References

1. Yoshida, H., Shiratori, Y., Moriyama, M. *et al.* (1999). Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. *Annals of Internal Medicine* **131**, 174–81.

2. Kasahara, A., Hayashi, N., Mochizuki, K. *et al.* (1998). Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatology* **27**, 1394–402.

3. Imai, Y., Kawata, S., Tamura, S. *et al.* (1998). Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. *Annals of Internal Medicine* **129**, 94–9.

4. Ikeda, K., Saitoh, S., Chayama, K. *et al.* (1999). Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* **29**, 1124–30.

5. Tanaka, H., Tsukuma, H., Kasahara, A. *et al.* (2000). 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. *International Journal of Cancer* **87**, 741–9.

6. Okanoue, T., Itoh, Y., Kirishima, T. *et al.* (2002). 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. *Hepatology Research* **23**, 62–77.

7. Okanoue, T., Itoh, Y., Minami, M. *et al.* (1999). Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. *Journal of Hepatology* **30**, 653–9.

8. Hino, K., Kitase, A., Satoh, Y. *et al.* (2002). Interferon retreatment reduces or delays the incidence of hepatocellular carcinoma in patients with chronic hepatitis C. *Journal of Viral Hepatitis* **9**, 370–6.

9. Donat, M. F., Arosio, E., Ninno, E. D. *et al.* (2001). High rates of hepatocellular carcinoma in cirrhotic patients with liver cell proliferative activity. *Hepatology* **34**, 523–8.

10. Hayashi, J., Aoki, H., Kajino, K. *et al.* (2000). Hepatitis C virus core protein activates the MAP/ERK cascade synergistically with tumor promoter TPA, but not with epidermal growth factor or transforming growth factor alpha. *Hepatology* **32**, 958–61.

11. Ito, Y., Sasaki, Y., Horimoto, M. *et al.* (1998). Activation of mitogen-activated protein kinase/extracellular signal-regulated kinases in human hepatocellular carcinoma. *Hepatology* **27**, 951–8.

12. Seger, R. & Krebs, E. G. (1995). The MAPK signaling cascade. *FASEB Journal* **9**, 726–35.

13. Talarmin, H., Rescan, C., Cariou, S. *et al.* (1999). The mitogen-activated protein kinase kinase/extracellular signal-regulated kinase cascade activation is a key signalling pathway involved in the regulation of G_1 phase progression in proliferating hepatocytes. *Molecular and Cellular Biology* **19**, 6003–11.

14. Cheng, M., Sexl, V., Sherr, C. J. *et al.* (1998). Assembly of cyclin D-dependent kinase and titration of $p27^{Kip1}$ regulated by mitogen-activated protein kinase kinase (MEK1). *Proceedings of the National Academy of Sciences, USA* **95**, 1091–6.

15. Balmanno, K. & Cook, S. J. (1999). Sustained MAP kinase activation is required for the expression of cyclin D1, $p21^{Cip1}$ and a subset of AP-1 proteins in CCL39 cells. *Oncogene* **18**, 3085–91.

16. Masaki, T., Shiratori, Y., Rengifo, W. *et al.* (2003). Cyclins and cyclin-dependent kinases: comparative study of hepatocellular carcinoma versus cirrhosis. *Hepatology* **37**, 534–43.

17. Huang, H., Fujii, M., Sankila, A. *et al.* (1999). Beta-catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C virus infection. *American Journal of Pathology* **155**, 1795–801.

18. Buendia, M. A. (2000). Genetics of hepatocellular carcinoma. *Seminars in Cancer Biology* **10**, 185–200.

19. Okuda, M., Li, K., Beard, M. R. *et al.* (2002). Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* **122**, 366–75.

20. Moriya, K., Nakagawa, K., Santa, T. *et al.* (2001). Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Research* **61**, 4365–70.

21. Kato, J., Kobune, M., Nakamura, T. *et al.* (2001). Normalization of elevated hepatic 8-hydroxy-2′-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Research* **61**, 8697–702.

22. Desmet, V. J., Garber, M., Hoofnagel, J. H. *et al.* (1994). Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* **19**, 1513–20.

23. Takayama, T., Makuuchi, M., Hirohashi, S. *et al.* (1998). Early hepatocellular carcinoma as an entity with a high rate of surgical cure. *Hepatology* **28**, 1241–6.

24. Ikeda, K., Arase, Y., Saitoh, S. *et al.* (2000). Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-a prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* **32**, 228–32.

25. David, M., Petricoin, E., III, Benjamin, C. *et al.* (1995). Requirement for MAP kinase (ERK2) activity in interferon alpha- and interferon beta-stimulated gene expression through STAT proteins. *Science* **269**, 1721–3.

26. Romerio, F. & Zella, D. (2002). MEK and ERK inhibitors enhance the antiproliferative effect of interferon-α2b. *FASEB Journal* **16**, 1680–2.

27. Murphy, D., Detjen, K. M., Welzel, M. *et al.* (2001). Interferon-α delays S-phase progression in human hepatocellular carcinoma cells via inhibition of specific cyclin-dependent kinases. *Hepatology* **33**, 346–56.

28. Chao, Y., Shi, Y. L., Chiu, J. H. *et al.* (1998). Overexpression of cyclin A but not Skp 2 correlates with the tumor relapse of human hepatocellular carcinoma. *Cancer Research* **58**, 985–90.

29. Dogukan, A., Akpolat, N., Celiker, H. *et al.* (2003). Protective effect of interferon-alpha on carbon tetrachloride-induced nephrotoxicity. *Journal of Nephrology* **16**, 81–4.

30. Lu, G., Shimizu, I., Cui, X. *et al.* (2002). Interferon-alpha enhances biological defense activities against oxidative stress in cultured rat hepatocytes and hepatic stellate cells. *Journal of Medical Investigation* **49**, 172–81.

31. Higueras, V., Raya, A., Rodrigo, J. M. *et al.* (1994). Interferon decreases serum lipid peroxidation products of hepatitis C patients. *Free Radical Biology & Medicine* **16**, 131–3.

32. Sakon, M., Nagano, H., Dono, K. *et al.* (2002). Combined intra-arterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* **94**, 435–42.

33. Takaoka, A., Hayakawa, S., Yanai, H. *et al.* (2003). Integration of interferon-α/β signaling to p53 responses in tumor suppression and antiviral defence. *Nature* **424**, 516–23.

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Types of human leukocyte antigen and decrease in HCV core antigen in serum for predicting efficacy of interferon-α in patients with chronic hepatitis C: analysis by a prospective study

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Background. A prospective study was conducted to evaluate the influence of host factors, including human leukocyte antigen (HLA), and viral factors, including hepatitis C virus (HCV) core antigen, on the response to interferon (IFN)-α. ***Methods.*** Natural IFN-α was given to 66 patients with chronic hepatitis C at a dose of 9 million units per day for 2 weeks, followed by 9 million units three times a week for 22 weeks. ***Results.*** Sustained virological response without detectable HCV RNA in serum 24 weeks after the end of IFN therapy was achieved in 21 patients, while it was not in 32 patients; the remaining 13 patients were not evaluated. HCV core antigen and HCV RNA started to decrease 1 and 4 weeks, respectively, after the commencement of IFN in responders ($P = 0.02$ and $P = 0.05$, respectively). On univariate analysis, age of 50 years or less ($P < 0.001$); lack of HLA DR6 ($P = 0.018$) or DR52 ($P < 0.041$); platelets more than $14 \times 10^4/mm^3$ ($P = 0.031$); HCV core antigen 500 fmol/l or less ($P = 0.001$); and HCV RNA 100 KIU/ml or less were predictive of response. On multivariate analysis, age 50 years or less (odds ratio [OR], 4.009; $P = 0.039$); lack of HLA DR6 (OR, 8.130; $P = 0.027$); IFN-naive (OR, 11.63; $P = 0.016$); HCV core antigen 500 fmol/l or less (OR, 10.61; $P = 0.007$); and genotypes other than 1b (OR, 8.929; $P = 0.010$) were predictive of response. ***Conclusions.*** Lack of HLA DR6 determined the response to IFN. HCV core antigen was useful in predicting and monitoring the response to IFN.

Key words: hepatitis C virus, interferon, core antigen, human leukocyte antigen

Introduction

There are 190 million people estimated to be infected with hepatitis C virus (HCV) in the world,[1] and in Japan alone, 1.5 million are infected with HCV. Persistent HCV infection can induce a spectrum of chronic liver disease, ranging from chronic hepatitis through liver cirrhosis to eventual hepatocellular carcinoma (HCC) during the lifetime.[2] Liver cancers, including HCC and cholangioma, rank as the fourth most frequent malignancy in Japan and cause more than 30000 deaths annually, and by far the greatest majority of liver cancers (>95%) are HCC.[3] In the individuals infected with HCV, it is necessary to diagnose chronic hepatitis and treat them without delay, in order to prevent the development of HCC.

