The effect of interferon on the long-term clinical outcome of patients with chronic hepatitis C remains unclear. This study included 594 patients with chronic hepatitis C who received interferon therapy (Interferon group) and 144 patients with chronic hepatitis C who did not receive interferon (Control group). The patients in the Interferon group were classified into the following three groups based on the result of the serum aminotransaminase level of the patient during and after completion of the therapy protocol: sustained responders (n = 175), transient responders (n = 165), and non-responders (n = 254). The age, sex, serum aminotransaminase level, platelet count, histological staging, hepatitis C virus (HCV) subtype, and HCV concentration at baseline were adjusted with the Cox proportional hazards model. The length of follow-up for the assessment of the risk for developing hepatocellular carcinoma (HCC) was 57.2 ± 13.9 months in the Interferon group and 67.7 ± 28.7 months in the Control group. Multivariate analysis showed that interferon therapy decreased the risk for developing HCC by 48% compared with that in the Control group (P = 0.064). The older the age, being male, having a low platelet count, and higher histological stage were independent factors associated with the development of HCC. The hazard rate ratio for development of HCC in the sustained responders, transient responders, and non-responders was 0.16 (95% confidence interval [CI]: 0.04–0.62), 0.27 (95% CI: 0.09–0.79), and 0.74 (95% CI: 0.37–1.48), respectively. The cumulative survival analysis showed that interferon therapy significantly lowered the incidence of HCC among patients with chronic hepatitis C with a sustained normal serum aminotransaminase level after completion of the therapy protocol, and interferon therapy improves the long-term survival of chronic hepatitis C patients who respond to this therapy, possibly by decreasing mortality from liver-related diseases. Int. J. Cancer 87:741–749, 2000.

Hepatitis C virus (HCV) has been identified as the major causal agent of chronic liver disease and greatly contributes to the etiology of hepatocellular carcinoma (HCC; IARC, 1994; Tanaka and Tsukuma, 1999). According to our statistical estimation using data on the prevalence of HCV infection among healthy individuals and data on the population-based incidence of HCC (Tanaka et al., 1994a), the lifetime risk for developing HCC in middle-aged HCV carriers is 30% in males and 6% in females. The cohort study of Japanese patients with chronic hepatitis C showed that the average annual incidence rate of HCC is 1.3–3.2% (Tsukuma et al., 1993; Takano et al., 1995; Ikeda et al., 1998). These figures indicate the importance of reducing the risk for developing HCC among patients with chronic hepatitis C.

In the early 1990s, interferon-α was introduced worldwide as a therapy for patients with chronic hepatitis C (Camma et al., 1999). This therapy induces normalization of aminotransferase levels and improvement of liver histological findings in 38–48% of chronic hepatitis C patients (Hoofnagle et al., 1986; Davis et al., 1989; Di Bisceglie et al., 1989; Marcellin et al., 1991, 1995; Causse et al., 1991; Shindo et al., 1995). However, over half of the patients who respond to interferon therapy relapse within 6 months after discontinuation of the therapy.

The effect of interferon on the long-term clinical outcome of patients with chronic hepatitis C remains unclear. To evaluate the effect of interferon therapy on the incidence of HCC among patients with chronic hepatitis C, it would be essential to conduct a randomized controlled trial. However, because interferon therapy is already established as a standard modality of radical therapy for chronic hepatitis C patients, a prospective randomized trial with untreated control patients is actually impossible. Only three retrospective studies using control patients who did not receive interferon therapy have been reported (Imai et al., 1998; Ikeda et al., 1999; Yoshida et al., 1999). Two of the studies (Imai et al., 1998; Ikeda et al., 1999) demonstrated that interferon significantly decreased the risk for developing HCC among patients with chronic hepatitis C who showed a persistent normal aminotransaminase level after completion of the therapy; however, interferon did not significantly reduce the risk for HCC among those who had transient normalization of aminotransaminase level for less than 6 months after discontinuation of the therapy (Imai et al., 1998; Ikeda et al., 1999). The effect of interferon-α on the incidence of HCC in patients with compensated type C cirrhosis has been assessed in randomized controlled trials (Nishiguchi et al., 1995;
Valla et al., 1999) and in observational studies using a control group (Mazzaletti et al., 1996; Fattovich et al., 1997; Benvegnu et al., 1998; Serfaty et al., 1998; Benvegnu et al., 1998). Two of these studies (Valla et al., 1999; Fattovich et al., 1997) concluded that interferon therapy does not have a significant beneficial effect in preventing the development of HCC in these patients. Three of the studies (Valla et al., 1999; Serfaty et al., 1998; Benvegnu et al., 1998) also evaluated the impact of interferon-α on mortality, but these results are controversial. Little is known about the effect of interferon therapy on the mortality of patients with chronic hepatitis C.

We therefore attempted to undertake a retrospective cohort study to (1) assess the impact of interferon on the incidence of HCC among patients with chronic hepatitis C with statistical adjustment using possible covariates that were explored in multivariate analysis; and (2) compare the mortality and causes of deaths of the interferon-treated and non–interferon-treated patients with chronic hepatitis C to assess the role of interferon therapy in the long-term survival of chronic hepatitis C patients.

**MATERIAL AND METHODS**

**Patients**

Between January 1980 and June 1996, the Osaka Hepatitis Research Group enrolled 738 patients with chronic hepatitis C who underwent liver biopsy at Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka University Hospital, Osaka National Hospital, Osaka Prefectural Hospital, Osaka Koseinenkin Hospital, and Osaka Roujai Hospital. The subjects were restricted to those who resided in the Osaka Prefecture at the time of diagnosis of chronic hepatitis C to increase the probability that follow-up data on the patients could be obtained. The initial serum sample of each subject was negative for hepatitis B surface antigen and positive for anti-HCV by either first or second-generation ELISA (Ortho Diagnostics, Tokyo, Japan). The sera of patients who had been diagnosed before anti-HCV testing became available in Japan (before 1988) had been frozen at -80°C and retrospectively assayed. Patients were excluded if they presented with HCC or other liver disease such as alcoholic liver disease, autoimmune hepatitis, or primary biliary cirrhosis at the time of liver biopsy.

