcellin P *et al.* Treatment of hepatitis C virus-related cirrhosis: a randomized, controlled trial of interferon alfa-2b versus no treatment. *Hepatology* 1999; 29: 1870–1875.
20 Serfaty L, Aumaitre H, Chazouilleres O *et al.* Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 1998; 27: 1435–1440.
21 Nishiguchi S, Shiomi S, Nakatani S *et al.* Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet* 2001; 357: 196–197.
22 Fattovich G, Giustina G, Degos F *et al.* Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; 112: 463–472.
23 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis C: results of a large prospective cohort study. *Hepatology* 1998; 28: 1687–1695.
24 Yoshida H, Arakawa Y, Sata M *et al.* Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology* 2002; 123: 483–491.
25 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: grading and staging. *Hepatology* 1994; 19: 1513–1520.
26 Statistics and Information Department, Japan Ministry of Health and Welfare. *Vital Statistics in Japan* (in Japanese). Tokyo: Health and Welfare Statistics Association, 2002.

J Gastroenterol 2004; 39:1069–1077
DOI 10.1007/s00535-004-1448-0

Journal of Gastroenterology

Interferon therapy for aged patients with chronic hepatitis C: improved survival in patients exhibiting a biochemical response

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Background. In Japan, generally, patients with chronic hepatitis C are aged. The aim of this study was to investigate the effect of interferon (IFN) therapy on the mortality of chronic hepatitis C patients over age 60. ***Methods.*** Seven-hundred and seven patients with histologically proven chronic hepatitis C were enrolled in this study; 649 received IFN therapy (IFN group) and 58 did not (control group). The standardized mortality ratio (SMR) and Cox proportional hazard regression analysis were used to evaluate the effect of IFN on the survival of the patients. ***Results.*** Mean follow-up periods in the IFN and control groups were 5.7 and 6.7 years, respectively. During follow-up, 13 patients in the control group died (7 of liver-related diseases) and 42 in the IFN group died (29 of liver-related diseases). The SMRs of the control and IFN groups were 1.40 (95% confidence interval [CI], 0.76–2.45) and 0.73 (95% CI, 0.52–0.98) for overall death, and 10.70 (95% CI, 4.29–22.05) and 5.05 (95% CI, 3.38–7.26) for liver-related death, respectively. Sustained and transient biochemical responders in the IFN group (SMR, 0.53; 95% CI, 0.01–2.97 and SMR, 3.25; 95% CI, 0.87–8.32, respectively) showed lower liver-related mortality compared with the control group. In patients with sustained virological response, liver-related mortality was also very low (SMR, 0.65; 95% CI, 0.01–3.61). The risk for liver-related death of sustained and transient biochemical responders was also low compared with that of the control group (adjusted risk ratios 0.10 [95% CI, 0.01–0.95] and 0.50 [95% CI, 0.11–2.21], respectively). ***Conclusions.*** These results suggest that IFN treatment could reduce liver-related mortality in chronic hepatitis C patients over age 60, notably in patients showing a biochemical response and in those showing a sustained virological response.

Key words: interferon, chronic hepatitis C, aged, liver-related mortality, standardized mortality ratio

Introduction

A high prevalence of hepatitis C virus (HCV) infection is observed in patients with hepatocellular carcinoma (HCC) in Japan.[1–4] In the early 1990s, interferon (IFN) was introduced, and it is now widely used worldwide, as well as in Japan, for the treatment of patients with chronic hepatitis C. Hitherto, many studies, including our own reports, have shown that IFN therapy reduced the incidence of HCC in patients with chronic hepatitis C.[5–10]

Recently, several groups have studied the effect of IFN therapy on survival in patients with chronic hepatitis C. Most of these studies reported that IFN therapy improved the survival of HCV-related chronic hepatitis and cirrhosis, although some studies did not find any efficacy of IFN therapy on survival.[10–19] We also reported the beneficial effect of IFN therapy on survival in chronic hepatitis C patients. In that report, we also