Interferon (IFN) is the only drug that can clear HCV infection. Not all patients with chronic hepatitis C, however, respond virologically, with the loss of HCV RNA from serum, and/or biochemically, with the normalization of alanine aminotransferase (ALT) levels in serum. A number of factors have been reported to influence the response to IFN. They include virological factors, such as HCV genotypes[4–8] and viral load;[4,6,7,9] as well as host factors, such as age,[4,8] sex,[9] pretreatment ALT levels,[8] and fibrosis of the liver.[4,5] Some of these factors are not unanimously agreed upon, while others have not yet been studied enough to be conclusive.

Human leukocyte antigen (HLA) has attracted attention for its possible influence on the response to IFN-α therapy.[10–17] Insofar as HLA is associated with the immune responses of the host, it may modify the pathogenesis of chronic hepatitis C that is mediated by the immunity of the host to HCV.[16,18–20] As such, HLA may influence the response to IFN-α for treatment of chronic hepatitis C. Because previous studies along this line are retrospective and controversial, we conducted a prospective study to evaluate HLA and other host factors to find their influence on response to IFN-α.

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Recently, an assay method for the immunological determination of the core antigen of HCV has been developed, and HCV core antigen was found to reflect the viral load.[21,22] Because the HCV RNA level is an important viral factor that influences the response to IFN-α,[4,6,7,9] the determination of HCV core antigen could be useful clinically, because the assay method for this purpose is easier and low-cost compared with the determination of HCV RNA levels.[21,22] Hence, the usefulness of an assay for HCV core antigen in predicting and monitoring the response to IFN-α was evaluated prospectively, in comparison with the determination of HCV RNA levels.

Patients and methods

Patients

In the period March 1998 through December 2001, 66 patients with chronic hepatitis C, aged from 23 to 73 years, and including 38 (58%) men were registered for treatment with IFN-α by members of Shinshu University Hospital and 17 institutions constituting the Nagano Interferon Treatment Research Group. The patients fulfilled the requirements that they were older than 20 years and positive for HCV RNA, with elevated alanine aminotransferase (ALT) levels during the previous 6 months. Exclusion criteria included the presence of hepatitis B surface antigen or antibody to human immunodeficiency virus type 1 in serum; leukocytes less than 3000/mm^3 or platelets less than 6×10^4/mm^3; autoimmune diseases, such as autoimmune hepatitis; daily intake of ethanol more than 80 g; psychiatric conditions, such as depression; decompensated liver cirrhosis; and pregnancy. Patients with HCC or a history thereof, as well as those who were judged inappropriate for entering the study by attending physicians, were excluded also. Treatment with glycyrrhizin products such as "sho-saiko-to" and Stronger Neo-Minophagen C (Minophagen Pharmaceutical, Tokyo, Japan) was withdrawn at least a month before the start of IFN-α, but ursodeoxycholic acid was continued. All but 3 patients (2 with hemophilia and 1 with von Willebrand disease) received liver biopsies 6 months before the commencement of IFN-α. Histological diagnosis was based on the criteria of Desmet et al.[23]

The study design conformed to the 1975 Declaration of Helsinki. Written consent was obtained from each patient in regard to IFN-α treatment, determination of HLA types, and use of their serum for the study.

IFN therapy

Natural IFN-α (Sumitomo Pharmaceuticals, Tokyo, Japan) was given to each patient, at a dose of 9 million units (MU) daily for 2 weeks, followed by the same dose given three times a week for 22 weeks; the total dose was 720 MU. HCV RNA was determined in sera collected before treatment and 1, 2, and 4 weeks after the start of IFN-α treatment, and every 4 weeks thereafter, until 48 weeks. The determination of HLA types was performed immediately before IFN-α therapy was begun.

Sustained virological response was defined by the loss of detectable HCV RNA from serum during IFN-α treatment that persisted until 6 months after the completion of therapy.

Determination of HCV markers

HCV RNA was detected with an AMPLICOR HCV test (Roche Diagnostics, Tokyo, Japan) with a sensitivity at 0.5 kilo IU (KIU)/ml, and quantified with an AMPLICOR HCV Monitor test, version 2 (Roche Diagnostics) over a range of 1.0–850 KIU/ml. The cutoff value of HCV RNA was set at 100 KIU/ml, because patients with baseline HCV RNA levels below this value have been reported to respond significantly better to IFN.[24] HCV core antigen was determined by a chemiluminescence enzyme immunoassay (EIA) with a high sensitivity.[21,22] The detection limit of HCV core antigen was set at 15 fmol/l in a previous report.[22] For predicting the response to IFN, a cutoff value of 500 fmol/l was determined by analysis of the receiver operating characteristic (ROC) curve.

Genotypes of HCV were determined with a commercial kit (Genotyping EIA; International Reagents, Kobe, Japan) which distinguishes between genotypes 1 and 2,[25] as well as by polymerase chain reaction (PCR) with type-specific primers that detect genotypes 1a, 1b, 2a, 2b, and 3a.[26] When genotype 1 or 1b was detected, the sequence of the IFN sensitivity-determining region (ISDR) in the non-structural 5A (NS5A) region was determined directly on extracted and amplified HCV RNA, by the method of Enomoto et al.[27] Based on the amino-acid sequence, three types were determined, i.e., wild-type, intermediate type, and mutant type.

HLA typing

Types of HLA-A, B, C, and DR and DQ loci were determined by micro-lymphocyte cytotoxicity, by the method of Terasaki and McClelland,[28] at the Special Reference Laboratory (Tokyo, Japan).

Statistical analyses

Univariate analysis for factors influencing the response to IFN-α was performed using the Mann Whitney *U*-test for quantitative data and the χ^2 test with Yates'

Table 1. Comparison of demographic, clinical, and virological characteristics between patients with and without sustained virological response to IFN-α

Features	Responders (n = 21)	Nonresponders (n = 32)	P value
Male	11 (52%)	18 (56%)	0.784
Median age (years)[a]	46 (30–72)	57 (31–66)	0.015
History of blood transfusion	8 (38%)	19 (59%)	0.133
History of IFN treatment	2 (10%)	7 (22%)	0.246
ALT (IU/l)[a]	94 (22–272)	83 (40–355)	0.877
Platelet count ($\times 10^4$/ml)[a]	167 (84–315)	144 (62–320)	0.047
Fibrosis (F1/F2/F3/ND)	11/5/3/0/2	13/11/4/3/1	0.472
HCV genotype (1b/2a or 2b/UC)	6/15/0	22/8/2	0.013
HCV RNA (KIU/ml)[a]	30 (0.5–330)	150 (0.5–850)	0.004
HCV core antigen (fmol/l)[a]	221 (3.0–14426)	3794 (112–19383)	0.001

IFN, interferon; KIU, kilo international units; ND, not determined; UC, unclassifiable
[a]Median value is shown, with the range in parentheses

correction for qualitative data. Fisher's exact test was used for comparison of small numbers. Multivariate analysis was performed using a logistic regression model, with a stepwise method, employing the statistical computer program known as SPSS 6.1J (SPSS, Chicago, IL, USA). Differences were evaluated by two-tailed analysis and considered significant for P values of less than 0.05.

Results

Sustained virological response to IFN-α therapy in patients with chronic hepatitis C

Of the 66 patients with chronic hepatitis C who were eligible for INF-α therapy, 4 dropped out and 9 were withdrawn from treatment. The 4 dropouts included 1 who did not comply with the treatment protocol and 3 who failed to visit hospitals by their own judgments. IFN-α was withdrawn because of a psychiatric condition (depression) in 4 patients, severe general malaise in 2, continuous fever in 1, pain in the neck and upper left arm in 1, and ophthalmagra in 1. Of the 9 patients in whom IFN-α was withdrawn, 3 were sustained virological responders and had completed more than 65% of the total regimen of 720MU. Their HCV genotypes were 1b, 2a, and unclassifiable, respectively.

A reduction of IFN-α dose was necessary in 2 of the 53 patients who completed the 24-week therapy, because of anorexia and fever, respectively. The dose was reduced from 9 to 6MU in the former patient, while 9MU was given twice a week instead of three times a week in the latter. Because these 2 patients had received more than 80% of the total dose of IFN-α, they were included in the study. The 53 patients eligible for the evaluation of virological response had a median age of 56.1 years (range, 30–72 years) and included 29 (55%) men, and 9 of them had been treated with IFN before. Liver biopsies performed before treatment revealed fibrosis of stage F1 in 24 (45%), F2 in 16 (30%), F3 in 7 (13%), and F4 in 3 (6%); liver biopsy was not performed in the remaining 3 (6%) patients.

The HCV genotype was 1 in 28 patients and 2 in 23; genotype was unclassifiable in 2 patients by the EIA genotype method.[25] Genotype 1 in all the 28 patients was found to be 1b by PCR with type-specific primers.[26] Of the 23 patients with genotype 2 determined by EIA, 13 had genotype 2a and 5 had 2b; subtypes of genotype 2 were not distinguishable in the remaining 5 patients. Genotypes in the 2 patients unclassifiable by EIA were not determined by PCR, either. Based on these results, genotypes of HCV were judged to be 1b in 28 (53%) patients, 2a or 2b in 23 (43%) patients, and unclassifiable in the remaining 2 (4%) patients.