Of the 738 patients with chronic hepatitis C, 594 received interferon therapy (Interferon group) after 1989, the year when interferon became available in Osaka. Interferon therapy was initiated within 14 days after the liver biopsy. Twelve patients who did not complete the interferon treatment protocol on an intention-to-treat basis were included in this group. The remaining 144 patients did not undergo interferon therapy or any other antiviral therapy, and were placed in the Control group. The Control group consisted of those who had been diagnosed with chronic hepatitis C before 1988 (n = 51) and those who had been diagnosed after 1989 (n = 93).

**Interferon therapy**

The 594 patients in the Interferon group were administered human lymphoblastoid interferon, recombinant interferon-α 2a, or recombinant interferon-α 2b for 6 months according to the standard protocol (Shindo et al., 1995; Hagiwara et al., 1996; Kuzushita et al., 1997). The median total interferon dose in the 594 patients was 480 megaunits (MU; range, 228–1,284 MU). No patient had received interferon therapy before entering the study nor were they retreated with interferon during the observed period. Contraindications to interferon treatment included pregnancy, presence of hepatitis B surface antigen, other types of liver disease (such as alcoholic liver disease and autoimmune liver disease), ultrasonic coarse-nodular cirrhosis, and any other serious illness.

The 594 patients who were treated with interferon were divided into the following three groups based on the change in serum alanine aminotransferase (ALT) level during and after completion of interferon therapy as described previously (Hagiwara et al., 1993): sustained responders included patients whose serum ALT level remained within the normal range for more than 24 weeks after the therapy was discontinued; transient responders included patients whose serum ALT level decreased to the normal range during therapy, but subsequently increased to an abnormal level within 24 weeks following completion of interferon therapy; and non-responders included patients whose ALT level either did not decrease during the therapy or fluctuated.

**Follow-up**

The starting date of follow-up of each patient in the Interferon and Control groups was defined as the date of liver biopsy. Biochemical examinations including α-fetoprotein and abdominal ultrasonography were routinely performed every 3–6 months at the outpatient clinic of the respective hospital. The diagnosis of HCC was confirmed by fine-needle biopsy of the surgically resected tumor specimen, or by liver-selective angiographic and/or computed tomographic imaging. The end of follow-up was the date of diagnosis of HCC, date of death, or the closing date of the study, July 31, 1997. The starting date of the Control group (1980–1996) was earlier than that of the Interferon group (1989–1996). Thirty-one control subjects were observed for over 100 months, which corresponds to the longest period of observation of those in the Interferon group. In these 36 subjects, only the follow-up data up to 100 months were considered. Follow-up data on the patients were obtained from the respective hospital. Follow-up data that were not available from the respective hospital were collected from the data at the Osaka Cancer Registry and the resident registries of the local municipal office. The Osaka Cancer Registry has been operating since 1962 and covers all of Osaka Prefecture which had a population of 8.8 million in 1995. It registers cancer cases using reports from hospitals and clinics and death certificates collected from the Osaka Prefectural Government (Hanai et al., 1997; Oshima et al., 1998; Tanaka et al., 1999). The underlying cause of death was judged from the death certificate of the individual and was classified according to the International Classification of Diseases, 9th Revision. This study protocol is in accordance with the Helsinki Declaration of 1975 (and with its revision of 1983), and was approved by the Ethical Committee of the Osaka Cancer Registry.

**Determination of HCV genome subtype and HCV RNA level**

HCV subtype was classified by either Okamoto’s method (Okamoto et al., 1992) or Kohara’s method (Tanaka et al., 1994b). The serological genotyping assay reported by Kohara (Tanaka et al., 1994b) has been widely and commercially used for classification of HCV subtypes in Japan. In the current study, we classified an individual with HCV genome subtype 1a or 1b as determined by group-specific PCR (Okamoto et al., 1992) or by serological genotype I as determined by ELISA (Tanaka et al., 1994b), as subtype 1; HCV genome subtype 2a or 2b, or serological genotype II, as classified as subtype 2, according to Simmonds classification (Simmonds et al., 1994).

The serum HCV RNA level was quantified using a branched DNA probe assay (version 1; Chiron, Dai-ichi Kagaku, Tokyo; Yuki et al., 1995; Lau et al., 1993), competitive RT-PCR (Hagiwara et al., 1993), or a combined RT-PCR assay (Amplicor-HCV monitor assay; Shiratori et al., 1997). It was designated as a high viral load if the serum HCV RNA level was more than 10⁶ Eq/mL by branched DNA probe assay, more than 10⁶ copies per milliliter serum by competitive RT-PCR, or more than 10⁷ copies per milliliter serum by Amplicor-HCV monitor assay, as described previously (Hagiwara et al., 1993; Yuki et al., 1995; Shiratori et al., 1997).

**Evaluation of histological findings**

The histological findings on liver biopsy specimens were analyzed by the pathologists at the respective institution. For assessment of histological staging, the fibrosis scoring system of Knodell et al. (1981): 0, no fibrosis; 1, fibrous portal expansion; 3, bridging...
fibrosis [portal-portal or portal-central linkage]; and 4, cirrhosis) was used. Unfortunately, Knodell's score could not be assigned to the liver biopsy specimens of 13 patients in the Interferon group and 34 patients in the Control group because their specimens had been evaluated according to other classification systems.

**Statistical analysis**

The chi-square test was used to compare the frequency of the baseline characteristics of sex, serum ALT level, histological staging, subtype of HCV, and serum HCV RNA level in the Interferon and Control groups. The difference in the age and platelet count, sex, serum ALT level, histological staging, HCV subtype, and serum HCV RNA level were included as variables. Univariate analysis with the Kaplan-Meier method and the log-rank test was used to compare the cumulative incidence of HCC and the log-rank test was used to compare the cumulative incidence of HCC in the Interferon and Control groups. Independent factors associated with the development of HCC were studied with Cox proportional hazards regression analysis. In the analysis, interferon therapy, age, sex, serum ALT level, platelet count, histological staging, HCV subtype, and serum HCV RNA level were assessed for significance by Student's t-test. The Kaplan-Meier method was used to calculate the cumulative incidence of HCC and the prevalence of high viral load were significantly higher in the Interferon group than in the Control group (P < 0.01).