Received: December 28, 2003 / Accepted: March 24, 2004
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showed that the effect of IFN therapy on survival was notable in the patients exhibiting sustained and transient biochemical responses, as well as in those showing sustained virological response.[20]

Many clinical trials showed that IFN therapy resulted in normalization of serum aminotransferase levels and eradication of serum HCV RNA, although a sustained virological response was achieved in a limited number of patients.[21-25] Recently, a combination therapy of ribavirin and IFN, or pegylated IFN, has been shown to have efficacy superior to IFN monotherapy for chronic hepatitis C.[26-28]

Patients in Japan with chronic hepatitis C are, generally, aged.[29,30] Also, patients with HCV-related HCC have been shown to be old, with a peak around age 70.[31] Despite the beneficial effects of IFN therapy or combination therapy of IFN and ribavirin for chronic hepatitis C patients, these treatments have several adverse effects which are not tolerable, especially for aged patients who have illnesses other than liver disease.[32] If IFN therapy does not prolong life expectancy in aged patients with chronic hepatitis C, the indications for IFN therapy in these patients may be very limited. Therefore, it is very important to investigate whether IFN therapy could improve survival in aged patients with chronic hepatitis C.

The aim of this study was to evaluate the effect of IFN therapy on mortality in aged patients with chronic hepatitis C. We conducted a multicenter, large-scale, retrospective cohort study of chronic hepatitis C patients over 60 years of age.

Patients and methods

Patients

We found previously that IFN therapy improved the survival in patients with chronic hepatitis C.[20] Of the 2954 patients with chronic hepatitis C in that study, we enrolled 707 patients over age 60 in the present study, to investigate the effect of IFN therapy on mortality in aged patients. Accordingly, the inclusion criteria were the same as those of the previous study: (1) histological diagnosis of chronic hepatitis or cirrhosis; (2) no history of clinical signs, at entry into the study, of complications of cirrhosis, i.e., ascites, jaundice, encephalopathy, or variceal bleeding; (3) no evidence of HCC at entry into the study, as assessed by ultrasonography and/or computed tomography; (4) absence of serum hepatitis B surface antigen; (5) absence of coexisting liver diseases, such as autoimmune hepatitis or primary biliary cirrhosis; (6) absence of excessive alcohol consumption (>80 g/day); and (7) absence of human immunodeficiency virus antibodies.[20]

The IFN group comprised 649 patients who had started IFN therapy between 1992 and 1997 and had received a 4- to 12-month course of IFN, which was initiated within 1 month after liver biopsy. None of the patients had received IFN therapy before entry into this study. The control group consisted of 58 patients who had received liver biopsies between 1986 and 1997, but who did not undergo IFN therapy.

Biochemical responses to IFN therapy were categorized as follows. Patients whose alanine aminotransferase (ALT) levels decreased to the normal range during therapy and remained normal for up to 24 weeks after the end of the therapy were considered to have a sustained biochemical response. Patients whose ALT levels decreased to the normal range by the end of therapy, remained normal during therapy, but returned to abnormal levels during the 24 weeks following the end of the IFN therapy were considered to have a transient biochemical response. All other ALT patterns were classified as showing biochemical non-response. A sustained virological response was defined as persistent HCV RNA negativity during IFN therapy and follow-up. Patients showing positive HCV RNA after IFN therapy were classified as virological non-responders.

Follow-up

Abdominal ultrasonography or computed tomography and biochemical examinations, including α-fetoprotein, were carried out before a liver biopsy and every 3 to 6 months during follow-up, equally in the IFN and control groups. The starting date of follow-up for patients in the control and IFN groups was defined as the date of liver biopsy. Follow-up data that were not available were collected from the resident registry of the local municipal office. In the patients residing in Osaka whose follow-up data were not obtained, the Osaka Cancer Registry was used, and the data were available until the end of 1999.[6] Therefore, it was decided to use the date of death or the end of 1999 as the end of follow-up. Because the longest observation period of the patients in the IFN group was 96 months, only the follow-up data for the first 96 months were considered in the control group. Causes of death were divided into liver-related and liver-unrelated deaths. Causes of liver-related death included HCC, liver failure, and esophageal variceal bleeding.

