CLINICAL RESEARCH STUDY

THE AMERICAN JOURNAL of MEDICINE®

# Necessities of Interferon Therapy in Elderly Patients with Chronic Hepatitis C

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## ABSTRACT

**BACKGROUND:** The significance of antiviral therapy for elderly patients with chronic hepatitis C virus (HCV) infection has not been elucidated.

**PATIENTS AND METHODS:** Among 5645 patients with HCV-related chronic liver disease, the prognosis of 1917 elderly patients aged 60 years or more was analyzed. A total of 454 patients underwent interferon (IFN) therapy. By using multivariate analysis, carcinogenesis and survival were analyzed according to initial findings.

**RESULTS:** At 10 and 15 years, cumulative survivals in untreated elderly patients were 90.7% and 72.7% in the high platelet ($\geq$150,000/mm$^3$) group, 78.6% and 47.8% in the intermediate (100,000-149,000/mm$^3$) group, and 52.5% and 25.0% in the low platelet group (<100,000/mm$^3$), respectively. At 5 and 10 years, hepatocarcinogenesis rates in the intermediate and low platelet groups were 10.9% and 21.6% in the IFN group (N = 217) and 19.5% and 43.0% in the untreated group (N = 459), respectively ($P = .0005$). IFN independently decreased carcinogenesis risk with a hazard ratio of 0.56 ($P = .035$). In the high platelet group, 5- and 10-year carcinogenesis rates were 3.7% and 8.3% in the IFN-treated group (N = 228) and 5.1% and 14.0% in the untreated group (N = 585), respectively ($P = .69$). IFN treatment significantly increased cumulative survivals in the lower platelet subgroup ($P = .0001$) but did not affect the higher platelet subgroup ($P = .08$). IFN was independently associated with a longer survival in the lower platelet subgroup (hazard ratio 2.33, $P = .005$).

**CONCLUSION:** In elderly patients with chronic HCV, IFN for a subgroup with intermediate and low platelet counts had significant advantages in regard to hepatocarcinogenesis and survival.

 • *The American Journal of Medicine (2009) 122, 479-486*

**KEYWORDS:** Chronic hepatitis C virus; Elderly; Hepatocellular carcinogenesis; Interferon; Survival

Hepatitis C virus (HCV) is one of the principal causes of hepatocellular carcinoma and often causes high morbidity and mortality in many countries.[1-5] Because interferon (IFN) has antiviral, antifibrotic, and anti-inflammatory actions, it is still a main arm in the treatment of chronic HCV.[6,7] Many authors have demonstrated that IFN prevents hepatocarcinogenesis and eventually prolongs the survival period of patients.[8-13] Radical eradication of HCV by IFN depends on viral load, HCV subtype, certain mutations of hepatitis virus gene, liver histology, modes of IFN administration, and various host factors, including a patient's age.[14-16] When a significant side effect occurs during IFN therapy, cessation or early withdrawal of the therapy often failed to attain a successful result. Early withdrawal and treatment failure are likely more common in elderly patients and patients with an advanced stage of liver disease.

The number and rate of elderly patients with HCV-positive chronic hepatitis are currently increasing in the United States and Japan[17-19] because of a significant decrease of new blood-borne HCV infections and an aging

**Funding:** This work was partly supported by a grant of Ministry of Health, Labor, and Welfare, Japan.

**Conflict of Interest:** None of the authors have any conflicts of interest associated with the work presented in this manuscript.

**Authorship:** All authors had access to the data and played a role in writing this manuscript.

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doi:10.1016/j.amjmed.2008.09.045

society, such as in Japan. In elderly patients with chronic hepatitis or cirrhosis type C, adverse effects of IFN are more prevalently found and hematologic disorders often disturb the completion of the therapy. As a result, IFN administration is considered less effective in elderly patients.[16,20-22] Because the fibrotic stage of liver disease is often correlated with a patient's age, an elderly patient naturally has a high risk of carcinogenesis and mortality. IFN is effective in reducing hepatocarcinogenesis and improving the survival of patients with HCV-related chronic hepatitis, but the clinical influence of IFN is considered less advantageous in elderly patients because of the short life expectancy. There has been little information on the prognosis of elderly patients with HCV-related chronic liver disease and the significance of antiviral therapy for elderly patients.

To clarify whether IFN had similar advantages between young and elderly patients, we analyzed a large cohort of HCV-positive elderly patients in regard to hepatocellular carcinogenesis and survival at a single institution. We also attempted to elucidate favorable indications and the best candidates for IFN therapy among elderly patients, if any.

**CLINICAL SIGNIFICANCE**

- Significant differences in hepatocarcinogenesis and survival exist among patients with HCV, according to initial platelet count.
- IFN for a subgroup with intermediate and low platelet counts had significant advantages in regard to hepatocarcinogenesis and survival of elderly patients with chronic HCV.
- Asymptomatic elderly patients with HCV should be observed carefully as to hepatocarcinogenesis by using ultrasonography when the platelet count is 150 × 1000/mm$^3$ or less.
- IFN therapy should be considered in elderly patients when they have intermediate and low platelet counts.
- In view of the side effects in elderly patients, treatment should be initiated as soon as possible after diagnosis of chronic HCV.

## PATIENTS AND METHODS

### Entire Population and Analyzed Cohorts

A total of 7235 patients were diagnosed with HCV-positive chronic liver disease with positive anti-HCV antibody and detectable HCV-RNA (nested polymerase chain reaction) and negative hepatitis B surface antigen from 1974 to 2004 at the Department of Hepatology, Toranomon Hospital, Tokyo. Anti-HCV and HCV-RNA were assayed using stored frozen sera. There were 4121 men and 3114 women, with a median age of 54 years (range, 1-92 years). We excluded 1144 patients with acute hepatitis, overt alcoholic liver disease or fatty liver, association of other types of liver disease (eg, primary biliary cirrhosis, autoimmune hepatitis), or association with hepatocellular carcinoma or other. We also excluded 446 patients with a short observation period (<6 months).

There were 3728 patients aged less than 60 years and 1917 patients aged 60 years or more. The diagnosis was established by peritoneoscopy or biopsy in 636 patients and by clinical data in 1281 patients. The ratio of women was higher (36.9% vs 54.4%, $P < .001$) and history of IFN therapy was lower (60.3% vs 23.7%, $P < .001$) in elderly patients. Median albumin value was lower (4.3 vs 4.1 g/dL, $P < .001$) and platelet count was lower (181,000 vs 155,000/mm$^3$, $P < .001$) in elderly patients. This study analyzed 1917 elderly patients with HCV: 454 patients (23.7%) with IFN therapy and 1463 patients (76.3%) without IFN therapy.

