ported similar results, assuming that the greatest dimension of occult HCC was 5 mm before IFN therapy.[34] We calculated the growth interval between a single HCC cell and an HCC measuring 1 cm in greatest dimension on the assumption that the DT of HCC was 90 days and concluded that the growth interval may be > 6 years.[8] Because smaller and well differentiated HCCs have a longer DT, the growth interval to reach 1 cm in greatest dimension may be much longer than 6 years. Therefore, it is probable that small HCC may have existed in the liver prior to IFN therapy in the current SR HCC patients.[35]

It cannot be determined with certainty how long SR patients should be followed after they complete IFN therapy. Judging from the results obtained in the current study, we recommend that, when SR patients are male, age > 50 years old, and have F3 or F4 histologic stage, they should be checked by US or CT at least twice per year for > 10 years. Other SR patients with less advanced disease should be checked at least once per year.

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Type I interferon receptor and response to interferon therapy in chronic hepatitis C patients: a prospective study

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SUMMARY. The type I interferon (IFN) receptor consists of at least two subunits, IFNAR1 and IFNAR2. We previously found a correlation between IFNAR1 and IFNAR2 expression in liver, and a correlation in IFNAR2 expression, but not in IFNAR1, between liver and peripheral blood mononuclear cells (PBMCs). The aim of this study was to prospectively assess whether IFNAR2 expression levels in PBMCs as well as in liver act as markers for predicting response to IFN therapy in chronic hepatitis C patients. Fifty-two Japanese patients with chronic hepatitis C, were enrolled. IFNAR2 mRNA was quantified using competitive polymerase chain reaction, in liver and PBMC specimens, and of the 52 patients assigned to receive a 6-month course of interferon-α therapy, 36 patients who received more than 300 million units of interferon were analysed. IFNAR2 mRNA expression levels were significantly higher in liver than in PBMCs in all 36 patients ($P = 0.016$). Seventeen sustained virologic responders showed lower pretreatment hepatitis C virus (HCV)-RNA levels ($P = 0.017$) in serum and higher pretreatment levels of IFNAR2 mRNA in liver ($P = 0.007$), but not in PBMCs, compared with nonsustained virologic responders. In multivariate analysis, these factors were independently associated with a sustained virologic response (i.e. HCV-RNA level: odds ratio 0.23, 95% CI 0.038–0.864; and IFNAR2 in liver: odds ratio 1.116, 95% CI 1.015–1.227). Hence, IFNAR2 expression levels in liver, but not in PBMCs, is predictive of response to IFN treatment in chronic hepatitis C patients.

Keywords: IFNAR1, IFNAR2, liver tissue, peripheral blood mononuclear cells, PBMCs.

INTRODUCTION

To date, interferon (IFN) with or without ribavirin is the only therapy known to eradicate the hepatitis C virus (HCV) and induce long-term normalization of aminotransferase levels in patients with chronic hepatitis C. However, this occurs in <50% of the treated patients. Therefore, the identification of prognostic factors predictive of response to IFN therapy is important and several factors have been reported [1–5]. As IFN elicits antiviral activity by binding to receptors on the cell surface [6,7], expression of the type I IFN receptor in liver tissue is likely to be involved in the pathogenesis of viral hepatitis and response to IFN therapy. In fact, recent studies have demonstrated a significant correlation between hepatic expression of the type I IFN receptor and response to IFN therapy in chronic hepatitis C patients [8–12]. A prospective study, however, has not been conducted.

The type I IFN receptor consists of at least two subunits: the IFNAR1 (IFNα receptor) and the IFNAR2 (IFNα/β receptor) [13,14], both of which have been cloned and are directly involved in signal transduction [13,15,16]. We have previously quantified mRNA levels of both subunits, using a competitive polymerase chain reaction (PCR) assay, in liver and peripheral blood mononuclear cells (PBMCs) from chronic hepatitis C patients [17]. We have demonstrated that levels of IFNAR1 expression are strongly correlated with IFNAR2 levels in liver, and further that IFNAR2 expression, but not IFNAR1 expression, in liver is related to that in

Abbreviations: IFN, interferon; IFNAR1, interferon receptor subunit 1; IFNAR2, interferon receptor subunit 2; PBMC, peripheral blood mononuclear cells.