Sustained virological response to IFN-α was achieved by 21 (40%) of the 53 patients. Table 1 compares demographic, clinical, and virological characteristics between the 21 responders and 32 nonresponders to IFN-α. Responders were significantly younger and had higher platelet counts than non-responders. Virologically, responders were significantly less frequently infected with HCV genotype 1b and had significantly lower levels of both HCV RNA and HCV core antigen.

HLA types were determined for loci with more than five patients testing positive for them (Table 2). Significant differences were observed only for DR6 and DR52, both of which were more frequency in nonresponders than responders.

Factors influencing the response to IFN-α therapy

The results of univariate analysis for evaluating factors predictive of sustained virological response to IFN-α

are shown in Table 3. Of host factors, age 50 years or less, lack of HLA DR6 and DR52, and platelet counts of more than $14 \times 10^4/mm^3$ were significantly predictive of the response. In virological aspects, HCV genotypes other than 1b, HCV core antigen of 500 fmol/l or less, and HCV RNA of 100 KIU/ml or less were significantly associated with the response to IFN-α. When each HLA type was evaluated by χ^2 analysis, a strong positive correlation with the response was found for DR6 and DR52. When these HLA types were subjected to multivariate analysis, DR6 was superior to DR52 in predicting the response to IFN on the multivariate analysis. Hence, DR6 was adopted for comparison with the other factors in multivariate analysis.

Table 4 shows the results of multivariate analysis of host and virological factors for influence on the response to IFN-α. Only age 50 years or less, history of IFN treatment, lack of HLA DR6, HCV genotypes other than 1b, and HCV core antigen of 500 fmol/l or less were significantly predictive of the virological response to IFN-α.

Table 2. HLA types in patients with chronic hepatitis C who did and who did not achieve sustained virolgical response to IFN-α treatment

HLA type	Responders (n = 21)	Nonresponders (n = 32)	P value
A2	7 (33%)	12 (38%)	0.7570
A11	3 (14%)	5 (16%)	1.0000
A24	10 (47%)	17 (53%)	0.6949
A26	5 (24%)	5 (16%)	0.4564
A33	3 (14%)	8 (25%)	0.4938
B7	2 (10%)	3 (9%)	1.0000
B35	3 (14%)	5 (16%)	1.0000
B44	2 (10%)	5 (16%)	0.6897
B51	2 (10%)	6 (19%)	0.4550
B52	2 (10%)	6 (19%)	0.4550
B54	3 (14%)	3 (9%)	0.6711
B60	1 (5%)	5 (16%)	0.3837
B61	1 (5%)	7 (22%)	0.1264
B62	4 (19%)	4 (13%)	0.6978
Cw1	6 (29%)	5 (16%)	0.2557
Cw3	9 (43%)	14 (44%)	0.9489
Cw7	6 (29%)	5 (16%)	0.2557
DQ1	11 (52%)	22 (69%)	0.2292
DQ3	7 (33%)	14 (44%)	0.4482
DQ4	4 (19%)	6 (19%)	1.0000
DR1	3 (14%)	4 (13%)	1.0000
DR2	8 (38%)	13 (41%)	0.8539
DR4	6 (29%)	8 (25%)	0.7730
DR6	3 (14%)	16 (50%)	0.0080
DR9	4 (19%)	8 (25%)	0.7433
DR52	5 (24%)	18 (56%)	0.0198
DR53	8 (38%)	15 (47%)	0.5282

Factors useful for early prediction of sustained virological response to IFN-α

Serial serum samples from before treatment to 24 weeks after the completion of IFN-α were available for 29 patients, including 14 responders and 15 nonresponders. Levels of HCV core antigen and HCV RNA, as well as frequency of elevated ALT levels (>45 IU/l) were compared between the responders and nonresponders (Fig. 1). HCV core antigen turned negative 1 week after the start of IFN-α in all the responders and stayed negative throughout follow-up (Fig. 1a). In some nonresponders, by contrast, HCV core antigen tested positive during IFN-α treatment. At 1 week after the start of IFN-α, HCV core antigen was detected significantly less often in responders than in nonresponders (0% vs 40%; P = 0.02). HCV RNA was cleared from serum from 4 weeks after the beginning of IFN-α treatment and stayed negative in all the responders (Fig. 1b). It was detected significantly less frequently in responders than in nonresponders at 1, 4, and 8 weeks after the start of

Table 3. Univariate analysis of factors for the association with sustained virological response to IFN-α in 53 patients with chronic hepatitis C

Factor	n (%)	OR	95% CI	P value
Age (≤50 years)	18 (34%)	8.78	2.39–32.15	0.001
Male	29 (55%)	0.86	0.28–2.58	0.782
HLA DR6-positive	19 (36%)	0.17	0.04–0.68	0.018
HLA DR52-positive	23 (43%)	0.24	0.07–0.83	0.041
Fibrosis score (1 or 2)	43 (81%)	1.12	0.99–1.26	0.276
Platelet count (≤14×10^4/ml))	18 (34%)	0.19	0.05–0.77	0.031
ALT level (≤135 IU/l)	42 (79%)	0.36	0.09–1.47	0.270
HCV genotype 1b	28 (53%)	0.18	0.05–0.61	0.004
ISDR (wild-type)[a]	5 (9%)	0.84	0.73–0.98	0.144
HCV core antigen (≤500 fmol/l)	16 (30%)	10.10	2.55–40.22	0.001
HCV RNA (≤100 KIU/ml)	31 (58%)	3.63	1.07–12.29	0.034

OR, odds ratio; CI, confidence interval

[a] Types of IFN sensitivity determining region (ISDR) were analyzed in the 28 patients infected with HCV genotype 1b only

Table 4. Multivariate analysis of factors for the association with sustained virological response to IFN-α in 53 patients with chronic hepatitis C

	n	OR	95% CI	*P* value
HCV core antigen				
>500 fmol/l	37	1.000		
≤500 fmol/l	16	10.610	1.924–58.53	0.007
HCV genotype				
1b	28	1.000		
Non-1b	25	8.929	1.681–47.62	0.010
History of IFN treatment				
Present	9	1.000		
Absent	44	11.630	1.570–83.33	0.016
HLA DR6				
Present	19	1.000		
Absent	34	8.130	1.269–52.63	0.027
Age				
>50 Years	35	1.000		
≤50 Years	18	4.009	1.073–15.66	0.039

OR, odds ratio; CI, confidence interval

IFN-α treatment. At the end of follow-up, both HCV core antigen and HCV RNA were negative in all the responders, while they were positive in all the nonresponders.

There were no significant differences in the frequency of elevated ALT levels (>45 IU/l) between responders and nonresponders during IFN-α treatment (Fig. 1c). Elevated ALT levels were observed less frequently in responders than in nonresponders 12 and 24 weeks after the completion of IFN-α treatment. The difference, however, was not clear-cut. There were sustained virological responders who kept elevated ALT levels, while some nonresponders did not possess them.

Discussion

Although IFN clears HCV infection in patients with chronic hepatitis C, sustained virological response is achieved in only 50% of these patients even with the most sophisticated combination therapy with pegylated IFN and ribavirin.[29] It remains difficult to treat patients who are infected with HCV genotype 1b with a high viral load. Because IFN can induce grave side effects, such as autoimmune thyroiditis and severe depression, patients who would be likely to respond need to be identified beforehand, to spare nonresponders unfruitful side effects. Many host and viral factors have been proposed to be predictive of the response to IFN.[4–9] Only a few of them, however, were evaluated in prospective studies.

In the present prospective study, various host and viral factors were evaluated as predictors of sustained virological response, focusing on HLA types and HCV core antigen. These factors were chosen because no agreement has been reached on the association of HLA types with the response to IFN,[10–17] and the determination of HCV core antigen by EIA is very handy and less expensive than PCR for testing HCV RNA.[21,22] In previous studies, there were many patients with low pretreatment viral loads, disproportional to the number of patients with chronic hepatitis C who receive IFN therapy. Patients with low baseline viral loads might have tended to be registered more frequently in studies than those with higher loads, because of a better response to IFN.

HLA DR6 and DR52 were predictive of the virological response by univariate analyses performed in 21 responders and 32 nonresponders to natural IFN-α who had a total dose of 720 MU. By multivariate analysis, only HLA DR6 was significantly predictive of the response, and this has not attracted attention in previous studies. Thus far, association with response has been reported for DRB1*0404 in Canda,[14] DRB1*07− in France,[15] DR2+ and DR3− in an Egyptian population living in Qatar,[12] and the DRB1*0701-DQA1*0202-DQB1*02 haplotype in Poland.[17] There are, however, reports showing no influence of HLA types on the response to IFN.[16] Inasmuch as HLA types represent anthropological markers and show distinct differences with different ethnicities, the HLA types have cohort effects in studies in which it is attempted to correlate therapeutic efficacy with HLA types. It would not be easy, therefore, to reconcile the results obtained in different countries.