Informed consent was obtained from each patient included in the study. The study protocol was in accordance with the Helsinki Declaration of 1975 (revised in 1983) and was approved by the Ethics Committee of the Osaka University Graduate School of Medicine.

Table 1. Baseline characteristics of the interferon and control groups

	Interferon group						
	Virological response		Biochemical response				
	Sustained response (n = 161)	Non-response (n = 484)	Sustained response (n = 206)	Transient response (n = 144)	Non-response (n = 299)	Total (n = 649)	Control group (n = 58)	P value
Age (years; mean ± SD)	63.6 ± 3.0	63.3 ± 2.9	63.8 ± 3.1	63.0 ± 2.8	63.1 ± 2.8	63.3 ± 2.9	64.1 ± 3.1	0.06
Age distribution (years; %)								
60–64	67.7	71.1	63.6	75.0	72.9	70.4	56.9	0.03
≥65	32.3	28.9	36.4	25.0	27.1	29.6	43.1	
Male/Female	110/51	272/212	134/72	80/64	171/128	385/264	31/27	0.38
Histologic staging score (%)								
0	0.6	0.2	0.5	0.0	0.3	0.3	5.2	0.06
1	24.8	18.2	27.7	25.0	12.4	20.0	31.0	
2	29.2	27.7	26.7	28.5	28.8	28.0	20.7	
3	39.8	46.9	40.3	39.6	50.5	44.8	31.0	
4	5.6	7.0	4.9	6.9	8.0	6.8	12.1	
ALT (IU/l; mean ± SD)	113 ± 82	107 ± 68	110 ± 86	87 ± 45	117 ± 69	108 ± 71	105 ± 80	0.75

Histological evaluation

In all patients, liver biopsy was undertaken before IFN therapy. Sections were stained with hematoxylin-eosin and Azan-Mallory and analyzed by two pathologists in a blinded manner. For the assessment of liver histology, the classification of Desmet et al.[33] was used.

Statistical analysis

To compare the distribution of age at liver biopsy and histological staging between the IFN and control groups, the Wilcoxon rank-sum test was used. Differences in age at liver biopsy and ALT between the two groups was assessed for significance by Student's t-test. The χ^2 test was used to compare sex differences. The Kaplan-Meier method was used to compare the cumulative survival rates in the IFN and control groups.

We compared the observed number of deaths with the expected number of deaths, which was calculated by applying sex-, 5-year age, 5-year calendar time, and cause-specific mortality rates for the general population in Japan, as prepared by the Statistics and Information Department, Japan Ministry of Health and Welfare.[34] The standardized mortality ratio (SMR) was expressed by dividing the observed number of deaths by the expected number of deaths. Survival was also analyzed by Cox proportional hazards regression. For analysis, age, sex, stage of liver fibrosis (stages 0,1/2/3/4), time of liver biopsy (until 1992/after 1993), and IFN therapy were used as variables. SMRs and hazard risk ratios were expressed with 95% confidence intervals (CIs).

Data analysis was performed with the SAS/PC statistical package (SAS Institute, Cary, NC, USA). All reported P values were two-sided, and a P value of less than 0.05 was considered to be significant.

Results

Baseline characteristics

In the IFN group, 206 patients (31.7%) had a sustained biochemical response, 144 (22.2%) had a transient biochemical response, and 299 patients (46.1%) were biochemical non-responders. Four sustained biochemical responders whose serum HCV RNA was not examined during follow-up were excluded from the analysis. Accordingly, 161 patients (25.0%) of the 645 IFN-treated patients were classified as sustained virological responders. Table 1 shows the baseline characteristics of the IFN and control groups. Age at entry, sex, histologic staging score, and serum ALT level did not differ between the two groups. The proportion of patients more than 65 years of age in the control group was higher than that in the IFN group (P = 0.03).