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PBMCs [17]. These results prompted us to determine whether IFNAR2 expression levels in PBMCs as well as in liver act as possible markers for predicting response to IFN therapy in chronic hepatitis C patients. Herein we prospectively examined the expression levels of IFNAR2 in liver and PBMCs in chronic hepatitis C patients and assessed whether expression levels were related to response therapy.

METHODS

Patients

From March 1999 to February 2001, 52 Japanese patients with chronic hepatitis C, who met the study's criteria, were enrolled in this prospective trial by Yamaguchi University Hospital and affiliated institutions. Criteria for enrollment included: persistently elevated serum alanine aminotransferase (ALT) levels for >6 months prior to enrollment; positive results for HCV-RNA in serum; liver histological examination upon informed consent (showing lesions compatible with chronic hepatitis) performed within a month of enrollment; absence of detectable hepatitis B virus surface antigen; exclusion of all other potential causes of chronic liver disease such as autoimmune hepatitis, primary biliary cirrhosis, drug-induced hepatitis, or metabolic liver disease; no history of alcohol abuse, defined as alcohol intake of ≥80 g/day for longer than 3 years; no pregnancy; platelet count ≥70 000/mm^3 and leukocyte count ≥3000/mm^3.

Study design

After providing informed consent, patients were assigned to receive 6–9 million units (MU) of recombinant IFN alpha-2b (Intron A, Schering-Plough, Osaka, Japan) intramuscularly, six times weekly for 2–4 weeks, followed by 6–9 MU three times weekly for 22–24 weeks (total 468–810 MU). Patients were followed up for >6 months after completion or cessation of therapy. Blood chemistry and blood cell counts were measured at the beginning of treatment, and then every 4 weeks during the treatment and follow-up period. HCV serotype and HCV-RNA levels were measured immediately upon collection of the initial sample at commencement of therapy. PBMC samples were available from all patients on the same day as liver biopsy. Expression levels of IFNAR2 mRNA were determined for both liver tissue and PBMCs, using a competitive PCR assay.

Patients were categorized according to IFN response: patients with a sustained virologic response were defined by the normalization of serum ALT during the 6-month period after completion of treatment and the absence of detectable serum HCV-RNA tested 6 months after completion of therapy; those categorized as nonsustained virologic responders did not meet these criteria. Informed consent in writing was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics committee.

Detection of serum HCV-RNA, HCV serotyping and quantification of HCV-RNA

Serum HCV-RNA was detected using the AMPLICOR HCV amplification kit (Roche Diagnostics, Tokyo, Japan). HCV serotypes were determined by the method of Tanaka *et al.* [18]. Serum viral load was quantified by either a branched DNA probe assay, version 1.0 (Quantiplex, Chiron, Emeryville, CA, USA) or AMPLICORE HCV Monitor test, version 1.0 (Roche Diagnostics), and expressed as the logarithm of copy numbers per millilitre. For statistical purposes, HCV-RNA levels below the detection limit of the branched DNA probe assay (350 000 genome equivalents per millilitre) were set at 350 000 genome equivalents per millilitre.