### Interferon Treatment and Judgment of Effect

Among 454 patients with IFN therapy, 413 received IFN monotherapy and 41 received IFN plus ribavirin combination therapy as an initial antiviral therapy. Of 413 patients with IFN monotherapy, 272 patients received IFN every day for the first 2 to 8 weeks and then 2 to 3 times per week for the following 16 to 96 weeks (median, 24 weeks), 108 patients received IFN 3 times per week for 24 to 104 weeks, and 33 patients received IFN for 4 to 8 weeks. Among 346 patients without viral elimination after initial IFN therapy, 186 patients underwent repeated IFN therapy including IFN plus ribavirin combination therapy. The age at the time of initiation of therapy ranged from 60 to 84 years, with a median of 64 years.

Most patients (N = 451) with IFN therapy showed varied degrees of influenza-like symptoms, leukocytopenia, and thrombocytopenia. Forty-three patients discontinued IFN therapy because of significant adverse reactions: depression in 10 patients, marked anorexia in 9 patients; psychosis, epilepsy, or loss of consciousness in 8 patients; ophthalmic diseases in 3 patients; severe cytopenia in 3 patients; interstitial pneumonia in 2 patients; and other conditions in 8 patients. No patients had decompensated liver disease with ascites, encephalopathy, jaundice, or variceal bleeding.

Judgment of IFN effect was classified according to elimination of HCV RNA and alanine aminotransferase for 6 months after the end of treatment. Sustained virologic response was defined as persistent disappearance of HCV RNA after therapy, biochemical response was defined as normal alanine aminotransferase values without elimination of HCV RNA for at least 6 months after therapy, and no response was defined as persistently abnormal or only transient normalization of alanine aminotransferase for less than 6 months. Because 12 patients (2.6%) were lost to follow-up and 49 patients (10.8%) were still in the course of IFN therapy, the judgment was made in 393 (86.6%) of 454 patients.

**Table 1** Profiles and Laboratory Data of 1917 Elderly Patients at the Initial Visit to Toranomon Hospital

| | No Therapy N = 1463 | IFN Therapy N = 454 | P[c] |
|---|---|---|---|
| Demography | | | |
| Sex (M/F) | 660/803 | 214/240 | .45 |
| Age (y)[a] | 65 (60-88) | 62 (60-80) | <.001 |
| Observation period (y)[a] | 5.91 (0.5-27.6) | 6.23 (0.5-17.6) | .23 |
| Lost to follow-up (y) | 165 (11.3%) | 12 (2.6%) | <.001 |
| Laboratory Data[b] | | | |
| Albumin (g/dL) | 4.1 (3.8-4.3) | 4.1 (3.9-4.3) | .11 |
| Bilirubin (mg/dL) | 0.6 (0.5-0.9) | 0.7 (0.5-0.8) | .14 |
| Aspartic aminotransferase (IU/L) | 51 (33-83) | 70 (46-106) | <.001 |
| Alanine aminotransferase (IU/L) | 56 (32-97) | 90 (56-148) | <.001 |
| Hemoglobin (g/dL) | 13.8 (12.9-14.7) | 14.2 (13.3-15.1) | <.001 |
| Platelet count (×1000/mm$^3$) | 157 (120-198) | 150 (122-195) | 0.12 |
| Alpha-fetoprotein (ng/mL) | 4 (3-6) | 4 (3-6) | .80 |
| HCV | | | |
| subtype 1 (1a/1b) | 714 (79.2%) | 154 (58.8%) | <.001 |
| subtype 2 (2a/2b) | 150 (16.6%) | 102 (38.9%) | |
| others | 38 (4.2%) | 6 (2.3%) | |

IFN = interferon; HCV = hepatitis C virus.
[a]Expressed by median (range).
[b]Expressed by median (25th percentile, 75th percentile).
[c]Mann–Whitney or chi-square test.

## Follow-up of and Diagnosis of Hepatocellular Carcinoma

Follow-up of patients was made on a monthly to trimonthly basis after the initial visit. Imaging diagnosis was made 1 or more times per year with ultrasonography, computed tomography, or magnetic resonance imaging.

## Statistical Analysis

Obtained clinical data were analyzed on an intention-to-treat basis. Nonparametric procedures were used for the analysis of background characteristics of the patients, including the Mann–Whitney *U*, Kruskal–Wallis, and chi-square tests.

Hepatocellular carcinogenesis and survival were calculated using the Kaplan–Meier test. The differences in carcinogenesis curves were tested using the log-rank test.[23] Independent factors associated with the appearance rate of hepatocellular carcinoma were studied using time-dependent Cox regression analysis.[24] The following 16 variables were analyzed for potential covariates for liver carcinogenesis at the initial hospital visit: age, sex, total alcohol intake, family history of liver disease, history of blood transfusion, association of diabetes, aspartic aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, albumin, bilirubin, hemoglobin, platelet count, serologic grouping of HCV, IFN administration, and effect of IFN treatment (time-dependent variable). A *P* value of less than .05 was considered significant. Statistical analysis was performed using the Statistical Package for the Social Sciences version 11.[25]

# RESULTS

## Demographics of Elderly Patients with or without Interferon Therapy

Table 1 summarizes the profiles and data of the 1917 elderly patients with or without IFN therapy during clinical course. The median age of the patients with IFN was younger by 3 years. Although aminotransferases were significantly higher in the treated group, albumin, bilirubin, and platelet count were not different between the 2 groups.

## Hepatocarcinogenesis and Survival without Interferon Therapy

Liver cancer developed in 285 (19.5%) of 1463 elderly patients without IFN therapy. Hepatocarcinogenesis rates were 13.1% at the end of 5 years, 29.9% at 10 years, 45.5% at 15 years, and 55.1% at 20 years. Carcinogenesis rates were calculated in subgroups according to initial platelet count: high (≥150,000/mm$^3$), intermediate (100,000-149,000/mm$^3$), and low (<100,000/mm$^3$). Cumulative carcinogenesis rates in the subgroups of high, intermediate, and low platelet counts were 5.1%, 14.2%, and 32.1% at 5 years, 14.0%, 34.2%, and 63.4% at 10 years, and 26.1%, 57.5%, and 74.9% at 15 years, respectively (Figure 1). The carcinogenesis rate was significantly different among the 3 subgroups ($P < .0001$).