Of the 594 patients in the Interferon group, 175 patients (30%) showed a sustained response, 165 patients (28%) showed a transient response, and 254 patients (42%) were non-responders to interferon therapy. Twelve patients could not complete the 6-month interferon treatment protocol mainly because of adverse effects, which included depression, severe general fatigue, and skin eruptions. Of the 12 patients who did not complete 6 months of interferon treatment, 2 showed a sustained response, 2 showed a transient response, and 8 were non-responders.

**Follow-up data on the patients in the interferon and control groups**

For assessment of the risk for developing HCC, the sustained responders were followed for a mean of 59.6 months, the transient responders were followed for 57.3 months, the non-responders were followed for 55.5 months, and the controls were followed for 67.7 months (Table II). Three sustained responders, 5 transient responders, 25 non-responders, and 19 control patients developed HCC. Among these, 3 cases (two non-responders, one control patient) who had been lost to follow-up at the respective institution were found to have HCC through the Osaka Cancer Registry.

The sustained responders, transient responders, non-responders, and controls were observed for a mean of 59.7, 58.1, 57.0, and 71.6 months, respectively, for assessment of the risk for mortality (Table II). Of all 738 subjects, we identified 35 subjects who died during the follow-up period: 25 deaths were identified from follow-up at the respective hospital; 6 deaths were identified from Osaka Cancer Registry data; and 4 deaths were identified by referring to the resident registries of the local municipal office. The 35 subjects who died consisted of 2 sustained responders, 1 tran-

---

**TABLE I - BASELINE CHARACTERISTICS OF THE SUBJECTS WITH CHRONIC HEPATITIS C AT ENTRY**

| Characteristic                  | Interferon group (n = 594) | Control group (n = 144) | Statistical analysis |
|--------------------------------|-----------------------------|-------------------------|---------------------|
| Age (year) (M ± SD)            | 51.7 ± 10.4                 | 52.2 ± 10.6             | p = 0.62            |
| Male/female                    | 410/184                     | 94/50                   | p = 0.39            |
| ALT level (U/L)                |                             |                         |                     |
| <100                           | 422 (71.3)                  | 75 (53.2)               | p < 0.01            |
| ≥100                           | 170 (28.7)                  | 66 (46.8)               |                     |
| Not available                  | 2                           | 3                       |                     |
| Platelet count (×10^9/μL) (M ± SD) | 15.9 ± 5.5                 | 15.7 ± 6.2              | p = 0.71            |
| Histological staging score     |                             |                         |                     |
| 0                              | 14 (2.4)                    | 11 (9.9)                | p < 0.05            |
| 1                              | 315 (54.2)                  | 53 (47.7)               |                     |
| 3                              | 235 (40.4)                  | 36 (32.4)               |                     |
| 4                              | 17 (2.9)                    | 11 (9.9)                |                     |
| Not available                  | 13                          | 33                      |                     |
| HCV subtype                    |                             |                         |                     |
| Subtype 1                      | 416 (74.7)                  | 79 (87.8)               | p < 0.01            |
| Subtype 2                      | 141 (25.3)                  | 11 (12.2)               |                     |
| Not tested                     | 37                          | 54                      |                     |
| HCV concentration              |                             |                         |                     |
| High viral load                | 294 (53.8)                  | 37 (38.1)               | p < 0.01            |
| Low viral load                 | 252 (46.2)                  | 60 (61.9)               |                     |
| Not tested                     | 48                          | 47                      |                     |
sient responder, 15 non-responders, and 17 patients who did not receive interferon therapy. None of the study subjects had undergone orthotopic liver transplantation. The average annual incidence rate of HCC and the average annual mortality rate in each group are presented in Table II.

Cumulative risk for HCC

Figure 1 shows the cumulative incidence of HCC in the Interferon and Control groups, which was estimated using the Kaplan-Meier method. The risk for developing HCC among the Interferon and Control patients was 3.2% and 3.5%, respectively, at the end of the third year; 6.1% and 9.9%, respectively, at the end of the fifth year; and 12.0% and 17.3%, respectively, at the end of the seventh year (log-rank test, \( P = 0.076 \)). Figure 2 presents the Kaplan-Meier estimates of the cumulative incidence of HCC among each of the sustained responders, transient responders, and non-responders to interferon therapy. In comparison with the non-responders, both the sustained and transient responders had significantly lower cumulative risk for developing HCC (log-rank test, \( P < 0.001 \) and \( P < 0.01 \), respectively). The 3-year cumulative incidence of HCC among the sustained responders, transient responders, and non-responders was calculated to be 0.6%, 2.4%, and 5.6%, respectively; the 5-year incidence was 1.2%, 3.7%, and 10.0%, respectively; and the 7-year incidence was 1.2%, 3.7%, and 22.4%, respectively.