Determination of IFNAR2 expression levels in liver tissue and PBMCs

Liver biopsy specimens were divided into two portions: one for light microscopy, and the other for measurement of IFNAR2 mRNA. This second portion was immediately put into acid guanidinium solution and frozen in liquid nitrogen. PBMC samples were separated from 10 mL of heparinized blood by density gradient centrifugation with Leuko PREP (Becton Dickinson, Lincoln Park, NJ, USA), washed three times with RPMI 1640 culture medium, and stored at −80 °C until use, as were liver specimens. Levels of IFNAR2 mRNA expression were quantified using a competitive PCR assay, as described previously [17]. In brief, competitive PCR fragments were designed to have deletions and to be amplified by the same PCR primers as those for the amplification of IFNAR2 cDNA. Competitive PCR fragments were quantified by the absorbance value at 260 nm of UV, and serial twofold dilutions were prepared. PCR primers were designed on the basis of the sequences published by Novik *et al.* [13] (Genebank X77722). RNA was extracted from the homogenized PBMCs and liver tissue using 900 μL of RNA zol^{TM}B (Biotex Laboratories Inc., Houston, TX, USA). One microgram of total RNA was reverse transcribed, and the resulting cDNA and a known volume (368, 184, 92, 46, or 23 copies) of competitive PCR fragments were co-amplified. The PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide and bands of expected size were measured visually under UV. The PCR products of competitive fragments for IFNAR2 cDNA were observed at lengths of 622 bp. The reverse-transcribed PCR products of IFNAR2 mRNA were observed at lengths of 759 bp. The quantity of IFNAR2 mRNA was determined by comparing the density of PCR product from PBMCs or liver with that from competitive fragments, and expressed as copy numbers per 10 ng β-actin mRNA amplified from the same specimen.

	Subjects	SVR	Non-SVR	P-value
Number of patients	36	17	19	
Age	49 ± 10	47 ± 9	50 ± 10	0.31
Gender (M/F)	23/13	12/5	11/8	0.43
HCV RNA level (log [copies])	5.5 ± 0.7	5.2 ± 0.7	5.7 ± 0.5	0.017
HCV serotype (1/2/1+2)	20/14/2	8/9/0	12/5/2	0.15
Staging of liver fibrosis (F0/F1/F2/F3/F4)	4/19/7/6/0	2/11/3/1/0	2/8/4/5/0	0.36
Total IFN dose (mega units)	679 ± 180	678 ± 186	679 ± 179	0.99
Expression level of IFNAR2 mRNA*				
Liver	1074 ± 913†	1494 ± 925	698 ± 737	0.007
PBMCs	592 ± 724	715 ± 928	481 ± 478	0.34

Table 1 Parameters of analysed patients with comparison between sustained virologic responders (SVR) and nonsustained virologic responders (non-SVR)

HCV, hepatitis C virus; IFN, interferon.
*Expressed as copy numbers per 10 ng of β-actin mRNA.
†$P = 0.016$ *vs* expression level of IFNAR2 mRNA in PBMCs.
Results were expressed as mean ± SD.

using a human β-actin competitive PCR set (Takara Biochemicals, Tokyo, Japan).

Statistical analysis

Results were expressed as mean ± SD. Differences in proportion were tested by the Fisher's exact test. Mean quantitative values were compared by the Student's *t*-test. Nonparametric data were compared using the Wilcoxon signed rank test. Multiple logistic regression analysis was performed using a multiple regression model to identify factors associated with a sustained virologic response to IFN therapy. Possible associated factors for the sustained virologic response to IFN therapy included eight variables: age, gender, total dose of interferon, HCV subtype, HCV-RNA level, staging for liver fibrosis, expression level of IFNAR2 in liver, and expression level of IFNAR2 in PBMCs. All reported *P*-values were two-tailed and a *P*-value of 0.05 was considered to be significant.

RESULTS

Completion of the assigned course of IFN

Three patients refused the assigned course of IFN therapy. Among the remaining 49 patients treated with IFN, seven were obliged to suspend or cease IFN treatment before completion of therapy because of adverse effects. One patient who completed the scheduled course of IFN therapy could not be followed up for more than 6 months. Liver or PBMCs specimens were not available from eight patients. The mean total dose of administered IFN was 609 ± 247 MU for all patients. Consequently, taking the standard total dose of IFN into consideration, 36 patients who received more than 300 MU of IFN were included in the analysis of factors predicting response to IFN.