Survival in the elderly patients without IFN therapy was 92.9% at 5 years, 76.6% at 10 years, 54.3% at 15 years, and 37.2% at 20 years. Survivals in the subgroups with high, intermediate, and low platelet counts were 97.9%, 95.9%,

and 86.8% at 5 years, 90.7%, 78.6%, and 52.5% at 10 years, and 72.7%, 47.8%, and 25.0% at 15 years, respectively (Figure 2). A significant difference was observed among the 3 subgroups ($P < .0001$).

## Adverse Effects and Effect of Interferon in the Elderly

Thirty-nine patients discontinued IFN therapy because of adverse effects: severe fatigue or anorexia in 10 patients (25.6%), depression in 10 patients (25.6%), hematologic disorder in 6 patients (15.4%), ophthalmic disorders in 4 patients (10.3%), and other side effects in 9 patients (23.1%). Duration of the therapy ranged from 2 weeks to 8.1 years, with a median of 24 weeks.

Among 393 patients with available judgment of IFN effect, 140 (35.6%) had a sustained virologic response, 80 (20.4%) had a biochemical response, and 173 (44.0%) had no response.

## Hepatocarcinogenesis Rates in Elderly Patients with or without Interferon

During observation, hepatocellular carcinoma developed in 334 (17.4%) of 1917 patients: 285 (19.5%) in the untreated group and 49 (10.8%) in the IFN group.

Hepatocarcinogenesis rates in the untreated and IFN groups were 13.1% and 7.0% at 5 years, 29.9% and 13.9% at 10 years, and 45.5% and 33.4% at 15 years, respectively. The carcinogenesis rate in the IFN-treated group was significantly lower than in the untreated group (log-rank test, $P < .0001$).

Carcinogenesis rates also were evaluated in the subgroups with sustained virologic response (N = 140), biochemical response (N = 80), and no response (N = 173). Cumulative carcinogenesis rates were 2.5%, 1.3%, and 9.1% at 5 years, 2.5%, 11.0%, and 18.1% at 10 years, and 2.5%, 39.6%, and 41.2% at 15 years, respectively. A significant difference was found among the 4 groups, including the untreated patient group ($P < .0001$).

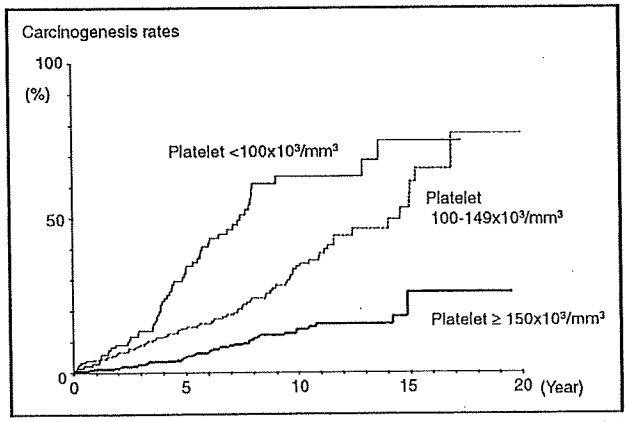


**Figure 1** Hepatocarcinogenesis rates in patients without IFN therapy, according to initial platelet count. The lower the initial platelet count was, the higher the hepatocellular carcinogenesis was in the untreated cohort ($P < .0001$).

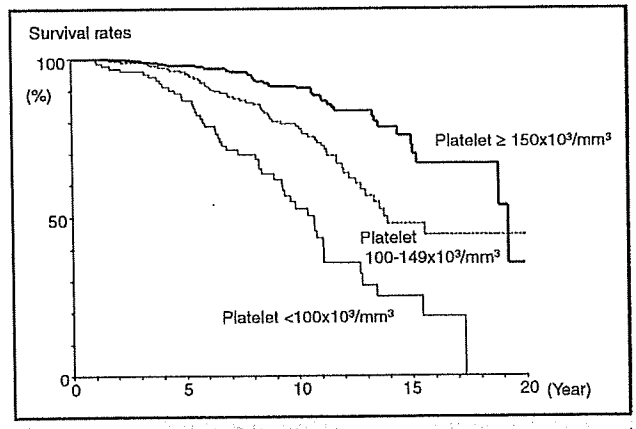


**Figure 2** Cumulative survival in patients without IFN therapy, according to initial platelet count. Survival of patients with high platelet count was significantly higher than those with a low or intermediate platelet count ($P < .0001$).

Carcinogenesis rates were compared between those with or without IFN treatment in a subgroup with a high platelet count of 150,000/mm$^3$ or more. Cumulative carcinogenesis rates in the untreated (N = 585) and treated groups (N = 228) were 5.1% and 3.7% at 5 years, 14.0% and 13.1% at 10 years, and 26.1% and 25.9% at 15 years, respectively. The carcinogenesis rate in the IFN therapy group was slightly lower than in the untreated group, but no statistical significance was found in the high platelet subgroup ($P = .69$). Next, carcinogenesis rates were analyzed between those with or without IFN in a combined subgroup with low and intermediate platelet counts of less than 150,000 mm$^3$. Carcinogenesis rates in untreated (N = 459) and treated (N = 217) groups were 19.5% and 10.9% at 5 years, 43.0% and 21.6% at 10 years, and 65.3% and 39.4% at 15 years, respectively (Figure 3). The carcinogenesis rate in the group with IFN therapy was significantly lower in the untreated group ($P = .0005$).

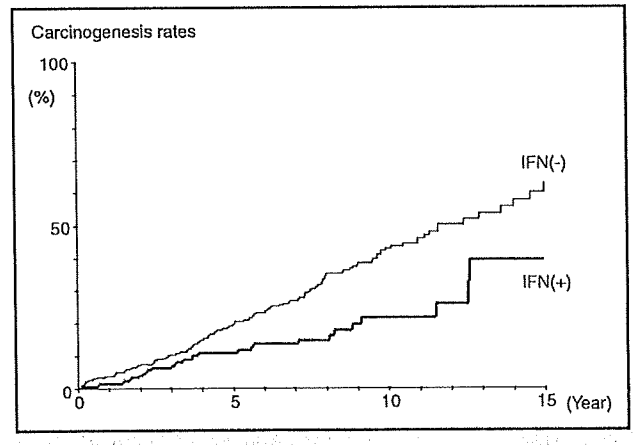


**Figure 3** Hepatocarcinogenesis rates in patients with a low or intermediate platelet count. Carcinogenesis rate of patients with IFN therapy was significantly lower than those without therapy ($P = .0005$). IFN = Interferon.