Table 2. Cumulative survival rate calculated from overall deaths

	Interferon group						Control group
	Virological response		Biochemical response			Total	
	Sustained response	Non-response	Sustained response	Transient response	Non-response		
Mean follow-up period (years; mean ± SD)	5.7 ± 1.6	5.7 ± 1.7	5.6 ± 1.7	5.7 ± 1.8	5.8 ± 1.6	5.7 ± 1.7	6.7 ± 1.7
4-Year survival rate	99.3%	96.2%	98.4%	99.2%	95.0%	97.0%	93.0%
8-Year survival rate	94.6%	86.8%	94.3%	93.0%	83.4%	88.7%	73.9%
P Value[a]	<0.001	0.0197	<0.001	0.0036	0.1212	0.0031	

[a]The log rank test was used to determine the difference against the control group

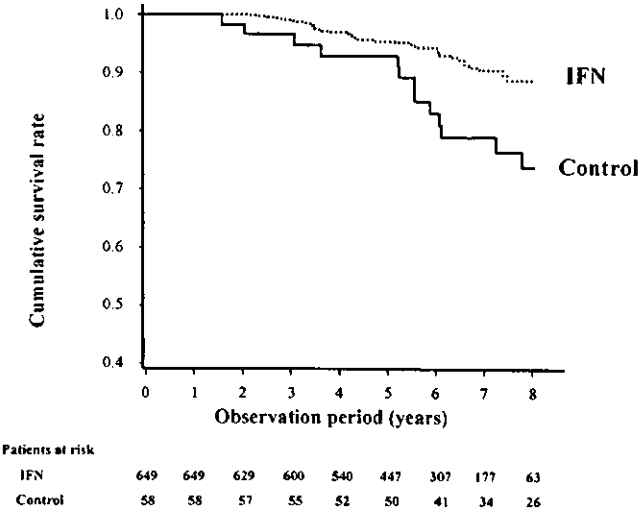

Fig. 1. Cumulative survival rates in the interferon (*IFN*; *dotted line*) and control (*solid line*) groups. Log-rank test of the two curves showed a significant difference between the two groups ($P = 0.003$)

Cumulative survival and cause of death

The mean follow-up periods of the IFN and control groups were 5.7 and 6.7 years, respectively. The mean follow-up periods of the patients with each response in the IFN group are shown in Table 2. Figure 1 shows the cumulative survival rates of the IFN and control groups, estimated by the Kaplan-Meier method. The 8-year survival rates of the IFN and control groups were 88.7% and 73.9%, respectively (log-rank test; $P = 0.003$; Table 2). The cumulative survival rates of sustained virological responders were significantly higher than those for virological non-responders (log-rank test; $P = 0.02$). The 8-year survival rates of sustained virological responders and virological non-responders were 94.6% and 86.8%, respectively (Table 2). The cumulative survival rates of both the sustained and transient biochemical responders were significantly higher than that of the biochemical non-responders (log-rank test; $P = 0.007$ and $P = 0.049$; Fig. 2). The 8-year survival rates of sustained and transient biochemical respon-ders and biochemical non-responders were calculated to be 94.3%, 93.0% and 83.4%, respectively (Table 2).

During follow-up, 42 of the 649 IFN-treated patients and 13 of the 58 control patients died. The numbers of liver-related and liver-unrelated deaths in the IFN and control groups are shown in Table 3. Liver-related deaths corresponded to 69% of all deaths (29/42) in the IFN group and 54% of all deaths (7/13) in the control group. HCC was the major cause of liver-related deaths in both groups. Only one liver-related death (17%) was found in the deaths of sustained biochemical respond-