Clinical and viral characteristics of patient population

Twenty-three men and 13 women (aged 25–64 years, with a mean age of 49 years) were examined in this study. According to the criteria for staging fibrosis (F0, F1, F2, F3, and F4) [19], four showed an F0 staging, 19 showed F1, seven showed F2, and six showed F3. HCV serotype 1 was detected in 20 patients (55.6%), serotype 2 in 14 (38.9%), and both were detected in two (5.5%). The mean logarithm of pretreatment HCV RNA levels was 5.5 ± 0.7 for analysed patients (Table 1).

Expression level of IFNAR2 in liver and PBMCs

Pretreatment IFNAR2 mRNA levels in liver and PBMCs were greater than the detection limit in 33 (92%) and 30 (83%) of the 36 patients examined, respectively. For statistical purposes, IFNAR2 mRNA levels below the detection limit of our assay were set at 23 copies/10 ng β-actin, as the maximum dilution in which competitive PCR fragments were constantly detected included more than 23 copies of cDNA. The mean levels of IFNAR2 mRNA expression were significantly higher in liver (1074 ± 913 copies/10 ng β-actin) than in PBMCs (592 ± 724 copies/10 ng β-actin) ($P = 0.016$) (Table 1) and were weakly correlated between liver and PBMCs ($r = 0.357$, $P = 0.032$) (Fig. 1). Expression levels of IFNAR2 in liver and PBMCs were not correlated with HCV-RNA levels in serum, and did not differ between HCV serotypes or between stagings based on degree of liver fibrosis.

Response to IFN and predictive factors of response

Seventeen patients (47.2%) treated with IFN therapy were sustained virologic responders. Sustained and nonsustained virologic responders did not differ significantly with respect

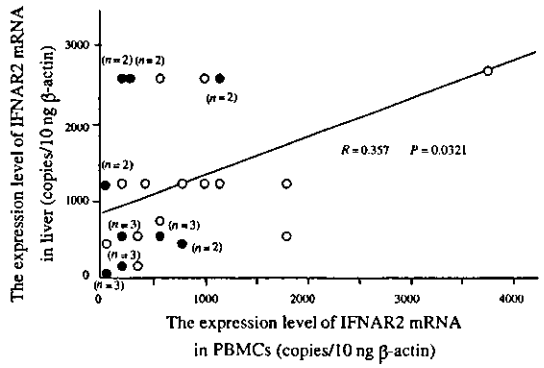

Fig. 1 The expression level of IFNAR2 mRNA was weakly correlated between liver and peripheral blood mononuclear cells in 36 patients analysed ($r = 0.357$, $P = 0.032$). Open circles represent individual patients and closed circles represent multiple patients (number indicated in parentheses).

to age, gender, pretreatment staging of liver fibrosis, HCV serotype or total dose of IFN. However, pretreatment HCV RNA levels were lower in sustained than in nonsustained virologic responders (5.2 ± 0.7 *vs* 5.7 ± 0.5, $P = 0.017$) (Table 1). In addition, pretreatment IFNAR2 mRNA levels in liver were significantly higher in sustained than in nonsustained virologic responders (1494 ± 925 copies/10 ng β-actin *vs* 698 ± 737 copies/10 ng β-actin, $P = 0.007$), but expression levels in PBMCs did not differ between groups (715 ± 928 copies/10 ng β-actin *vs* 481 ± 478 copies/10 ng β-actin, $P = 0.34$) (Table 1). HCV-RNA levels [odds ratio 0.23, 95% confidence interval (CI) 0.038–0.864, $P = 0.057$] and IFNAR2 mRNA levels in liver (odds ratio 1.12, 95% CI 1.015–1.227, $P = 0.023$) were both shown by multivariate analysis to be predictive factors of response to IFN therapy (Table 2). This implies that with each increase of 100 copies of IFNAR2 mRNA, a sustained virologic response is likely to occur 1.12 times more often.