**Table 2** Independent Factors Associated with Hepatocellular Carcinogenesis in Elderly Patients with Hepatitis C Virus-related Chronic Liver Disease

| Factors | (Category) | Hazard Ratio (95% CI) | *P* |
|---|---|---|---|
| Platelet count | 1: ≥150,000/mm$^3$ | 1 | |
| | 2: 100,000-149,000/mm$^3$ | 2.42 (1.71-3.40) | <.001 |
| | 3: <100,000/mm$^3$ | 5.64 (3.88-8.22) | <.001 |
| Alanine aminotransferase | 1: <75 IU/L | 1 | |
| | 2: ≥75IU/L | 2.02 (1.48-2.77) | <.001 |
| Gender | 1: Female | 1 | |
| | 2: Male | 1.79 (1.35-2.37) | <.001 |
| IFN | 1: No therapy | 1 | |
| | 2: No response | 0.74 (0.44-1.25) | .26 |
| | 3: Biochemical response | 0.52 (0.17-1.65) | .27 |
| | 4: Sustained virologic response | 0.063 (0.009-0.449) | .006 |

CI = confidence interval; IFN = interferon.

## Factors Affecting Hepatocellular Carcinogenesis

In the first proportional hazard analysis using IFN therapy factor as a time-dependent covariate, factors associated with carcinogenesis were explored in the entire elderly cohort. Hepatocarcinogenesis is independently associated with low platelet count ($P < .001$), high alanine aminotransferase value ($P < .001$), male sex ($P < .001$), and IFN therapy (hazard ratio = 0.67, $P = .045$).

Next, multivariate analysis was performed using factors of each IFN effect: sustained virologic response, biochemical response, no response, and no IFN therapy. Carcinogenesis was significantly associated with platelet count, male sex, alanine aminotransferase value, and sustained virologic response after IFN therapy (Table 2). Patients with low and intermediate platelet counts showed high hazard ratios and high alanine aminotransferase value; male gender showed high hazard ratios. Sustained virologic response significantly decreased the hazard ratio to 0.063 ($P = .006$).

The role of IFN treatment factor was not significant (hazard ratio 0.87, $P = .67$) in the high platelet group (≥150,000/mm$^3$), but it was significant (hazard ratio 0.56, $P = .035$) in the low or intermediate platelet group (<150,000/mm$^3$).

## Survival of Elderly Patients

A total of 276 patients (14.4%) died during observation: 255 (17.4%) in the untreated group and 21 (4.6%) in the treated group. Crude survivals in the untreated and IFN groups were 92.9% and 98.7% at 5 years, 76.6% and 92.6% at 10 years, and 54.3% and 70.4% at 15 years, respectively. Survival in the IFN-treated group was significantly higher ($P < .0001$).

When a subgroup with high platelet counts (≥150,000/mm$^3$) was analyzed, survivals in the untreated and IFN groups were 97.9% and 99.6% at 5 years, 90.7% and 94.5% at 10 years, and 72.7% and 76.9% at 15 years, respectively. Survival was not significantly different ($P = .08$). Survival also was analyzed in a subgroup with low or intermediate platelet count (<150,000/mm$^3$). Cumulative survivals in the untreated and treated groups were 93.2% and 97.5% at 5 years, 70.8% and 89.9% at 10 years, and 41.2% and 64.9% at 15 years, respectively (Figure 4). Survival in the IFN therapy group was significantly higher than in the untreated group ($P = .0001$).

## Factors Affecting Survival in the Elderly

Independent factors associated with survival were explored in all the elderly patients. Multivariate hazard analysis disclosed that survival is independently associated with low platelet count ($P < .001$), male sex ($P < .001$), older age ($P < .001$), and IFN therapy (hazard ratio = 0.56, $P = .041$).

In the high platelet group (≥150,000/mm$^3$), only gender and age were independently associated with survival. The factor of IFN therapy only showed a hazard ratio for death of 0.70 in the multivariate analysis. In the low or intermediate platelet group (<150,000/mm$^3$), platelet count, age,

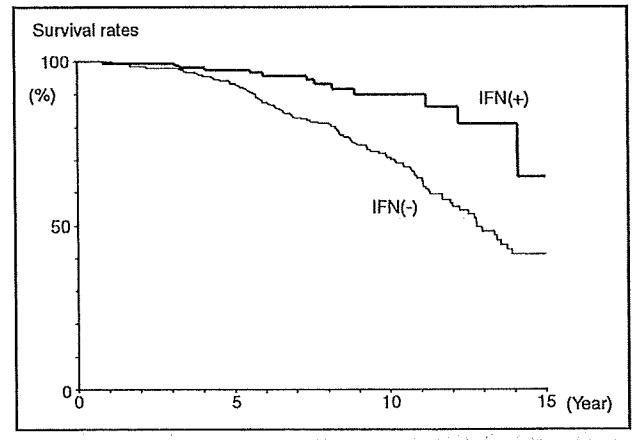


**Figure 4** Cumulative survival in patients with a low or intermediate platelet count. Survival of patients with IFN therapy was significantly higher than those without therapy ($P = .0001$). IFN = Interferon.

**Table 3** Independent Factors Associated with Survival Period in Elderly Patients with Hepatitis C Virus-related Chronic Liver Disease

| Factors | (Category) | Hazard Ratio (95% CI) | *P* |
|---|---|---|---|
| Subgroup with High Platelet Count (≥150,000/mm$^3$) | | | |
| Gender | 1: Female | 1 | |
| | 2: Male | 2.81 (1.46-5.41) | .002 |
| Age | by 1 y | 1.11 (1.04-1.18) | .002 |
| IFN | 1: No | 1 | |
| | 2: Yes | 0.70 (0.32-1.18) | .39 (NS) |
| Subgroup with Low or Intermediate Platelet Count (<150,000/mm$^3$) | | | |
| Platelet count | 1: 100,000-149,000/mm$^3$ | 1 | |
| | 2: <100,000/mm$^3$ | 3.14 (2.19-4.50) | <.001 |
| Age | by 1 y | 1.09 (1.05-1.13) | <.001 |
| IFN | 1: No | 1 | |
| | 2: Yes | 0.43 (0.24-0.77) | .005 |
| Gender | 1: Female | 1 | |
| | 2: Male | 1.56 (1.09-2.22) | .015 |

CI = confidence interval; IFN = interferon; NS = not significant.