Factors affecting the development of HCC

Cox proportional hazards analysis was performed in all 738 chronic hepatitis C patients with the eight variables noted in Table III to identify factors that contribute to the development of HCC. In the analysis, the platelet counts of the subjects were classified into three groups containing an equivalent number of subjects: $\leq 17.5 \times 10^4/\mu L$; $12.8 \leq 17.5 \times 10^4/\mu L$, and $> 17.5 \times 10^4/\mu L$. The 25 patients with no fibrotic change (score 0) were combined with the patients with fibrous portal expansion (score 1); this grouping was used as the reference category in histological staging score. Dummy variables were used for the missing data in histological staging score, HCV subtype, and HCV concentration. There was a significant positive correlation between the age at diagnosis of chronic hepatitis C and risk for developing HCC (\( P = 0.001 \)). Male chronic hepatitis C patients were significantly more likely to develop HCC than female patients (\( P = 0.04 \)). An inverse relationship between platelet count and risk for HCC was found (rate ratio 3.92, \( P = 0.01 \) for $\leq 12.8 \times 10^4/\mu L$; rate ratio 1.92, \( P = 0.23 \) for $12.8 < 17.5 \times 10^4/\mu L$ and $< 12.8 \times 10^4/\mu L$). The 25 patients who had no fibrotic change (score 0) were combined with the patients with fibrous portal expansion (score 1); this grouping was used as the reference category in histological staging score. Dummy variables were used for the missing data in histological staging score, HCV subtype, and HCV concentration. There was a significant positive correlation between the age at diagnosis of chronic hepatitis C and risk for developing HCC (\( P < 0.001 \)). Male chronic hepatitis C patients were significantly more likely to develop HCC than female patients (\( P = 0.04 \)). An inverse relationship between platelet count and risk for HCC was found (rate ratio 3.92, \( P < 0.01 \) for $< 12.8 \times 10^4/\mu L$; rate ratio 1.92, \( P = 0.23 \) for 12.8 $\leq < 17.5 \times 10^4/\mu L$). Patients who had more severe fibrosis at diagnosis had a greater risk for developing HCC (rate ratio 2.04, \( P = 0.04 \) for score 3; rate ratio 5.46, \( P < 0.001 \) for score 4), whereas the ALT level at the time of entry did not significantly influence the development of HCC (rate ratio 1.15, \( P = 0.66 \) for $< 100 \text{ U/L}$). Patients who had HCV subtype 1 had a higher risk for developing HCC than those who had HCV subtype 2 at a marginally significant level (rate ratio

### TABLE II – FOLLOW-UP DATA ON THE STUDY SUBJECTS

|                      | Interferon group | Control group |
|----------------------|------------------|---------------|
|                      | Sustained responder | Transient responder | Non-responder | Total | Control group |
| (n = 175) | (n = 165) | (n = 254) | (n = 594) | (n = 144) |
| Mean period of observation (months) | 59.6 | 57.3 | 55.5 | 57.2 | 67.7 |
| (SD) | (12.6) | (12.6) | (15.2) | (13.9) | (28.7) |
| No. of HCC cases | 3 | 5 | 25 | 33 | 19 |
| Average annual incidence rate | 0.35% | 0.63% | 2.13% | 1.17% | 2.34% |
| All causes of death | Mean period of observation (months) | 59.7 | 58.1 | 57.0 | 58.1 | 71.6 |
| (SD) | (12.5) | (11.7) | (13.6) | (12.8) | (26.5) |
| No. of deaths | 2 | 1 | 15 | 18 | 17 |
| Average annual mortality rate | 0.23% | 0.13% | 1.25% | 0.63% | 1.98% |

\[1\] Calculated by using the person-years method.

**FIGURE 1** – Cumulative incidence of HCC among the 594 interferon-treated chronic hepatitis C patients and among the 144 patients who were not treated with interferon using the Kaplan-Meier method and the log-rank test.
2.16, P = 0.083), whereas the serum HCV concentration was not associated with the risk for developing HCC (rate ratio 0.89, P = 0.71). Interferon therapy lowered the risk for developing HCC by 48% (rate ratio 0.52, 95% CI: 0.26–1.04, P = 0.064), although it did not reduce the risk by a statistically significant degree (Table III).

### Risk of carcinogenesis according to the level of response to interferon

The rate ratio for developing HCC among the sustained responders, transient responders, and non-responders in comparison with the Control patients was determined with adjustment for age and sex (Table IV). Only the sustained responders had a significantly lower risk for developing HCC (rate ratio 0.18, 95% CI: 0.05–0.61, P = 0.006) in the analysis. Second,

![Figure 2](image)

**TABLE III** - Factors associated with the development of HCC in patients with chronic hepatitis C according to Cox proportional hazard analysis (n = 738)

| Variable                        | Rate ratio | 95% CI   | p value |
|---------------------------------|------------|----------|---------|
| Age (by every 1 year)           | 1.09       | 1.04–1.13| <0.001  |
| Female                          | 1.00       |          |         |
| Male                            | 2.00       | 1.03–3.87| 0.04    |
| ALT level (U/L)                 |            |          |         |
| ≥100                            | 1.15       | 0.63–2.11| 0.66    |
| <100                            | 1.00       |          |         |
| Platelet count                  |            |          |         |
| 17.5 × 10^9/μL                  | 1.00       |          |         |
| 12.8 ≤ 17.5 × 10^9/μL           | 1.92       | 0.66–5.57| 0.23    |
| <12.8 × 10^9/μL                 | 3.92       | 1.47–10.45| <0.01  |
| Histological staging score      |            |          |         |
| 0 or 1                          | 1.00       |          |         |
| 3                               | 2.04       | 1.03–4.02| 0.04    |
| 4                               | 5.46       | 2.23–13.39| <0.001 |
| Not available                   | 0.44       | 0.10–2.09| 0.30    |
| HCV subtype                     |            |          |         |
| Subtype 2                       | 1.00       |          |         |
| Subtype 1                       | 2.16       | 0.90–5.19| 0.083   |
| Not tested                      | 0.45       | 0.09–2.38| 0.35    |
| HCV concentration               |            |          |         |
| Low viral load                  | 1.00       |          |         |
| High viral load                 | 0.89       | 0.49–1.63| 0.71    |
| Not tested                      | 0.44       | 0.14–1.32| 0.14    |
| Control group                   | 1.00       |          |         |
| Interferon group                | 0.52       | 0.26–1.04| 0.064   |

1Age, sex, ALT level, platelet count, histological staging, HCV subtype, and HCV concentration at entry were adjusted in the Cox proportional hazard analysis.

we calculated the ratio with adjustment for all of the variables mentioned above. The risk for developing HCC among the sustained and transient responders to interferon therapy was significantly lower than the risk among the Control group (rate ratio 0.16, 95% CI: 0.04–0.62, P = 0.007; rate ratio 0.27, 95% CI: 0.09–0.79, P = 0.02, respectively). In contrast, the HCC risk among the non-responders and that among the controls did not differ significantly (rate ratio 0.74, 95% CI: 0.37–1.48, P = 0.39).