In Japan, Kikuchi et al.[13] reported detecting B54 and A24-B54-DR4 more frequently in nonresponders. Miyaguchi et al.[11] found B55, B62, Cw3, and Cw4 more

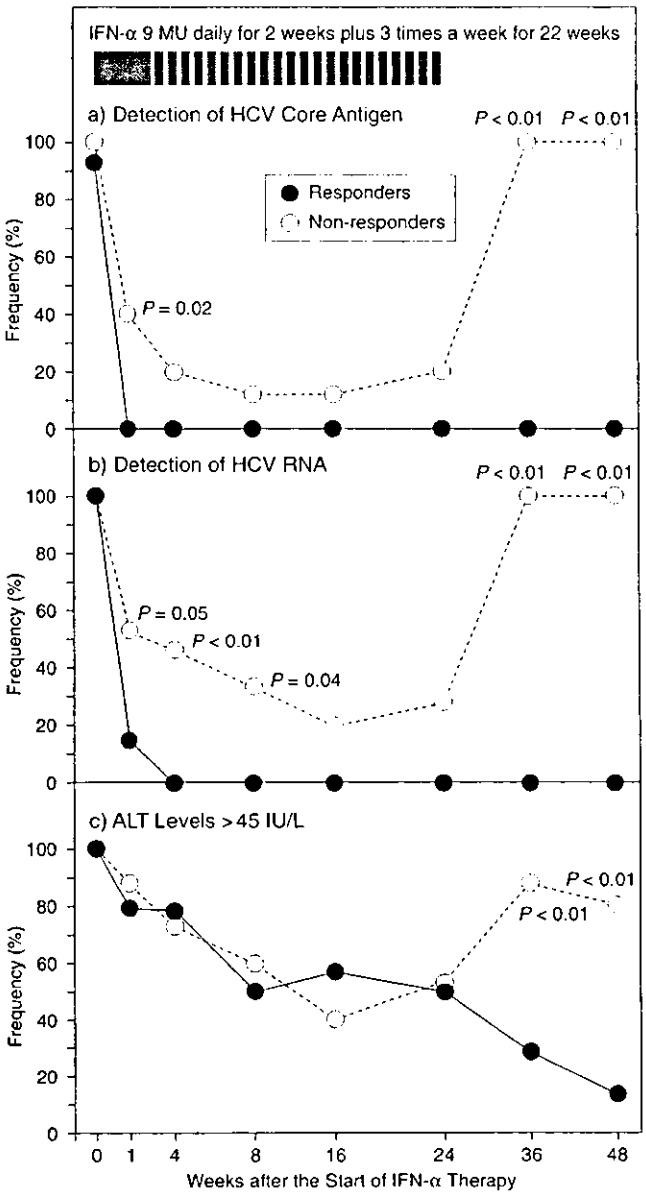

Fig. 1a–c. Follow-up of viral markers and elevated alanine aminotransferase (*ALT*) levels during and after *interferon-α* (*IFN-α*) treatment in patients with chronic hepatitis C. Frequencies of the detection of hepatitis C virus (*HCV*) core antigen (**a**), as well as the persistence of HCV RNA (**b**) and elevated ALT levels (**c**) were compared between the 14 patients who achieved virological response and the 15 patients who did not; before treatment, during IFN-α treatment, and until 24 weeks after the completion of the therapy. Duration of IFN-α treatment is indicated by *gray bars* at *the top above a*.

often in responders, who also had lower HCV RNA levels in serum than nonresponders. On that basis, they deduced that HLA types would modulate the replication of HCV. Their results are not consistent and stand at variance with the association of HLA DR6 and DR52 with the virological response to IFN-α observed in the present study. Knowing that all the patients studied were Japanese, with no remarkable differences in therapeutic regimens of IFN used, the discrepancy between their results and ours is hard to explain. Marked diversity in HLA haplotypes even among Japanese individuals, and difficulties in examining all of them, could be among the reasons for these different results in Japanese patients. Multicenter collaborative studies are required to confirm the previously obtained results to elucidate the influence of HLA types on the response of patients with chronic hepatitis C to IFN therapies.

The influence of HCV genotypes and HCV RNA levels on the response to IFN has been established.[4–6,8,9] The HCV genotypes were evaluated in association with the response to IFN, along with HCV core antigen, which has a close correlation with HCV RNA.[21,22] On univariate analysis, both HCV RNA and HCV core antigen, as well as HCV genotypes, were significantly associated with the response to IFN. On multivariate analysis, however, HCV genotypes and HCV core antigen remained significantly predictive, while HCV RNA did not. The cutoff level of HCV core antigen at 500 fmol/l was found to be optimal for distinguishing between response and nonresponse, based on the ROC curve (data not shown), and this could have been the reason for the better performance of HCV core antigen than HCV RNA in the present study.

HCV core antigen was useful, also, for the early prediction of the response to IFN-α. It tested negative in all the 14 individuals who were responders at 1 week after the start of IFN-α, in contrast to its detection in 6 of the 15 (40%) nonresponders at that time point. HCV RNA behaved similarly to HCV core antigen during IFN-α treatment, except that it was till detectable in responders at week 1 of therapy. Because all the responders were negative for both HCV core antigen and HCV RNA in serum throughout follow-up until 24 weeks after the completion of IFN-α treatment, HCV core antigen, as well as HCV RNA, will be instrumental in monitoring for the persistence of response. The advantage of using HCV core antigen as a parameter of response is that it can be determined by EIA with less of a burden and lower cost than PCR for determining HCV RNA. Therefore, we believe that the determination of HCV core antigen will find a number of applications in predicting and monitoring response to IFN treatments in patients with chronic hepatitis C in future.

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References

1. Cohen J. The scientific challenge of hepatitis C. Science 1999;285: 26–30.
2. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, 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;12:671–5.
3. Kiyosawa K, Tanaka E. Characteristics of hepatocellular carcinoma in Japan. Oncology 2002;62:S5–7.
4. Hino K, Sainokami S, Shimoda K, Iino S, Wang Y, Okamoto H, et al. Genotypes and titers of hepatitis C virus for predicting response to interferon in patients with chronic hepatitis C. J Med Virol 1994;42:299–305.
5. Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, et al. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. Hepatology 1994;19:1088–94.
6. Shiratori Y, Kato N, Yokosuka O, Imazeki F, Hashimoto E, Hayashi N, et al. Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Gastroenterology 1997;113: 558–66.
7. Fried MW, Shiffman M, Sterling RK, Weinstein J, Crippin J, Garcia G, et al. A multicenter, randomized trial of daily high-dose interferon-alfa 2b for the treatment of chronic hepatitis C: pretreatment stratification by viral burden and genotype. Am J Gastroenterol 2000;95:3225–9.
8. Ebeling F, Lappalainen M, Vuoristo M, Nuutinen H, Leino R, Karvonen AL, et al. Factors predicting interferon treatment response in patients with chronic hepatitis C: late viral clearance does not preclude a sustained response. Am J Gastroenterol 2001;96:1237–42.
9. Izopet J, Payen JL, Alric L, Sandres K, Charlet JP, Vinel JP, et al. Baseline level and early suppression of serum HCV RNA for predicting sustained complete response to alpha-interferon therapy. J Med Virol 1998;54:86–91.
10. Alric L, Fort M, Izopet J, Vinel JP, Charlet JP, Selves J, et al. Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. Gastroenterology 1997: 113:1675–81.
11. Miyaguchi S, Saito H, Ebinuma H, Morizane T, Ishii H. Possible association between HLA antigens and the response to interferon in Japanese patients with chronic hepatitis C. Tissue Antigens 1997;49:605–11.
12. Almarri A, El Dwick N, Al Kabi S, Sleem K, Rashed A, Ritter MA, et al. Interferon-alpha therapy in HCV hepatitis: HLA phenotype and cirrhosis are independent predictors of clinical outcome. Hum Immunol 1998;59:239–42.
13. Kikuchi I, Ueda A, Mihara K, Miyanaga O, Machidori H, Ishikawa E, et al. The effect of HLA alleles on response to interferon therapy in patients with chronic hepatitis C. Eur J Gastroenterol Hepatol 1998;10:859–63.
14. Sim H, Wojcik J, Margulies M, Wade JA, Heathcote J. Response to interferon therapy: influence of human leucocyte antigen alleles in patients with chronic hepatitis C. J Viral Hepatol 1998;5: 249–53.
15. Alric L, Izopet J, Fort M, Vinel JP, Fontenelle P, Orfila C, et al. Study of the association between major histocompatibility complex class II genes and the response to interferon alpha in patients with chronic hepatitis C infection. Hum Immunol 1999;60:516–23.
16. Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC. Influence of MHC class II genotype on outcome of infection with hepatitis C virus. Lancet 1999;354:2119–24.
17. Wawrzynowicz-Syczewska M, Underhill JA, Clare MA, Boron-Kaczmarska A, McFarlane IG, Donaldson PT. HLA class II genotypes associated with chronic hepatitis C virus infection and response to alpha-interferon treatment in Poland. Liver 2000;20: 234–9.
18. Kuzushita N, Hayashi N, Katayama K, Hiramatsu N, Yasumaru M, Murata H, et al. Increased frequency of HLA DR13 in hepatitis C virus carriers with persistently normal ALT levels. J Med Virol 1996;48:1–7.
19. Kuzushita N, Hayashi N, Moribe T, Katayama K, Kanto T, Nakatani S, et al. Influence of HLA haplotypes on the clinical courses of individuals infected with hepatitis C virus. Hepatology 1998;27:240–4.
20. Mangia A, Gentile R, Cascavilla I, Margaglione M, Villani MR, Stella F, et al. HLA class II favors clearance of HCV infection and progression of the chronic liver damage. J Hepatol 1999;30:984–9.
21. Aoyagi K, Ohue C, Iida K, Kimura T, Tanaka E, Kiyosawa K, et al. Development of a simple and highly sensitive enzyme immunoassay for hepatitis C virus core antigen. J Clin Microbiol 1999;37:1802–8.
22. Tanaka E, Ohue C, Aoyagi K, Yamaguchi K, Yagi S, Kiyosawa K, et al. Evaluation of a new enzyme immunoassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating that of genomic amplification of HCV RNA. Hepatology 2000;32:388–93.
23. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–20.
24. Kawai S, Yokosuka O, Imazeki F, Saisho H, Mizuno C. Evaluation of the clinical usefulness of COBAS Amplicor HCV Monitor assay (ver 2.0): Comparison with Amplicor HCV Monitor assay (ver 1.0) and HCV core protein level. J Med Virol 2002;68:343–51.
25. Yamada G, Tanaka E, Miura T, Kiyosawa K, Yano M, Matsushima T, et al. Epidemiology of genotypes of hepatitis C virus in Japanese patients with type C chronic liver diseases: a multi-institution analysis. J Gastroenterol Hepatol 1995;10:538–45.
26. Okamoto H, Kobata S, Tokita H, Inoue T, Woodfield GD, Holland PV, et al. A second-generation method of genotyping hepatitis C virus by the polymerase chain reaction with sense and antisense primers deduced from the core gene. J Virol Methods 1996;57:31–45.
27. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.
28. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. Nature 1964;204:998–1000.
29. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–82.