IFN therapy, and sex were independently associated with hepatocellular carcinogenesis. IFN significantly decreased the hazard of death by 0.43 in the subgroup of low or intermediate platelet count ($P = .005$) (Table 3).

## DISCUSSION

This retrospective study was undertaken to evaluate whether IFN therapy could decrease hepatocellular carcinogenesis and increase survival in HCV-positive elderly patients aged 60 years or more at the initial hospital visit. Because it seemed to require at least 5 years to obtain a statistical difference in carcinogenesis rates and survival between IFN-treated and untreated groups, a prospective randomized trial with untreated control patients is difficult to perform from both ethical and medical viewpoints. We therefore attempted to carry out this retrospective study to show an impact of IFN treatment with a statistical adjustment and stratification using a large number of patients under a long-term observation period.

There were significant differences in carcinogenesis and survival among patients with HCV, according to initial platelet count. Because this study dealt with all patients with HCV-related hepatitis who visited Toranomon Hospital irrespective of IFN treatment, evaluation of liver histology was performed in approximately two thirds of the patients. Platelet count has been considered a simple indicator for the progression of hepatitis, and the patients without liver biopsy were well stratified by the initial platelet count in our study. From statistics of the nationwide census for the longevity of each age group in 2003, the life expectation was 21.9 and 27.5 years for 60-year-old Japanese men and women, respectively, and 18.0 and 23.07 years for 65-year-old Japanese men and women, respectively. In view of the median age (65 years) of the untreated cohort with HCV infection, the survival of patients with high platelet counts was almost the same as that of the general population in Japan (Figure 2). Physicians should consider the longevity without IFN therapy and the cost, side effects, and risks caused by IFN for more stratified age groups of the elderly.

Although several authors have shown that effects of both IFN monotherapy[20,26,27] and IFN plus ribavirin combination therapy[28,29] were not different between elderly and younger patients with chronic HCV in regard to viral elimination and normalization of transaminase, recent reports[16,21] have shown lower virologic response rates. A possible low response rate in the elderly was closely associated with a high rate of adverse reactions,[16,20,21] and hematologic side effects seemed significant in the elderly group.[22] The low discontinuation rate (43/454, 9.5%) in the current study was partly attributable to the low rate of IFN plus ribavirin combination therapy. Horiike et al,[27] Floreani et al,[16] and Koyama et al[21] recommended IFN therapy for select patient groups with a low HCV RNA titer, non-genotype 1, or relatively young age of less than 65 years.

We previously reported a high carcinogenesis rate in elderly patients with chronic HCV who underwent IFN therapy.[30] When crude hepatocarcinogenesis rates were compared between untreated and IFN-treated groups in the current study, IFN significantly decreased the carcinogenesis rate in the elderly patients with varied severity of liver disease. As was found in the general results of patients, including the younger age group,[13] carcinogenesis in patients with sustained virologic response was significantly lower than that of patients with no response or without IFN therapy. The carcinogenesis rate was low for several years after cessation of IFN administration and increased gradually after 8 years in the group with a biochemical response (Figure 3). The cancer appearance curve of the biochemical response group implied that the normal and stable hepatitis

state in the early years contributed to suppress the process of carcinogenesis, and that reactivation of hepatitis induced the progression of hepatic oncogenesis in the later years.

Among patients with a high platelet count and mild liver disease, IFN did not decrease the rate of hepatocarcinogenesis. IFN significantly decreased the carcinogenesis rate in patients with a low or intermediate platelet count. In view of the less effective rate and high adverse reaction rate by IFN in elderly patients, IFN therapy should be considered primarily for those with a low platelet count of 150,000/mm$^3$ or less. Because low platelet count was closely associated with advanced disease and high risk for carcinogenesis, treatment efficacy appeared prominent in the subgroup with low and intermediate platelet counts. The best candidates for IFN therapy were those with a low platelet count, also in regard to cost-effectiveness. Because a low platelet count is closely associated with advanced stages of liver disease, IFN therapy should be avoided for elderly patients with decompensated cirrhosis or severely decreased platelet count of less than 50,000/mm$^3$. A sustained virologic response improves clinical symptoms in decompensated cirrhosis,[31] but IFN often induces severe complications even in young patients with decompensated cirrhosis.[32] An elderly patient with hepatitis without decompensation can be a candidate for IFN therapy if careful, close hematologic monitoring is performed. Low-dose, intermittent, long-term IFN therapy also should be considered for these patients to obtain a sustained biochemical response without creating profound and irreversible side effects. Because elderly patients generally showed some difficulties with IFN treatment, our current study demonstrated practical information about carcinogenesis and the life expectancy of elderly patients with HCV and the order of priority in management of IFN for these patients. IFN administration is preferably considered and initiated at the age of 60 years or less to reduce side effects.

## CONCLUSIONS

IFN for a subgroup with low and intermediate platelet counts had significant advantages in regard to hepatocarcinogenesis and survival of elderly patients with chronic HCV.

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*Journal of Viral Hepatitis*, 2009, 16, 437–443 doi:10.1111/j.1365-2893.2009.01085.x

# Occult hepatitis B virus infection increases hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis: a cohort study

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*Received and accepted for publication February 2008*

SUMMARY. An impact of serum hepatitis B virus (HBV) DNA on hepatocarcinogenesis has not been investigated in a cohort of patients with non-B, non-C cirrhosis. Eighty-two consecutive Japanese patients with cirrhosis, who showed negative hepatitis B surface antigen and negative anti-hepatitis C virus, were observed for a median of 5.8 years. Hepatitis B virus core (HBc) region and HBx region were assayed with nested polymerase chain reaction. Both of HBc and HBx DNA were positive in 9 patients (11.0%) and both were negative in 73. Carcinogenesis rates in the whole patients were 13.5% at the end of the 5th year and 24.6% at the 10th year. The carcinogenesis rates in the patients with positive DNA group and negative DNA group were 27.0% and 11.8% at the end of the 5th year, and 100% and 17.6% at the 10th year, respectively ($P = 0.0078$). Multivariate analysis showed that men ($P = 0.04$), presence of HBc and HBx DNA (hazard ratio: 8.25, $P = 0.003$), less total alcohol intake ($P = 0.010$), older age ($P = 0.010$), and association of diabetes ($P = 0.005$) were independently associated with hepatocellular carcinogenesis. Existence of serum HBV DNA predicted a high hepatocellular carcinogenesis rate in a cohort of patients with non-B, non-C cirrhosis.