### Survival analysis

To compare the overall survival rates of the Interferon and Control groups, survival analysis was performed by the Kaplan-Meier method, under the condition that the distributions of age and sex of the two groups were quite similar (Table I). During the first 5 years after diagnosis, the survival curves of the two groups were virtually identical (Fig. 3). The 3-year survival rates of the Interferon and Control groups were 96.7% and 97.0%, respectively. Beyond 5 years from diagnosis, the survival rate in the Control group declined, whereas the survival rate in the Interferon group did not, as demonstrated by the finding that the 8-year survival rates of the Interferon and Control groups were 96.7% and 81.0%, respectively. The cumulative survival of the patients in the Interferon and Control groups differed by a marginally significant degree (P = 0.061,

![Figure 3](image)
log-rank test). These trends were similar to those observed in the liver disease-specific survival analysis, which showed that the 5-year survival rates of the Interferon and Control groups were 98.0% and 99.2%, respectively, and that the 8-year rates were 98.0% and 87.7%, respectively (P = 0.32; Fig. 4).

**Causes of death**

Table V shows the number of deaths according to the underlying cause of death. The underlying cause of death of five cases (two in the Interferon group and three in the Control group) is unknown because these individuals moved outside of the Osaka Prefecture after entry and their death certificates could not be obtained. In the Interferon group, 10 patients died of liver disease (8 of primary liver cancer, 2 of liver cirrhosis). No sustained or transient responder in the Interferon group died of liver disease. There was no significant difference in the proportion of deaths that were due to liver disease in the Interferon and Control groups (Interferon group 10 of 18, Control group 10 of 17, P = 0.69 by Fisher’s exact test). Six patients in the Interferon group died of other causes; one death each was attributed to stomach cancer, breast cancer, leukemia, and viral pneumonia, and two deaths were suicides. One of the patients who died of suicide was a sustained responder who committed suicide 9 months after completion of interferon therapy.
The other was a non-responder, who also died 10 months after the completion of interferon therapy. The median total interferon dose in the six patients who died of non-liver-related causes was 451 MU (range, 252–480 MU), which is nearly equivalent to the median total interferon dose in all Interferon subjects (480 MU). Of the 17 deaths in the Control group, 4 patients died of other underlying causes (stomach cancer, pancreas cancer, leukemia, and cerebral infarction). Mortality from non-liver-related causes in Interferon and Control groups did not differ significantly (P = 0.14, log-rank test). When the five cases of unknown cause of death were regarded as cases of non-liver-related causes of death in the respective groups, mortality from non-liver-related causes in the Interferon and Control groups did not differ significantly (P = 0.10).

DISCUSSION

Because interferon therapy is already widely used as a standard modality of radical therapy for chronic hepatitis C, a prospective randomized trial with untreated control patients is actually impossible in Japan. We, therefore, attempted to perform this retrospective study with statistical adjustment for possible covariates using the proportional hazards model. The proportion of patients who had liver cirrhosis with histological staging score 4 (9.9%) and the proportion who were infected with HCV subtype 1 (mainly genotype 1b; 87.8%) in the Control group were higher than the respective proportion in the Interferon group (2.4%, 74.7%, respectively), possibly because of their lower indication for interferon therapy for its short-term efficacy. These differences in the baseline characteristics of the two groups were adjusted in the statistical evaluation of the effect of interferon therapy, although the possibility of inequality between the two groups could not be fully excluded. Misscategorization of the histological grade of liver biopsy specimens due to the dependence on different pathologists may have occurred non-differentially in the two groups; this would have underestimated the effect of interferon on the incidence of HCC. Compliance with long-term medical follow-up may have been different among the patients in the Interferon and Control groups. This inequality may have caused a detection bias. To reduce the detection bias, we collected incidence data on HCC from the Osaka Cancer Registry to compensate for incomplete information from each hospital. In the Control group, patients who had been diagnosed after 1989 may have included patients who could not receive interferon therapy because of their poor physical condition, which may have overestimated the effect of interferon on overall survival. However, the survival curves of the two groups were quite similar over the first 5 years, which indicates that the difference in the potential survivorship of the patients in the Interferon and Control groups at entry must have been small, if it existed.

In our retrospective analysis of patients with chronic hepatitis C, treatment with interferon lowered the risk for developing HCC (rate ratio = 0.52, P = 0.064), although it did not reduce the risk by a statistically significant degree. One of the reasons why statistical significance was not obtained in this study is the wide CI (95% CI: 0.26–1.04) which resulted from a relatively small number of study subjects in the multiple regression analysis. Another reason is that the proportion of non-responders in the Interferon group (42%) was higher than we had expected based on earlier studies conducted in Japan (Imai et al., 1998; Hino et al., 1994; Tsubota et al., 1994). In general, a high percentage (70–80%; Tanaka et al., 1998) of Japanese patients with chronic hepatitis C has genotype 1b, expressing subtype 1. It has been reported that interferon is less effective in normalizing liver inflammation in patients with type 1b chronic hepatitis C than in patients with other genotypes of chronic hepatitis C (Shiratori et al., 1997; Hino et al., 1994; Tsubota et al., 1994). Therefore, interferon therapy in Japanese chronic hepatitis C patients would show a smaller prophylactic effect against HCC than interferon therapy in other populations of patients with a smaller percentage of type 1b.