Characteristics of Patients with Chronic Hepatitis C who Develop Hepatocellular Carcinoma after a Sustained Response to Interferon Therapy

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BACKGROUND. The objective of the current study was to determine the characteristic features of sustained responders who develop hepatocellular carcinoma after treatment with interferon for chronic hepatitis C.

METHODS. This study included 3626 patients with chronic hepatitis C who had received interferon monotherapy. Cox proportional hazards analysis was used to compare sustained responders who did and did not develop hepatocellular carcinoma, and nonsustained responders who developed hepatocellular carcinoma in a multicenter, retrospective cohort study.

RESULTS. Among 1197 sustained responders, 27 patients developed hepatocellular carcinoma (2.3%). Compared with sustained responders who did not develop hepatocellular carcinoma, patients who developed disease more often were male ($P = 0.0212$), were older ($P = 0.0068$), and had advanced-stage histologic disease before interferon therapy ($P = 0.0345$). Conversely, compared with patients with hepatocellular carcinoma who were not sustained responders, patients who were sustained responders tended to be older at the time of the initiation of interferon therapy ($P = 0.0552$) and at the time hepatocellular carcinoma was detected ($P = 0.0593$), and they also were predominantly male ($P = 0.0507$). The histologic staging and serum aminotransferase levels at the initiation of interferon therapy, the interval to the detection of tumor, and the tumor size showed no significant differences between the two groups.

CONCLUSIONS. Sustained responders in the group at high risk for developing hepatocellular carcinoma after interferon therapy were older, more often were male, and had more advanced histologic disease stage. Such patients should be followed carefully periodically for > 10 years after they complete interferon therapy. *Cancer* **2004;101:1616–22.** *© 2004 American Cancer Society.*

KEYWORDS: chronic hepatitis type C, hepatocellular carcinoma, interferon, sustained responder.

In Japan, chronic hepatitis C (CH-C) with advanced histologic staging often progresses to hepatocellular carcinoma (HCC),[1] although patients who are seropositive for antihepatitis C virus (anti-HCV) antibodies or for HCV RNA do not always progress to cirrhosis or HCC.[2,3] Risk factors for developing HCC in patients with CH-C are advanced histologic stage, irregular regeneration of hepatocytes, heavy drinking, higher serum alanine aminotransferase (ALT) levels or lower serum albumin levels, male gender, and older age.[1,4–7] Since 1992, patients with CH-C commonly have been treated with interferon α (IFN-α) or IFN-β, which are covered by public health insurance in Japan. Because IFN improves hepatic inflammation and inhibits the progression of hepatic fibrosis, it

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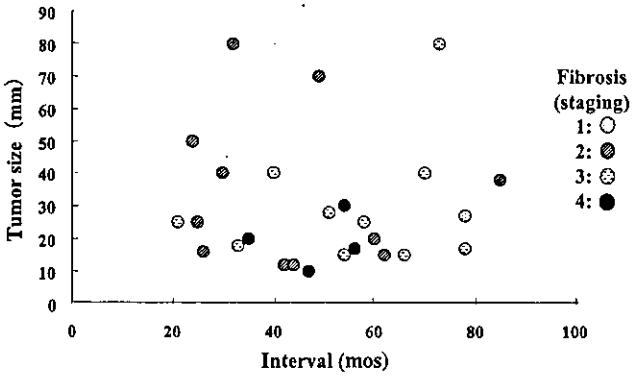

FIGURE 1. The interval from the completion of IFN therapy to the detection of SR HCC statistically did not correlate significantly with the tumor size or hepatic staging.

has been suggested that the incidence of HCC may be reduced by IFN treatment. In fact, IFN therapy reportedly was effective not only for improving liver biochemistry and eliminating HCV RNA but also for reducing the inflammation/fibrosis scores and lowering the risk of HCC, especially in sustained responders (SR patients).[8–14]

Although a significant decrease in the incidence of HCC has been observed in SR patients after IFN therapy,[9–14] HCC is detected in some of them.[15–25] The clinical features of SR patients who develop HCC (SR HCC patients) and the long-term incidence of HCC in SR patients remain unclear, and the optimal duration and frequency of follow-up have not been established. Therefore, we analyzed SR HCC patients to determine their characteristic features compared with SR patients who did not develop HCC (SR non-HCC patients) and non-SRs who developed HCC (non-SR HCC patients).

MATERIALS AND METHODS

Patients

For this study, 3626 patients with CH-C were enrolled (2344 males and 1282 females) who had received IFN therapy between January 1990 and November 2001. Data from these patients were collected from 6 institutions and related hospitals, including 1371 patients from Kyoto Prefectural University of Medicine, 1478 patients from Osaka University, 497 patients from Miyazaki Medical College, 130 patients from Nagoya University, 102 patients from Shinsyu University, and 48 patients from Yamaguchi University. All patients were seropositive for anti-HCV antibodies, positive for serum HCV RNA, and seronegative for hepatitis B virus surface antigen. We excluded patients who had coexisting liver diseases, such as autoimmune hepatitis or primary biliary cirrhosis, and confirmed that they did not abuse alcohol (daily alcohol intake > 60 g of ethanol). No patients were infected with human immunodeficiency virus (HIV). At the time of entry into this study, no patients showed evidence of HCC, as determined by ultrasonography (US) and/or computed tomography (CT) studies. In principle, patients underwent liver biopsy prior to IFN therapy, and the histologic diagnoses were reached according to the classification of Desmet et al.[26] The gender, mean age, and histologic disease stage at the initiation of IFN therapy are shown in Table 1.

TABLE 1
Characteristics of Patients with Chronic Hepatitis C who were Treated with Interferon[a]

Characteristic	Sustained responder	Nonsustained responder	*P* value[b]
No. patients	1197	2429	—
Male:female ratio	776:421	1568:861	0.8826
Age (yrs, mean ± SD)	49.4 ± 11.9	51.2 ± 10.6	< 0.0001
Histologic staging score: No. of patients (%)			
F1	385 (38.6)	522 (25.8)	
F2	322 (32.3)	613 (30.3)	< 0.0001
F3	262 (26.3)	782 (38.6)	
F4	29 (2.9)	109 (5.4)	
Not available	199	403	

SD: standard deviation; IFN: interferon.
[a] All data were determined before interferon therapy.
[b] *P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.