*Keywords*: hepatitis B virus, hepatocellular carcinogenesis, liver cirrhosis, occult hepatitis B virus infection, proportional hazard model.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of death in many parts of sub-Saharan Africa and Asia [1,2]. It is also one of the most common neoplasms in Japan [3]. Hepatitis B virus (HBV) infection is the primary cause of cirrhosis and HCC and one of the major causes of death globally [4]. Needless to say, a cohort of patients with HBV-related chronic hepatitis and cirrhosis has a significantly high risk for HCC development [5–7]. In our retrospective cohort studies concerning HBV-related disease, cumulative hepatocellular carcinogenesis rates in chronic hepatitis ($n = 610$) and cirrhosis ($n = 180$) were 2.1% and 7.2% at the end of the 5th year, and 4.9% and 27.2% at the 10th year, respectively [5,7]. Abundant epidemiological and molecular biological evidence shows that HBV is an important factor in the development of HCC [8–10], but the precise role of HBV in the oncogenesis is still unknown.

HBV infection is usually diagnosed when the circulating hepatitis B surface antigen (HBsAg) is detected. However, the availability of highly sensitive molecular biology techniques has allowed the identification of HBV infection in HBsAg-negative individuals with or without circulating antibodies to HBsAg and/or hepatitis B core antigen (anti-HBc) [11–16]. Much evidence suggests that this so-called occult HBV infection is highly prevalent in a number of patient subgroups including those with HCV infection [16,17], cryptogenic advanced liver fibrosis [18] and HCC [17,19–27]. Although Marusawa *et al.* [28] and Uetake *et al.* [29] described the relationship between anti-HBc and HCC appearance rate in each study, impact of occult HBV infection on carcinogenesis cannot be evaluated because of lack of HBV DNA assay. As all the previous studies were performed as a pilot study or a case-controlled one, actual risk ratio of occult HBV infection for hepatocellular carcinogenesis has not been reported in a cohort study until now.

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartic transaminase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PCR, polymerase chain reaction.

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We, therefore, analysed a retrospective cohort of consecutive patients with cirrhosis for a long period, in order to elucidate the influence of occult HBV infection on the carcinogenesis rate from non-B, non-C cirrhosis.

## PATIENTS AND METHODS

### *Patients*

Among 103 consecutive patients diagnosed as having non-B, non-C cirrhosis by peritoneoscopic liver biopsy at Toranomon Hospital, Tokyo, Japan in the period from 1976 to 1998, initial frozen sera at the time of the diagnosis of cirrhosis were available for the assay of HBV DNA in 82 patients (79.6%). The cohort of 82 patients was retrospectively observed for a long period. All the patients showed negative HBsAg, negative anti-hepatitis C virus (HCV) and negative HCV RNA. Patients with a possible association of HCC at the time of the diagnosis of cirrhosis were strictly excluded from this study. No patient received interferon or other antiviral therapy after the diagnosis of cirrhosis.

### *Background and laboratory data of the patients*

There were 67 men and 15 women aged 34–80 with a median age of 58 years. A total of 47 patients (57.3%) had a history of alcohol intake of more than 500 kg until the diagnosis of liver cirrhosis. Fifteen patients (18.3%) had decompensated cirrhosis with ascites, a history of encephalopathy, or both. The median value of indocyanine green retention rate at 15 min (ICG R15) was 33% (range, 7–75%), and total bilirubin concentration was 1.3 mg/dL (range 0.4–20.9 mg/dL).

### *Measurement of hepatitis virus markers*

Hepatitis virus markers were assayed using frozen sera at −80 °C. All sera were tested for HBsAg (radioimmunoassay, Dainabot, Tokyo, Japan), anti-HCV (second-generation anti-HCV, enzyme-linked immunosorbent assay, Dainabot), and HCV RNA with reverse transcription-nested polymerase chain reaction (PCR).

HBV DNA was analysed for the region of HBc and HBx by sensitive nested PCR according to Yotsuyanagi *et al.* [30]. Fifty microlitres of STE solution [100 mmol/L Tris–HCl (pH 8.0), 100 mmol/L NaCl, 2 mmol/L ethylenediaminetetraacetic acid (pH 8.0), and 0.2% sodium dodecyl sulphate] with 20 μg of proteinase K (Boehringer, Mannheim, Germany) were added to serum samples. Mixed samples were then incubated for 2 h at 55 °C. DNA was extracted twice with phenol/chloroform, once with chloroform, and precipitated with ethanol. The DNA pellet was dissolved in 25 μL of TE buffer [10 mmol/L Tris–HCl (pH 8.0) and 1 mmol/L ethylenediaminetetraacetic acid (pH 8.0)].

Prepared DNA was subjected to amplification using nested PCR technique. HBV DNA was amplified using two independent pairs of primers, with each primer complementary to sequences in the X or core region of the HBV genome [30]. Amplification was performed using a thermal cycler for a total of 40 cycles, with each cycle consisting of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, in 100 μL of reaction mixture containing 200 mmol/L of each dNTP, 1X PCR buffer [50 mmol/L KCl, 10 mmol/L Tris–HCl (pH 8.3), 1.5 mmol/L $MgCl_2$ and 0.001% (w/v) gelatine], and 2 units of Ampli-Taq polymerase (Perkin Elmer Cetus Corp., Norwalk, CT, USA). The PCR products were separated in a 2% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Dassel, Germany). The membrane was then probed with digoxigenin-labelled oligonucleotides, which hybridize specifically with the core or X gene. Results were considered valid only if the same results were obtained in at least two separate experiments.

We considered the cases with positivity in at least two different viral genomic regions as HBV DNA positive. Appropriate negative controls were included in each PCR. The limit of sensitivity of our nested PCR methods ranged from 10 to 1 genome equivalents/mL.

### *Follow-up of patients*

Follow-up of the patients was made on a monthly or bimonthly basis after diagnosis of cirrhosis by monitoring alpha-fetoprotein (AFP) and other biochemical data. Imaging diagnosis was made at least once a year for each patient with CT or US. After 1988, in order to detect HCC earlier, imagings were done three or more times per year in a majority of patients.

No patient underwent interferon therapy after the diagnosis of cirrhosis, but some of the patients received an oral or intravenous administration of medicinal herbs during the follow-up period.