DISCUSSION

This is the first report to prospectively evaluate whether type I IFN receptor expression levels in liver and PBMCs are predictive of response to IFN therapy in chronic hepatitis C patients. Despite the consecutive recruitment of patients in the present study, our patient population varied somewhat from previous reports [1–5], with respect to lower levels of serum HCV-RNA and the relatively high prevalence of HCV serotype 2, which may have resulted in a higher proportion of sustained virologic responders (47.2%).

Table 2 Predictive factors associated with response to interferon therapy in multivariate analysis

Variables	Odds ratio	95% CI	*P*-value
HCV RNA level (log [copies])	0.23	0.038–0.864	0.057
Expression level of IFNAR2 mRNA in liver*	1.12	1.015–1.227	0.023

HCV, hepatitis C virus.
*Expressed as copy numbers per 10 ng of β-actin mRNA.

The sensitivity and reproducibility of the competitive PCR assay utilized herein is believed to be reliable. We have previously confirmed the quantitative nature of our competitive PCR assays using IFNAR2 cDNA derived from HepG2 cells as a control [17]. However, whether IFNAR2 mRNA expression in homogenized liver tissue directly reflects expression solely in hepatocytes needs to be further examined, as cellular components of liver such as hepatocytes, fibroblasts, and infiltrating lymphocytes may express differing amounts of IFNARs. Moreover, it has not been determined whether levels of IFNAR2 mRNA reflect its protein expression levels in hepatocytes. To clarify this point, we compared expression levels of IFNAR2 mRNA in liver with those of IFNAR2 protein in hepatocytes which were quantified by a labelling index for antigen positive cells in immunohistochemical analysis, and found a weak, but significant correlation ($r = 0.46$, $P = 0.009$) between hepatic expression of mRNA and its protein expression in hepatocytes [20]. Therefore, we believe that the present competitive PCR method can be used to estimate the expression level of IFNAR2 protein in hepatocytes. As we did not establish the flow cytometric analysis of IFNAR protein in PBMCs [21] at the beginning of this prospective study and considered that IFNAR2 expression in liver and PBMCs should be assayed in the same method, we measured IFNAR2 mRNA, not IFNAR2 protein, in the present study.

The IFN molecule itself has no antiviral effect; the binding of IFN to its receptor is an essential first step required to exhibit antiviral activity. However, whether upregulation of the type I IFN receptor results in enhanced antiviral activity by IFN therapy is not confirmed. Mizukoshi *et al.* [22] have demonstrated upregulation of the type I IFN receptor by IFN-γ in HepG2 cells and that the 2-5 adenylate synthetase activity induced by combined treatment with type I IFN and IFN-γ was significantly higher than by type I IFN alone. These results are in agreement with our present observation that higher IFNAR2 mRNA expression in liver was a predictive factor associated with sustained virologic response to IFN therapy in chronic hepatitis C patients. A lower level of IFNAR2 expression in PBMCs may in part account for the poor sensitivity to IFN of HCV in PBMCs that has been demonstrated as persistence of HCV-RNA in PBMCs during IFN therapy, in spite of the disappearance of HCV-RNA from serum [23,24]. However, as IFN has not only an antiviral but also an antiproliferative effect [25,26], lower expression of the IFN receptor may be convenient for lymphocytes that are the effectors of the immune response to viral hepatitis.

The present results have revealed no correlation between IFNAR2 levels in PBMCs and patterns of IFN response. Although we have demonstrated a correlation in IFNAR2 expression levels between liver and PBMCs in the present study, the correlation coefficient was low. Also, the expression level of IFNAR2 was significantly higher in liver than in PBMCs. These results may account for the lack of association between IFNAR2 expression levels in PBMCs and response to IFN therapy. That hepatocytes act as the main replication site of HCV suggests that the antiviral activity in hepatocytes is closely related to the outcome of IFN therapy in chronic hepatitis C patients. Therefore, the present results that hepatic expression levels of IFNAR2, but not in PBMCs, were predictive for response to IFN therapy is in agreement with previous findings.

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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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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.

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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 ($P = 0.18$).