Natural IFN-α, recombinant IFN-α-2a, and recombinant IFN-α-2b were used in this study. In general, the IFN treatment protocol was within the range covered by public health insurance in Japan, namely, 3–10 MU of IFN-α for 24 weeks (daily for 2 weeks and 3 times per week for 22 weeks). In a few patients, administration of IFN-α was prolonged to 52 weeks. In some patients who suffered from severe side effects, the therapy period was shortened. In addition, patients for whom the total dose of IFN was < 200 MU were excluded from the study. Patients who had been treated with peginterferon or IFN/Ribavirin also were excluded. There was no difference noted with regard to the treatment protocol among the institutions and their related hospitals. We checked the laboratory findings at the end of IFN therapy and 6 months later. SR patients were defined as those who demonstrated continuous normal serum ALT levels for 6 months after finishing IFN therapy. The remaining patients were regarded as non-SR patients. The patient population included 1197 SR patients and 2429 non-SR patients.

We followed all patients for at least 1 year after the end of IFN therapy. The mean ± standard deviation

(SD) follow-up was 5.9 years ± 1.9 years. In SR patients, in general, we performed biochemical examinations, which sometimes included α-fetoprotein, every 3–12 months after confirming a sustained response. US and/or CT studies were performed at least once annually. However, because the incidence of HCC in non-SR patients—especially those with advanced-stage disease (fibrotic scores of F3 or F4)–was expected to be higher than that in SR patients, US and/or CT studies were performed every 3–6 months in non-SR patients. This strategy was similar in all of the institutions, and the frequency of radiographic examination was calculated to avoid unnecessary cost and not to miss HCC. However, some SR patients and non-SR patients who skipped or stopped visiting the outpatient clinic and some patients who were followed by their home physicians were not followed sufficiently. The diagnosis of HCC was based on appropriate radiologic findings (hepatic angiography, dynamic CT, magnetic resonance imaging).[27] When it was difficult to determine a final diagnosis with the radiologic findings, a histologic diagnosis was reached by tumor biopsy. In 17 of 27 SR HCC patients, a histologic diagnosis of HCC was obtained by the examination of resected hepatic tumors or biopsied tumor specimens. Patients who were diagnosed with HCC within 1 year after the end of IFN therapy were excluded from this study because of the possibility that a small but detectable HCC was missed before IFN therapy. Written informed consent to receive IFN therapy and to participate in this follow-up study was obtained from all patients, and the ethical committees of the participating institutions approved this study.

Statistical Analysis

Statistical analysis was performed using the SAS/PC statistical package (SAS Institute, Cary, NC). The Fischer exact probability test was used to compare the frequencies of gender. The Wilcoxon two-sample test was used to compare age, histologic staging, serum ALT level, interval between the end of IFN therapy and the detection of HCC, and the size of HCC. The independent risk factors for developing HCC in SR patients were examined by Cox proportional-hazards analysis; the variables were gender, age, histologic stage, and serum ALT level. Patients who had missing data were excluded from this analysis. Each variable was transformed into categorical data comprised of two-sample, ordinal numbers for multivariate analysis. P values were two-sided, and P values < 0.05 were considered statistically significant.

RESULTS

Characteristic Features of SR HCC Patients

During the observation of 3626 patients, HCC was detected in 259 patients; however, 19 patients were excluded, because HCC was detected within 1 year after they completed IFN therapy. The distribution of the remaining 240 HCC patients among the 6 institutions was as follows: 109 patients from Kyoto Prefectural University of Medicine (HCC incidence, 8.0%), 102 patients from Osaka University (HCC incidence, 6.9%), 3 patients from Miyazaki Medical College (HCC incidence, 0.6%), 15 patients from Nagoya University (HCC incidence, 11.5%), 8 patients from Shinsyu University (HCC incidence, 7.8%), and 3 patients from Yamaguchi University (HCC incidence, 6.3%). The incidence of HCC did not differ significantly among the institutions, except for Miyazaki Medical College. partly because hepatic fibrosis was less advanced in patients from this institution compared with patients from the other five institutions. Of 240 patients, 27 were SR patients, and 213 were non-SR patients. The ages of the 240 patients at the initiation of IFN therapy ranged from 37–77 years (mean age ± SD, 59.1 years ± 6.6 years) and varied from 39–83 years (63.6 years ± 6.8 years) at the time HCC was detected.

Among the 27 SR HCC patients, 5 patients consumed ≈ 50 g of ethanol daily. By evaluating liver specimens and biochemical examinations, including γ-glutamyl transferase, we excluded the possibility of alcoholic liver diseases in these patients. Serum HCV RNA was evaluated in the SR HCC patients by reverse transcriptase-polymerase chain reaction analysis. Twenty-six SR HCC patients were complete responders (seronegative for HCV RNA both at the end of IFN therapy and 6 months later), and 1 SR HCC patient was a biochemical responder (seropositive for HCV RNA at the end of IFN therapy). In 1 complete responder who developed HCC, serum HCV RNA became positive 12 months after completing IFN therapy.

No correlation could be found between the interval before HCC was detected, tumor size, or hepatic histologic stage among the SR HCC patients (Fig. 1). HCC that was detected long after discontinuing IFN therapy was not always large, and the patients with large HCC did not always show more advanced stage according to liver histology. The greatest dimensions of the 2 largest SR HCC tumors were 80 mm and were detected 32 months and 73 months after the end of IFN therapy. The greatest dimension of SR HCC found after the longest interval (85 months) was 38 mm.

Tumor tissue samples could be examined from 18 of 27 SR HCC patients. Two samples were categorized

TABLE 2
Comparisons between Sustained Responders with and without Hepatocellular Carcinoma[a]

Characteristic	SR HCC	SR non-HCC	*P* value[b]
No. of patients	27	1170	
Male:female ratio	25:2	751:419	0.0016
Age (yrs, mean ± SD)	60.7 ± 7.5	50.2 ± 12.4	< 0.0001
Serum ALT (IU/L, mean ± SD)	111.7 ± 67.7	122.6 ± 109.9	0.7267
Histologic staging score: No. of patients (%)			
F1	1 (3.7)	384 (39.6)	
F2	11 (40.7)	310 (32.0)	< 0.0001
F3	10 (37.0)	252 (26.0)	
F4	5 (18.5)	24 (2.5)	

SR: sustained responder; HCC: hepatocellular carcinoma; SD: standard deviation; ALT: alanine aminotransferase; IFN: interferon.

[a] All data were determined before interferon therapy.

[b] *P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.

TABLE 3
Factors Associated with the Development of Hepatocellular Carcinoma in Sustained Responders[a]

Characteristic	Risk ratio	95% CI	*P* value
Male vs. female	5.498	1.290–23.439	0.0212
Age	7.378	1.737–31.326	0.0068
Stage of liver disease	2.344	1.064–5.164	0.0345
Serum ALT	1.331	0.606–2.923	0.4768

95% CI: 95% confidence interval; ALT: alanine aminotransferase.

[a] All data were determined before interferon therapy. Statistical analysis was performed using the Cox proportional hazards test. The variable for age was set at < 50 years or ≥ 50 years, the variable for stage was set < F3 or ≥ F3, and the variable for the serum alanine aminotransferase level was set at < 88 UI/L or ≥ 88 UI/L. The variables age and serum alanine aminotransferase level were determined as median data. The variable for stage was set to obtain the largest hazard ratio.

as well differentiated HCC, 11 samples were moderately differentiated HCC, 2 samples were poorly differentiated HCC, and 2 samples were undifferentiated HCC. One sample was the necrotic tissue after transcatheter arterial embolization therapy (TAE). Nontumorous liver tissue samples from 18 patients were evaluated for their fibrosis scores in resected HCC or tumor biopsy specimens. Liver fibrosis scores improved in nine patients, did not change significantly in eight patients, and worsened in one patient.

Sixteen of 27 SR HCC patients underwent partial hepatectomy, and 10 patients were treated with TAE and/or percutaneous ethanol injection therapy. Because one patient changed his hospital after the diagnosis of HCC, we could not know his prognosis.

Comparison between SR HCC Patients and SR Non-HCC Patients

We compared 27 SR HCC patients with 1170 SR non-HCC patients. The SR HCC patients included 25 males (92.6%) and 2 females (7.4%), and the SR non-HCC patients included 751 males (63.5%) and 419 females (35.8%). At the time IFN therapy was initiated, the mean age of the SR HCC patients was 60.7 years ± 7.5 years (range, 37–70 years), whereas the mean age of the SR non-HCC patients was 50.2 years ± 12.4 years (range, 17–73 years). Thus, the SR HCC patients more often were male ($P = 0.0016$) and were older ($P < 0.0001$) compared with the SR non-HCC patients (Table 2).