All patients were finally evaluated in November 2004. The cases lost to follow-up were 13 (15.9%). The median observation period of the total patients was 5.8 years with a range of 0.1–34.8 years.

### *Statistical analysis*

Differences of background features and laboratory data between the patients with and without HBV DNA were analysed by chi-square test, Fisher's exact test and Mann–Whitney's *U*-test. The time between diagnosis of cirrhosis and appearance of HCC was analysed using the Kaplan–Meier technique [31] and differences in curves were tested using log-rank test [32]. Those patients who had been lost to follow-up were regarded as censored data at the time of missing in the statistics. Independent risk factors associated with the appearance rate of HCC were studied using the stepwise Cox regression analysis [33]. Potential risk factors

assessed for hepatocellular carcinogenesis included the following 18 variables: age, sex, association of diabetes mellitus, total alcohol intake, history of cigarette smoking, family history of liver disease, history of blood transfusion, state of cirrhosis (presence of ascites and/or a history of encephalopathy), HBc DNA, HBx DNA, aspartic transaminase (AST), alanine transaminase (ALT), albumin, bilirubin, globulin, AFP, platelet, and ICG R15. A probability less than 0.05 was considered as significant. Data analysis was performed using computer program SPSS version 11 [34].

## RESULTS

### *HCC appearance rate in all the patients*

During the observation period, HCC appeared in 16 patients (19.5%). Median interval between the diagnosis of cirrhosis and HCC was 5.6 years (range 0.7–15.6 years) in the patients with HCC development. The cumulative HCC appearance rate in the 82 patients was 13.5% at the end of the fifth year after the diagnosis of cirrhosis, 24.6% at the end of tenth year, 33.3% at the 15th year, and 41.6% at the end of 20th year.

### *HCC appearance rates according to serum HBV DNA*

Among the 82 patients, 9 patients (11.0%) showed positive serum HBV DNA and 73 (89.0%) negative HBV DNA. The former 9 patients had both HBc DNA and HBx DNA, and the latter 73 had neither of them. Table 1 summarizes the profiles and laboratory data of each group. There was no demographic difference between the two groups. There was also no statistically significant difference between them except for ALT value, which was lower in the patient group with positive HBV DNA ($P$ = 0.028).

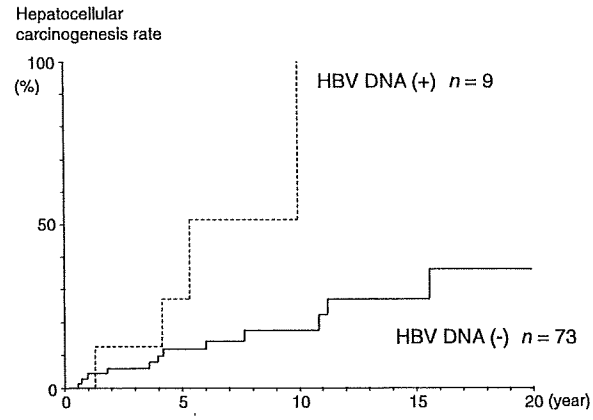


**Fig. 1** Hepatocellular carcinogenesis curves of the patients with and without serum hepatitis B virus DNA. Carcinogenesis rates were 12.5% and 6.0% at the end of the third year, 27.0% and 11.8% at the fifth year, and 100% and 17.6% at the tenth year, respectively.

Figure 1 shows the curves of crude HCC appearance rate in the two patients group with and without serum HBV DNA. The third-year HCC appearance rates in the patients with and without DNA were 12.5% and 6.0%, the 5th-yr rates 27.0%, 11.8%, the tenth-yr rates 100% and 17.6%, respectively. The HCC appearance rate of the patient group

**Table 1** Demography and laboratory data of patients with and without serum hepatitis B virus DNA

| | HBV DNA* | | |
|---|---|---|---|
| | Positive ($n$ = 9) | Negative ($n$ = 73) | $P$ |
| Demographic and background features | | | |
| Sex – men/women | 8/1 | 59/14 | 0.55 |
| Age (median, range) | 51 (45–68) | 58 (34–80) | 0.44 |
| History of transfusion | 1 (11.1%) | 14 (19.4%) | 0.55 |
| Alcohol intake of 500 kg or more | 5 (55.6%) | 42 (58.3%) | 0.87 |
| Diabetes mellitus | 3 (33.3%) | 15 (20.8%) | 0.40 |
| Observation period (years) | 5.7 (1.0–21.0) | 6.1 (0.1–34.8) | 0.92 |
| Laboratory data (median, range) | | | |
| ICG R15 (%) | 34 (12–51) | 32.5 (7–75) | 0.78 |
| AST (IU/L) | 32 (17–86) | 40.5 (14–184) | 0.26 |
| ALT (IU/L) | 16 (9–43) | 28.5 (4–160) | 0.028 |
| Albumin (g/dL) | 3.8 (2.6–4.5) | 3.6 (1.7–5.2) | 0.20 |
| Bilirubin (mg/dL) | 0.9 (0.5–2.8) | 1.3 (0.4–20.9) | 0.14 |
| Platelet (×1000/mm$^3$) | 142 (67–232) | 104 (27–647) | 0.18 |
| AFP (ng/mL) | 5 (3–9) | 6 (1–98) | 0.38 |

ICG R15, indocyanine green retention rate at 15 min; AST, aspartic transaminase; ALT, alanine transaminase; AFP, alpha-fetoprotein. *HBV DNA was assessed for HBc and HBx DNA using polymerase chain reaction

of positive HBV DNA was slightly higher than that of negative DNA ($P = 0.0078$, log-rank test).

### *Significance of serum HBV DNA in hepatocellular carcinogenesis*

Cox proportional hazard model was performed for analysis of risk factors for liver carcinogenesis, using the 18 variables as mentioned above.

In the last step of stepwise regression analysis, the following five variables entered the model and could not be removed: sex ($P = 0.005$), serum HBV DNA ($P = 0.003$), past history of alcohol intake ($P = 0.003$), age ($P = 0.035$), and association of diabetes mellitus ($P = 0.022$) (Table 2). Accordingly, these five factors were significantly associated with hepatocellular carcinogenesis in the patients with non-B, non-C cirrhosis. Among them, gender was the strongest predictor of future HCC occurrence rate, indicating that male patients had 15.4 times as high carcinogenesis hazard as women patients. Similarly, positive HBV DNA (hazard ratio, 8.25) and little alcohol consumption of less than 500 kg (hazard ratio, 7.19) were the second and third strongest predictors for carcinogenesis, respectively. When the background factors of the cases were adjusted with the other significant factors, positive test for HBV DNA was significantly associated with the hepatocellular carcinogenesis rate.