Our multiple regression analysis clearly showed that the risk for developing HCC among chronic hepatitis C patients who showed a sustained response to interferon was 84% lower than that among the Control patients (rate ratio 0.16, P = 0.007). In 76% of the patients with chronic hepatitis C who had a sustained response to interferon therapy in the study of Kasahara et al. (1998), serum HCV RNA became undetectable at 3–6 months after completion of treatment. The significant reduction in the risk for developing HCC among the sustained responders may be due to alleviation of liver fibrosis and inflammation by eradication of HCV and/or normalization of ALT (Moreno and Muriel, 1995). However, in our study, a total of three sustained responders developed HCC, of which two developed HCC within 39 months after completion of interferon therapy. These patients may have had a small, invisible HCC on various imaging at the time of interferon therapy. This suggests the need for further follow-up of sustained responders for at least several years after completion of interferon therapy.

The effect of interferon therapy on the incidence of HCC among transient responders has been poorly defined. Our findings demonstrated that the risk for developing HCC among the transient responders, who had re-elevation of their ALT within 24 weeks after completion of interferon therapy, was 73% lower than that among the controls (rate ratio 0.27, P = 0.02) over an observation period of a mean of 57.3 months. This finding is worthwhile in considering the implications of interferon therapy in chronic hepatitis C patients, because approximately one third of patients who respond to interferon relapse within 24 weeks after completion of interferon therapy. However, upon re-elevation of their ALT after completion of interferon therapy, the active carcinogenic process would resume and the risk for developing HCC in the later period would increase. Therefore, management of liver inflammation and screening for HCC are still necessary in transient responders to interferon therapy.

### TABLE V – UNDERLYING CAUSES OF DEATH OF THE PATIENTS WITH CHRONIC HEPATITIS C

| Cause of death         | Sustained responder (n = 2) | Transient responder (n = 1) | Non-responder (n = 15) | Total (n = 18) | Control group (n = 17) | p value |
|------------------------|-----------------------------|----------------------------|-----------------------|---------------|-----------------------|---------|
| Liver disease          | 0                           | 0                          | 10                    | 10            | 10                    | 0.32    |
| Primary liver cancer   | 0                           | 0                          | 8                     | 8             | 8                     |         |
| Liver cirrhosis        | 0                           | 0                          | 2                     | 2             | 2                     |         |
| Other causes           | 1                           | 1                          | 4                     | 6             | 4                     | 0.14    |
| Other cancers          | 0                           | 0                          | 2^2                   | 3             | 3^3                   |         |
| Other diseases         | 0                           | 0                          | 1^4                   | 1             | 1^5                   |         |
| Suicide                | 1^6                         | 0                          | 1^7                   | 2             | 0                     |         |
| Unknown                | 1                           | 0                          | 1                     | 1             | 3                     |         |

1Breast cancer. 2One from stomach cancer, one from leukemia. 3Viral pneumonia. 4Cerebral infarction. 5Committed suicide 9 months after the completion of interferon therapy. 6Committed suicide 10 months after the completion of interferon therapy. The p values are for comparison of the cumulative survival rate of the Interferon and Control groups by the log-rank test.
Although the effects of interferon therapy on the development of HCC in patients with chronic hepatitis C have been reported in retrospective studies, whether or not interferon therapy contributes to reducing mortality has not yet been well studied. One prospective study conducted in Germany (Niederau et al., 1998) suggested that interferon therapy improved survival among patients with chronic hepatitis C, although interferon-treated patients did not have a reduced HCC risk. In the present study, we compared the cumulative overall survival rate of the Interferon group with that of the Control group in univariate analysis, under the conditions that the distributions of age and sex in the two groups were quite similar. The survival curves showed that there was no difference in the cumulative mortality of the Interferon and Control groups over the first 5 years. Beyond 5 years from diagnosis, the survival rate in the Control group declined mainly due to a rise in mortality from liver disease (liver cirrhosis or primary liver cancer), whereas the survival rate in the Interferon group did not decline. None of the patients who had a sustained or transient response to interferon therapy in our study died of liver disease, although the cause of death of one patient who had sustained response could not be determined. These findings support the notion that interferon therapy in chronic hepatitis C patients suppresses hepatocellular inflammatory and regenerative processes, which subsequently reduce mortality from liver diseases and contribute to improvement of overall survival mainly in the responders. In other words, it may be realistic to consider that interferon therapy has a beneficial effect on the overall survival of patients with chronic hepatitis C, of which a large proportion die of liver disease (i.e., 10 of 17 in the Control group). To confirm this notion, chronic hepatitis C patients who receive interferon therapy need to be observed over a longer period of time.

The short-term adverse effects of interferon therapy include depression, severe weakness, thyroid dysfunction, thrombocytopenia, and interstitial pneumonia (Ikeda et al., 1999; Pariente et al., 1999), whereas the chronic or long-term serious effects have not yet been evaluated. Our study showed that mortality due to causes other than liver disease in the Interferon group did not significantly differ from that in the Control group. Two patients who had received interferon therapy committed suicide 9–10 months after the completion of therapy. In this condition, it is less likely to consider that depression subsequent to completion of interferon therapy triggered the suicidal attempts of these two patients.

In conclusion, our study demonstrated that interferon therapy significantly lowered the incidence of HCC among patients with chronic hepatitis C who showed sustained normalization and among patients who showed transient normalization of the serum aminotransferase level after completion of the interferon therapy. This finding supports the notion (Kasahara et al., 1998) that the aim of interferon therapy for chronic hepatitis C should be not only HCV eradication but also ALT normalization during the therapy to reduce the incidence of HCC. The survival analyses and determination of causes of death of the chronic hepatitis C patients suggested that interferon therapy improves the long-term survival in patients who responded to the therapy, possibly by decreasing mortality from liver-related diseases.

Acknowledgements

This study was financially supported, in part, by the Osaka Prefectural Government and a grant for the New Ten-Year Strategy for Cancer Control, Prevention of Cancer, from the Ministry of Health and Welfare of Japan. The authors thank Mr. Matsuji Deguchi and Ms. Yasue Kotani for their technical assistance.

References

Benvenuto, L., Chemello, L., Novella, F., Fattovich, G., Pontessi, P., and Alberti, A. Retrospective analysis of the effect of interferon therapy on the clinical outcome of patients with viral cirrhosis. Cancer, 83, 901–909 (1998).