The fibrotic scores in biopsied liver specimens before IFN therapy for the SR HCC patients included 1 F1 specimen (3.7%), 11 F2 specimens (40.7%), 10 F3 specimens (37.0%), and 5 F4 specimens (18.5%); and the fibrotic scores for the SR non-HCC patients included 384 F1 specimens (39.6%), 310 F2 specimens (32.0%), 252 F3 specimens (26.0%), and 24 F4 specimens (2.5%). The 2 female SR HCC patients both had F4 specimens. Among the total SR population, SR HCC patients had more advanced-stage disease ($P < 0.0001$). The mean serum ALT level at the initiation of IFN therapy was 111.7 IU/L ± 67.7 IU/L in the SR HCC patients and 122.6 IU/L ± 109.9 IU/L in the SR non-HCC patients (Table 2).

Cox proportional-hazards analysis of factors associated with the development of HCC in the SR patients was performed with four variables (gender, age, histologic stage, and serum ALT level). In this analysis, the hazard ratios for age, stage, and serum ALT level were calculated between the two groups. The age variable was set at < 50 years or ≥ 50 years, the fibrotic score (stage) variable was set at < F3 or ≥ F3, and the variable for serum ALT level was set at < 88 IU/L or ≥ 88 IU/L. The variables age and serum ALT level were determined as median data. We chose the variable for stage to obtain the greatest hazard ratio. The SR HCC patients more often were male ($P = 0.0212$, 95%CI, 1.290–23.439), were older ($P = 0.0098$, 95%CI, 1.737–31.326), and had advanced-stage disease according to liver histology ($P = 0.0345$; 95%CI, 1.064–5.164) before IFN therapy. Gender, age, and histologic stage before IFN therapy were considered independent risk factors for the development of HCC (Table 3).

Comparison between SR HCC Patients and Non-SR HCC Patients

We compared the clinical characteristics of the 27 SR HCC patients with the 213 non-SR HCC patients. The non-SR HCC patients included 161 males (75.6%) and 52 females (24.4%). The mean age of the non-SR HCC patients at the initiation of IFN therapy was 58.9 years ± 6.5 years (range, 40–77 years), and the mean age at

TABLE 4
Comparisons between Sustained Responders and Nonsustained Responders among Patients with Hepatocellular Carcinoma

Characteristic	SR	Non-SR	*P* value[a]
No. of patients	27	213	
Male:female ratio	25:2	161:52	0.0507
Age at the initiation of IFN (yrs, mean ± SD)	60.7 ± 7.5	58.9 ± 6.5	0.0552
Age at the detection of HCC (yrs, mean ± SD)	65.1 ± 7.8	63.4 ± 6.7	0.0593
Serum ALT (IU/L)[b]	111.7 ± 67.7	120.5 ± 56.4	0.2027
Histologic staging score: No. of patients (%)[b]			
F1	1 (3.7)	12 (5.6)	
F2	11 (40.7)	36 (16.9)	0.1861
F3	10 (37.0)	135 (63.4)	
F4	5 (18.5)	30 (14.1)	
Interval (mos, mean ± SD)[c]	49.3 ± 18.2	49.7 ± 24.8	0.7484
Tumor size (mm, mean ± SD)	31.2 ± 20.1	21.3 ± 9.9	0.1573

SR: sustained responder; IFN: interferon; SD: standard deviation; HCC: hepatocellular carcinoma; ALT: alanine aminotransferase.
[a]*P* values were calculated with the Fisher exact probability test and the Wilcoxon two-sample test.
[b]Data were determined before interferon therapy.
[c]The interval was between the completion of interferon therapy and the detection of hepatocellular carcinoma.

time HCC was detected was 63.2 years ± 6.7 years (range, 44–83 years). The mean serum ALT level in the non-SR HCC patients at the start of IFN therapy was 120.5 IU/L ± 56.4 IU/L. The fibrotic scores of biopsied liver specimens obtained from the non-SR HCC patients before IFN therapy included 12 F1 specimens (5.6%), 36 F2 specimens (16.9%), 135 F3 specimens (63.4%), and 30 F4 specimens (14.1%). Thus, concerning gender and age, the SR HCC patients tended to be predominantly male ($P = 0.0507$) and were older (both at the initiation of IFN therapy [$P = 0.0552$] and at the time HCC was detected [$P = 0.0593$]) compared with the non-SR HCC patients; however, the serum ALT levels and the histologic stage before IFN therapy among the SR HCC patients did not differ significantly compared with the non-SR HCC patients (Table 4).

The mean interval between the end of IFN therapy and the detection of HCC for the SR HCC patients was 49.3 months ± 18.2 months (range, 21–85 months), which was not significantly different from that for the non-SR HCC patients (49.7 months ± 24.8 months; range, 12–141 months). The mean greatest dimension of SR HCC was 31.2 mm ± 20.1 mm, which was slightly greater than, but not significantly different from, the mean greatest dimension of non-SR HCC (21.3 mm ± 9.9 mm) (Table 4).

DISCUSSION

In the current study, we compared the clinical characteristics of SR HCC patients with the characteristics of SR non-HCC patients to determine the characteristic features of SR HCC. The incidence of HCC among the 1197 SR patients was 2.3%, and the incidence among the 2429 non-SR patients was 8.8% during the mean follow-up of 5.9 years. In patients with CH-C, aging and advanced hepatic histologic stage reportedly are major risk factors for HCC development.[1,4] This was true for the SR population in our current investigation, because the risk ratio for developing HCC was > 7 times greater in older patients (≥ 50 years) and was more than twice as high in patients who had advanced histologic stage disease (fibrotic score ≥ F3) according to a Cox proportional-hazards analysis. Khan et al. also reported that male gender is an important risk factor for HCC development.[5] In the current study, males were more than five times more likely to develop HCC in the SR population. Thus, older male patients with advanced hepatic fibrosis were considered to be a high-risk group for developing HCC among the SR population (Table 3).

Conversely, compared with the non-SR HCC patients, the SR HCC patients were older at the initiation of IFN therapy ($P = 0.0552$) and at the detection of HCC ($P = 0.0593$), and they were predominantly male ($P = 0.0507$). Although these characteristics may not have differed significantly in the current study, a study of even larger size may show that this indeed is a trend. The histologic staging, the serum ALT level at the initiation of IFN therapy, the interval for the detection of HCC, and the tumor size did not differ significantly between the two groups. The tumor size in SR HCC patients was slightly greater compared with the tumor size in non-SR HCC patients, most likely because of the extended interval of screening for HCC after patients attained a sustained response to IFN therapy (Table 4).

Some previous articles reported that HCV RNA may survive in the hepatic tissues of SR HCC patients[28–30] and may be involved in the carcinogenesis or growth of HCC. Although we could not demonstrate the presence of HCV RNA in tumors and surrounding hepatic tissues from SR HCC patients, eradication of HCV from these tissues, along with the nontumorous hepatic tissues, was confirmed in several previous studies,[15–21] suggesting that the persistence of HCV is not essential for the growth of HCC in SR patients.

To ascertain the time of HCC occurrence, several studies were performed that examined the doubling time (DT) of HCC. Two studies from Japan reported that the DT of HCC measuring < 3 cm in greatest dimension was 93.0 days ± 57.4 days or 195.0 days ± 171.0 days.[31,32] Barbara et al. reported that the DT of HCC measuring < 5 cm in greatest dimension was 204.2 days ± 135 days.[33] Recently, Toyoda et al. re-

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.