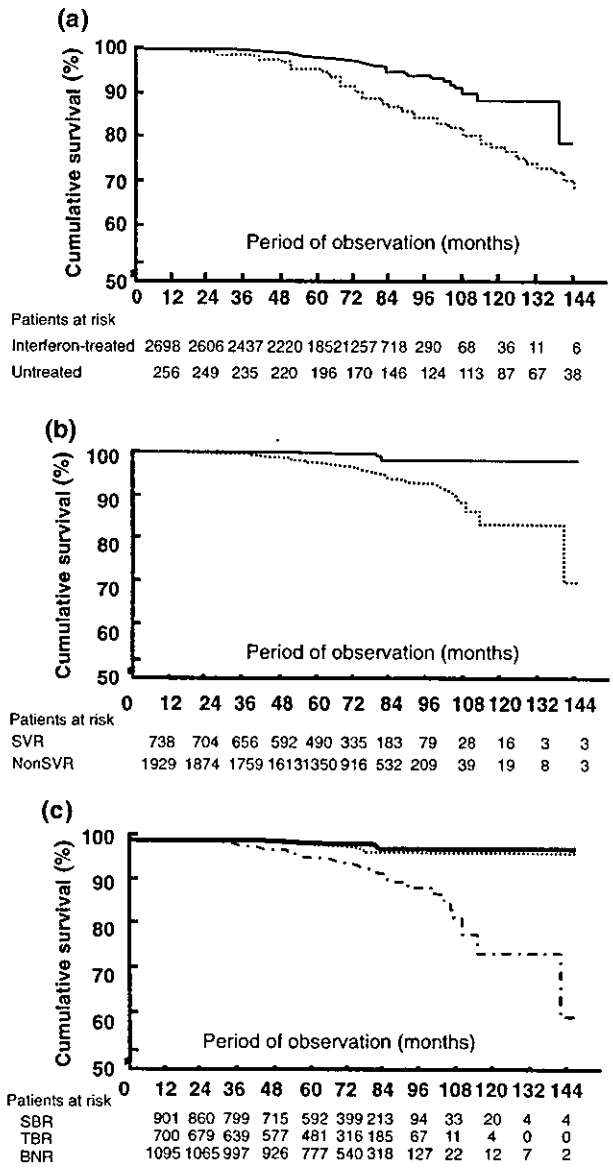

Fig. 1 Cumulative survival rates from all causes of death for patients with chronic hepatitis C. (a) For interferon-treated patients (solid line) and untreated patients (dotted line). (b) According to the virological response to interferon therapy; sustained virological responders (SVR) (solid line) 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.

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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

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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					
	Virological response		Biochemical response			
	Sustained response	Non-response	Sustained response	Transient response	Non-response	Total	Control group
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

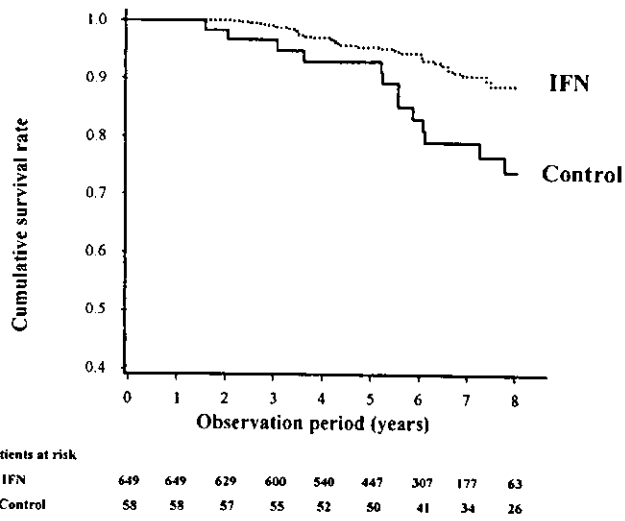

Fig. 1. Cumulative survival rates in the interferon (*IFN*; *dotted line*) and control (*solid line*) groups. Log-rank test of the two curves showed a significant difference between the two groups ($P = 0.003$)

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-