Brunetto, M.R., Oliveri, F., Kohler, M., Zahm, F., Bonino, F. and the International Interferon-α Hepatocellular Carcinoma Study Group. Effect of interferon-α on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. Lancet, 351, 1535–1539 (1998).

Camma, C., Giunta, M., Pinzello, G., Morabito, A., Verderio, P. and Pagliaro, L. Chronic hepatitis C and interferon alpha: conventional and cumulative meta-analyses of randomized controlled trials. Amer. J. Gastroenterol., 94, 581–586 (1999).

Caussé, X., Godinot, H., Chevallier, M., Cosségeos, P., Zoulim, F., Ouzan, D., Heyraud, J.P., Fontanges, T., Albright, J. and Meschkevitz, C., Comparison of 1 or 3 MU of interferon α-2b and placebo in patients with chronic non-A, non-B hepatitis. Gastroenterology, 101, 497–502 (1991).

Davis, G.L., Balart, L.A., Schiff, E.R., Lindsay, K., Bodenheimer, H.C. Jr., Perrillo, R.P., Carey, W., Jacobson, I.M., Payne, J. and Dienstag, J.L., Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Intervventional Therapy Group. N. Engl. J. Med., 321, 1501–1506 (1989).

Di Bisceglie, A.M., Martin, P., Kassianides, C., Lesker-Melman, M., Murray, L., Waggoner, J., Goodman, Z., Banks, S.M. and Hoofnagle, J.H., Recombinant interferon alpha therapy for chronic hepatitis C: A randomized, double-blind, placebo-controlled trial. N. Engl. J. Med., 321, 1506–1510 (1989).

Fattovich, G., Giustina, G., Dedios, F., Diodati, G., Tremolada, F., Nevens, F., Almasio, P., Solinas, A., Brouwer, J.T., Thomas, H., Realld, G., Corrocher, R. and Schaffiti, S.W., Effectiveness of interferon α-2a on incidence of hepatocellular carcinoma and decompen-
sation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EURO-HEP). J. Hepatol., 27, 201–205 (1997).

Hagiwara, H., Hayashi, N., Kasahara, A., Oshita, M., Kataya, K., Kato, M., Masuzawa, M., Fusamato, H., Sakurai, M. and Kamada, T., Treatment with recombinant interferon-α 2a for patients with chronic hepatitis C: predictive factors for biochemical and virologic response. Scand. J. Gastroenterol., 31, 1021–1026 (1996).

Hagihara, H., Hayashi, N., Mita, E., Takehara, T., Kasahara, A., Fusamato, H. and Kamada, T., Quantitative analysis of hepatitis C virus RNA in serum during interferon alfa therapy. Gastroenterology, 104, 877–883 (1993).

Hanai, A., Aiki, W., Tsukuma, H., Oshima, A. and Fujimoto, I., Osaka Cancer Registry. In D.M. Parkin, S.L. Whelan, J. Ferlay, L. Raymond, and J. Young (eds.), Cancer incidence in five continents, vol. VII, no. 32, pp. 394–401, IARC, Lyon (1997).

Hino, K., Sainokami, S., Shioda, K., Ino, S., Wang, Y., Okamoto, H., Miyakawa, Y. and Mayumi, M., Genotypes and titers of hepatitis C virus for predicting response to interferon in patients with chronic hepatitis C. J. Med. Virol., 42, 299–305 (1994).

Hoofnagle, J.H., Muller, K.D., Jones, D.B., Rustgi, V., Di Bisceglie, A., Peters, W., Waggoner, J.G., Park, Y. and Jones, E.A., Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon. A preliminary report. N. Engl. J. Med., 315, 1575–1578 (1986).

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, IARC monographs on the evaluation of carcinogenic risks to humans. Hepatitis C virus, vol. 59, IARC, Lyon (1994).

Ikeda, K., Saitoh, S., Arase, Y., Chayama, K., Suzuki, Y., Kobayashi, M., Tsutoba, A., Nakamura, I., Murashima, N., Komada, H. and Kawashima, M., 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, 1124–1130 (1999).

Ikeda, K., Saitoh, S., Suzuki, Y., Kobayashi, M., Tsutoba, A., Koida, I., Arase, Y., Fukuda, M., Chayama, K., Murashima, N. and Kamada, H., Disease progression and hepatocellular carcinogenesis in patients with chronic hepatitis C: a prospective observation study of 2215 patients. J. Hepato-

tol., 28, 930–938 (1998).

Imai, Y., Kawata, S., Tamura, S., Yabuuchi, L., Noda, S., Inada, M., Maeda, Y., Shirai, Y., Fukuzaki, T., Kaji, I., Ishikawa, M., Matsuoka, Y., Nishikawa, M. and 2 Others, Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Aon. intern. Med., 129, 94–99 (1998).

Kasahara, A., Hayashi, N., Mochizuki, K., Takayama, M., Yoshoka, K., Kakumi, S., Irima, A., Urushihara, A., Kiyosawa, K., Okuda, M.,...
EFFECT OF INTERFERON THERAPY ON HCV INFECTION

Hino, K. and Okita, K., Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. *Hepatology*, 27, 1394–1402 (1998).

Knodel, R.G., Ishak, K.G., Black, W.C., Chen, T.S., Craig, R., Kaplowitz, N., Kientz, T.W. and Wollman, J., Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology*, 1, 431–435 (1981).

Kuzushita, N., Hayashi, N., Katayama, K., Kanto, T., Oshita, M., Hagiwara, H., Kasahara, A., Fusamoto, H. and Kamada, T., High levels of serum interleukin-10 are associated with a poor response to interferon treatment in patients with chronic hepatitis C. *Scand. J. Gastroenterol.*, 32, 169–174 (1997).

Lau, J.Y., Davis, G.L., Kniffen, J., Qian, K.P., Ureeda, M.S., Chan, C.S., Mizokami, M., Neuwald, P.D. and Wilher, J.C., Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet*, 341, 1501–1504 (1993).