REFERENCES

1. 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.
2. Kenny-Walsh E, for the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med.* 1999;340: 1228–1233.
3. Alberti A, Noventa F, Benvegnu L, Boccato S, Gatta A. Prevalence of liver disease in a population of asymptomatic persons with hepatitis C virus infection. *Ann Intern Med.* 2002;17:961–964.
4. Aizawa Y, Shibamoto Y, Takagi I, et al. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. *Cancer.* 2000;89:53–59.
5. Khan MH, Farrell GC, Byth K, et al. Which patients with hepatitis C develop liver complications? *Hepatology.* 2000; 31:513–520.
6. Shibata M, Morizane T, Uchida T, et al. Irregular regeneration of hepatocytes and risk of hepatocellular carcinoma in chronic hepatitis and cirrhosis with hepatitis-C-virus infection. *Lancet.* 1998;351:1773–1777.
7. Kasahara A, Hayashi N, Mochizuki K, et al. Clinical characteristics of patients with chronic hepatitis C showing biochemical remission, without hepatitis C eradication, as a result of interferon therapy. The Osaka Liver Disease Study Group. *J Viral Hepatol.* 2000;7:343–351.
8. 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 in 1148 patients. *J Hepatol.* 1999; 30:653–659.
9. 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. Osaka Liver Disease Study Group. *Hepatology.* 1998;27:1394–1402.
10. Okanoue T, Itoh Y, Kirishima 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;23:62–77.
11. 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.
12. 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.
13. Ikeda K, Saitoh S, Arase Y, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology.* 1999;29:1124–1130.
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. Hirashima N, Mizokami M, Orito E, et al. Development of hepatocellular carcinoma in a patient with chronic hepatitis C infection after a complete and sustained response to interferon-alpha. *J Gastroenterol Hepatol.* 1996;11:955–958.
16. Inoue M, Ohhira M, Ohta T, et al. Hepatocellular carcinoma developed in a patient with chronic hepatitis C after the disappearance of hepatitis C virus due to interferon therapy. *Hepatogastroenterology.* 1999;46:2554–2560.
17. Miyano S, Togashi H, Shinzawa H, et al. Case report: occurrence of hepatocellular carcinoma 4.5 years after successful treatment with virus clearance for chronic hepatitis C. *J Gastroenterol Hepatol.* 1999;14:928–930.
18. Tamori A, Kuroki T, Nishiguchi S, et al. Case of hepatocellular carcinoma in the caudate lobe detected after interferon caused disappearance of hepatitis C virus. *Hepatogastroenterology.* 1996;43:1079–1083.
19. Kim SR, Matsuoka T, Maekawa Y, et al. Development of multicentric hepatocellular carcinoma after completion of interferon therapy. *J Gastroenterol.* 2002;37:663–668.
20. Okamura K, Yamazaki K, Ohmura T, et al. A resected case of hepatocellular carcinoma with sustained response to interferon for five years. *Acta Hepatol Jpn.* 2000;41:43–47.
21. Yamada M, Ichikawa M, Matsubara A, Ishiguro Y, Yamada M, Yokoi S. Development of small hepatocellular carcinoma 80 month after clearance of hepatitis C virus with interferon therapy. *Eur J Gastroenterol Hepatol.* 2000;12:1029–1032.
22. Nagano K, Fukuda Y, Nakano I, et al. A case of the development of two hepatocellular carcinoma and a cholangiocarcinoma with cirrhosis after elimination of serum hepatitis C virus RNA with interferon therapy. *Hepatogastroenterology.* 2000;47:1436–1438.
23. Sugo H, Kitayama N, Iwata T, et al. Development of hepatocellular carcinoma in a patients with chronic hepatitis C after a complete response to interferon therapy. *Acta Hepatol Jpn.* 2000;41:195–198.
24. Sugiura N, Sakai Y, Ebara M, et al. Detection of hepatocellular carcinoma after interferon therapy for chronic hepatitis C: clinical study of 26 cases. *J Gastroenterol Hepatol.* 1996;11:535–539.

25. Kubo S, Nishiguchi S, Tamori A, et al. Resected cases of hepatocellular carcinoma detected after interferon therapy for chronic hepatitis C. *Hepatogastroenterology.* 2000;47: 1100–1102.
26. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology.* 1994;19:1513–1520.
27. Okuda K, Kondo Y. Primary carcinoma of the liver. In: Haubrich WS, Schaffner F, Berk JE, editors. Bockus gastroenterology. 5th edition (3), Philadelphia: WB Sanders Company, 1995:2468–2472.
28. Larghi A, Tagger A, Crosignani A, et al. Clinical significance of HCV RNA in patients with chronic hepatitis C demonstrating long-term sustained response to interferon-alpha therapy. *J Med Virol.* 1998;55:7–11.
29. Reichard O, Glaumann H, Fryden A, et al. Two-year biochemical, virological, and histological follow-up in patients with chronic hepatitis C responding in a sustained fashion to interferon alfa-2b treatment. *Hepatology.* 1995;21:918–922.
30. Balart LA, Perrillo R, Roddenberry J, et al. Hepatitis C RNA in liver of chronic hepatitis C patients before and after interferon alfa treatment. *Gastroenterology.* 1993;104:1472–1477.
31. Majima Y. Growth rate of hepatocellular carcinoma by ultrasonography and its clinical significance. *Acta Hepatol Jpn.* 1984;25:754–765.
32. Ebara M, Ohto M, Shinagawa T, et al. Natural history of minute hepatocellular carcinoma smaller than three centimeters complicating cirrhosis. A study in 22 patients. *Gastroenterology.* 1986;90:289–298.
33. Barbara L, Benzi G, Gaiani S, et al. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of growth rate and patient survival. *Hepatology.* 1992;16:132–137.
34. Toyoda H, Kumada T, Honda T, et al. Analysis of hepatocellular carcinoma tumor growth detected in sustained responders to interferon in patients with chronic hepatitis C. *J Gastroenterol Hepatol.* 2001;16:1131–1137.
35. Okanoue T, Itoh Y. Hepatocellular carcinoma in sustained responders of interferon treated chronic hepatitis C. *J Gastroenterol Hepatol.* 2003;18:121–123.

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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

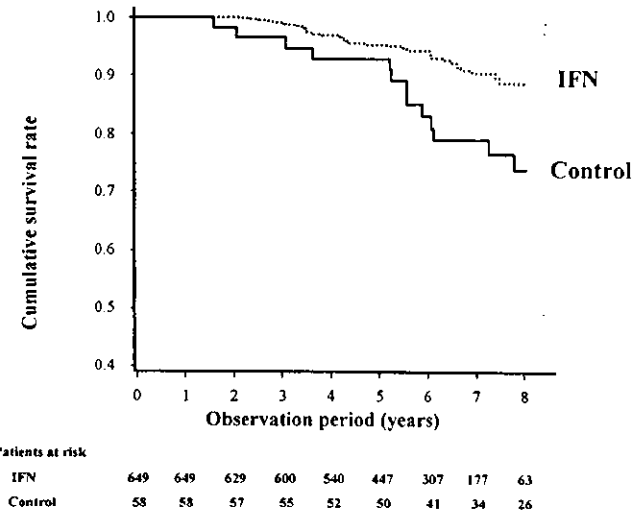

Fig. 1. Cumulative survival rates in the interferon (*IFN; dotted line*) and control (*solid line*) groups. Log-rank test of the two curves showed a significant difference between the two groups ($P = 0.003$)

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-

Table 3. Causes of death in the interferon and control groups

	Interferon group					Control group (n = 58)
	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)
All deaths (n)	4	38	6	6	30	42	13
Liver-related deaths (n)	1	28	1	4	24	29	7
Hepatocellular carcinoma	1	25	1	3	22	26	5
Other causes	0	3	0	1	2	3	2
Liver-unrelated deaths (n)	3	10	5	2	6	13	6

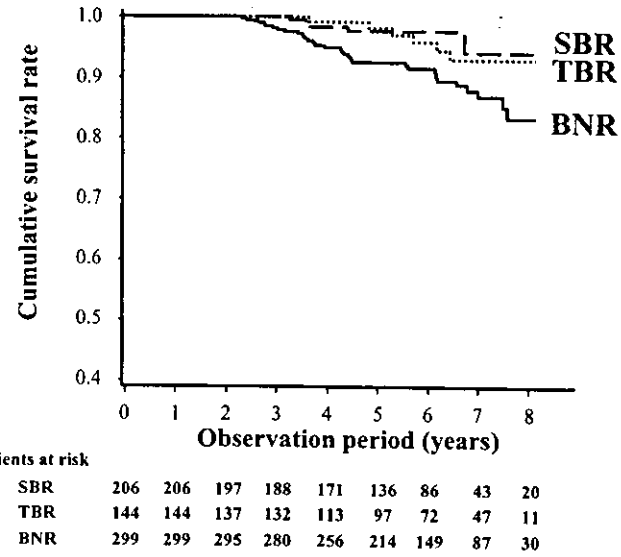

Fig. 2. Cumulative survival rates in the IFN-treated patients, categorized by sustained biochemical response (*SBR*; *dashed line*), transient biochemical response (*TBR*; *dotted line*), and biochemical non-response (*BNR*; *solid line*). Log-rank test showed significant differences between SBR and BNR (P = 0.007) and between TBR and BNR (P = 0.049)

ers. In the control group, 6 patients died of causes other than liver disease; 2 patients died of stomach cancer; 1 patient each died of lung cancer, colon cancer, and cerebral infarction; and in 1 patient, the cause of death was a traffic accident. In the IFN group, we identified 13 liver-unrelated deaths; 4 patients died of stomach cancer; 3 died of lung cancer; and 1 each died of breast cancer, colon cancer, esophageal cancer, pneumonia, chronic renal failure, and multiple myeloma.

Cox proportional hazard regression analysis

Cox proportional hazard regression analysis revealed that the risk of overall death in the IFN group was lower than that in the control group, with a marginally significant difference (risk ratio, 0.37; 95% CI, 0.13–1.05; Table 4). The patients with a sustained virological response had a low risk of overall death (risk ratio, 0.15; 95% CI, 0.04–0.59) compared with the control group. Sustained and transient biochemical responders also showed low risks of overall death (risk ratio, 0.18; 95% CI, 0.05–0.65; and risk ratio, 0.24; 95% CI, 0.07–0.87). The risk of liver-related death in the IFN group was similar to that in the control group (Table 4). However, the patients with sustained virological and biochemical response had a low risk of liver-related death compared to the control group (risk ratio, 0.12; 95% CI 0.01–1.16 and risk ratio, 0.10; 95% CI, 0.01–0.95, respectively). In transient biochemical responders, the risk ratio for liver-related deaths was 0.50 (95% CI, 0.11–2.21).