Marcellin, P., Boyer, N., Glostra, E., Couroucé, A.M., Degos, F., Coppere, H., Cales, P., Couzigou, P. and Benhamou, J.P., Recombinant human alpha-interferon in patients with chronic non-A, non-B hepatitis: a multicenter randomized controlled trial from France. *Hepatology*, 13, 393–397 (1991).

Marcellin, P., Pouteau, M., Martinot-Peignoux, M., Degos, F., Duchatelie, V., Boyer, N., Lemonnier, C., Degott, C., Erlinger, S. and Benhamou, J.P., Lack of benefit of escalating dosage of interferon alfa in patients with chronic hepatitis C. *Gastroenterology*, 109, 156–165 (1995).

Mazzella, G., Accogli, E., Sottill, S., Fesit, D., Orsini, M., Salzetta, A., Novelli, V., Cipolla, A., Fabbrini, C., Pizzolli, A. and Roda, E., Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. *J. Hepatol.*, 24, 141–147 (1996).

Moreno, M.G. and Muriel, P., Remission of liver fibrosis by interferon alpha 2b. *Biochem. Pharmacol.*, 50, 515–520 (1995).

Niederau, C., Lange, S., Heintges, T., Erhard, A., Buschkamp, M., Hütter, D., Nawkocki, M., Kruska, L., Hensel, F., Petit, W. and Häusseringer, D., Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology*, 28, 1687–1695 (1998).

Nishiguchi, S., Kuroki, T., Nakatani, S., Morimoto, H., Takeda, T., Nakajima, S., Shimoi, S., Seki, S., Kobayashi, K. and Otani, S., Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet*, 346, 1051–1055 (1995).

Okamoto, H., Sugiyama, Y., Okada, S., Kurai, K., Akahane, Y., Sugai, Y., Tanaka, T., Sato, K., Tsuda, F. and Miyakawa, Y., Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J. gen. Virol.*, 73, 673–679 (1992).

Oshima, A., Aiki, W., Tanaka, H. and Tsukuma, H., Significance and usefulness of cancer registries. *Int. J. clin. Oncol.*, 3, 343–350 (1998).

Pariante, C.M., Germaina, Orru, M., Batia, A., Farci, M.G. and Carpinello, B., Treatment with interferon-α in patients with chronic hepatitis and mood or anxiety disorders. *Lancet*, 354, 131–132 (1999).

Piron, L., Aumaître, H., Chazouillères, O., Bonnard, A.M., Rosmorduc, O., Poupon, R.E. and Poupon, R., Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology*, 27, 1435–1440 (1998).

Shindo, M., Arak, K., Sokawa, Y. and Okuno, T., Hepatic hepatitis C virus RNA as a predictor of a long-term response to interferon-α therapy. *Ann. intern. Med.*, 122, 586–591 (1995).

Shiratori, Y., Kato, N., Yokosuka, O., Imazeki, F., Hashimoto, E., Hayashi, N., Nakamura, A., Asada, M., Kuroda, H., Tanaka, N., Arakawa, Y. and Omata, M., Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. *Gastroenterology*, 113, 558–566 (1997).

Simmonds, P., Alberti, A., Alter, H.J., Bonino, F., Bradley, D.W., Brechot, C., Brouwer, J.T., Chan, S.W., Chayama, K. and Chen, D.S., A proposed system for the nomenclature of hepatitis C viral genotypes. *Hepatology*, 19, 1321–1324 (1994).

Takano, S., Yokosuka, O., Imazaki, F., Tagawa, M. and Omata, M., Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology*, 21, 650–655 (1995).

Tanaka, H., Hyama, T., Tsukuma, H., Fujimoto, I., Yamano, H., Okubo, Y. and Kitada, A., Cumulative risk of hepatocellular carcinoma in hepatitis C virus carriers: statistical estimations from cross-sectional data. *Jpn. J. Cancer Res.*, 85, 485–490 (1994a).

Tanaka, T., Tsukiyama-Kohara, K., Yamaguchi, K., Yagi, S., Tanaka, S., Hasegawa, A., Ohta, Y., Hattori, N. and Kohara, M., Significance of specific antibody assay for genotyping of hepatitis C viruses. *Hepatology*, 19, 1347–1553 (1994b).

Tanaka, H. and Tsukuma, H., Hepatitis C virus. In J. Tooze (ed.), *Cancer survey*, vol. 33, Cold Spring Harbor Laboratory Press, New York (1999).

Tanaka, H., Tsukuma, H., Masaoka, T., Aiki, W., Koyama, Y., Kinoshita, N., Haseo, S. and Oshima, A., Suicide risk among cancer patients: experiences at one medical center in Japan, 1978–1994. *Jpn. J. Cancer Res.*, 90, 812–817 (1999).

Tanaka, H., Tsukuma, H., Yamano, H., Okubo, Y., Inoue, A., Kasahara, A. and Hayashi, N., Hepatitis C virus infection & noncirrhotic patients with chronic hepatitis C in Japan. *Hepatology*, 24, 328, 1797–1801 (1993).

Valla, D.C., Chevallier, M., Marcellin, P., Payen, J.L., Tripo, C., Funck, M., Boulliere, M., Boucher, E., Miguet, J.P., Parlier, D., Lemonnier, C. and Opolon, P., Treatment of hepatitis C virus-related cirrhosis: a randomized, controlled trial of interferon alfa-2b versus no treatment. *Hepatology*, 29, 1870–1875 (1999).

Yoshida, H., Shiratori, Y., Moriyama, M., Arakawa, Y., Ide, T., Sata, M., Inoue, O., Yano, M., Tanaka, M., Furuya, S., Nishiguchi, S., Kuroki, T., Imazeki, F. and Tani, F., 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.*, 131, 174–181 (1999).

Yuki, N., Hayashi, N., Kasahara, A., Hagiwara, H., Takehara, T., Oshita, M., Katayama, K., Fusamoto, H. and Kamada, T., Pretreatment viral load and response to prolonged interferon-α course for chronic hepatitis C. *J. Hepatol.*, 22, 457–463 (1995).