Curves of carcinogenesis rates were generated from the multivariate analysis in an imaginary positive DNA group and an imaginary negative DNA group, with average sex ratio, average alcohol intake, average age and average association rate of diabetes (Fig. 2). The difference of the carcinogenesis curves indicated 'pure' impact of positive serum HBV DNA upon the carcinogenesis, which was adjusted with significant covariates assuming a standardized study group.

**Table 2** Independent factors associated with liver carcinogenesis in the patients with non-B, non-C cirrhosis

| Factors | Category | Hazard ratio (95% confidence interval) | *P* |
|---|---|---|---|
| Sex | Women | 1 | |
| | Men | 15.4 (2.24–111.1) | 0.005 |
| Serum HBV DNA* | Negative | 1 | |
| | Positive | 8.25 (2.01–33.93) | 0.003 |
| Total alcohol intake | ≥500 kg | 1 | |
| | <500 kg | 7.19 (1.98–26.32) | 0.003 |
| Age | <60 years | 1 | |
| | ≥60 years | 3.98 (1.10–14.42) | 0.035 |
| Diabetes mellitus | No | 1 | |
| | Yes | 3.89 (1.22–12.47) | 0.022 |

*Positive HBV DNA: positive for both HBc DNA and HBx DNA.

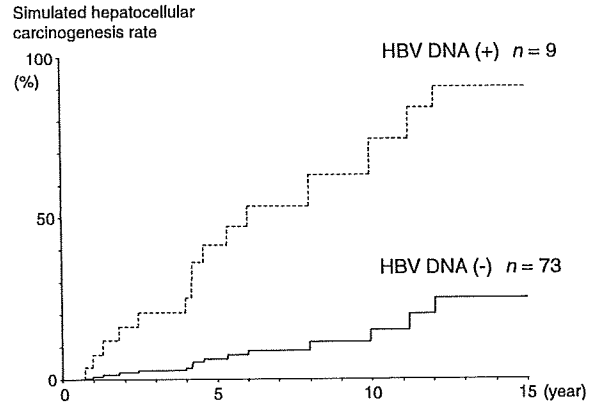


**Fig. 2** 'Adjusted' hepatocellular carcinogenesis rates in the positive HBV DNA group and the negative DNA group. Cox proportional hazard analysis showed that the carcinogenesis rate in the positive DNA group was significantly higher than that of the negative DNA group, when the other significant covariates were substituted with the same average parameters in the two groups.

### *Mortality and causes of death*

During the observation period, 36 (43.9%) of 82 patients died: 5 (55.6%) of 9 patients in the positive DNA group and 31 (42.5%) of 73 patients in the negative DNA group. Cumulative survival rates in patients with and without HBV DNA were 78.8% and 74.1% at the end of the fifth year, 54.4% and 44.4% at the tenth year, 38.4% and 29.6% at the 15th year, and 33.6% and 29.6% at the 20th year, respectively. Although the survival rate in the positive HBV DNA group was lower than in the negative group, statistical significance was not shown.

Causes of death included liver failure due to liver cirrhosis in 21 (4 in positive DNA group and 17 in negative DNA group), progression of HCC in 7 patients (all in negative DNA group), and other causes in 8 (one in positive DNA group and 7 in negative DNA group).

## DISCUSSION

Epidemiological and molecular virological studies in the 1970s and early 1980s established a strong aetiological association between chronic HBV infection and the hepatocellular carcinogenesis [35]. We also estimated annual carcinogenesis rates as 0.5% in chronic hepatitis and 3% in cirrhosis, from cohorts of biopsy-proven HBV disease [5,7].

Integration of HBV DNA has been reported in the majority of HBsAg positive HCCs since 1980s, and the fact suggested HBV might be oncogenic. Up to now, there is no evidence

that HBV DNA is directly oncogenic and the mechanism by which chronic HBV infection leads to carcinogenesis remains unclear. Integration of HBV DNA may stimulate cellular pro-oncogenes or suppress growth-regulating genes [36]. Integration of HBV DNA, however, has been found in varied regions of the host chromosomes and no preferential and specific site has been identified until now. The other authors suggested that integration of HBV DNA could also induce carcinogenesis via transactivation of other oncogenes [37]. Both HBx protein and the truncated pre-S/S protein are potent transactivators and are commonly found in HCC tissue but their precise role in hepatocarcinogenesis remains unknown.

Occult HBV infection is generally defined as the detection of HBV DNA in the serum or liver tissue of patients who test negative for hepatitis B surface antigen [38–41]. Occult HBV infection was first reported in the early 1980s when hybridization techniques for the detection of HBV DNA became available. These studies showed that HBV DNA could be detected in HBsAg negative patients with HCC [42]. Recent studies using more sensitive techniques confirmed the close correlation between chronic occult HBV infection and carcinogenesis. Many authors demonstrated the relationship between occult HBV infection and hepatocellular carcinogenesis, mainly by a pilot study or a case–control study [17,19–27]. Shiota *et al.* [24] reported in their case studies without control group that serum of 18 out of 26 HCC patients without HBsAg and anti-HCV were positive for either S, C, or X region on PCR and southern blotting. Pollicino *et al.* [26] described that viral DNA was detected in 68 of 107 cases of HCC tissue (63.5%) and in 63 of 192 cases of chronic hepatitis tissue (32.8%), and concluded that occult HBV is a risk factor for development of HCC. The other authors also emphasized the high incidence of HBV DNA in either serum or HCC tissue compared with that of cases without HCC development. All the literatures, except one [43] from Taipei where HBV infection was endemic and prevalent, concluded that occult HBV infection was closely associated HCC development. However, precise risk or hazard ratio for carcinogenesis has not been reported.

Current study on this topic provided strong evidence of an association between occult HBV infection and HCC. In the patient cohort of non-B, non-C cirrhosis, occult HBV infection increased the future carcinogenesis rate with a hazard ratio of 8.25 (95% confidence interval, 2.01–33.93). It has been proposed that diagnosis of occult HBV infection be made only when HBV DNA can be detected using at least two sets of primers from different areas of the HBV genome in duplicate assay [38,39]. Appropriate negative controls must be included in each assay and specificity of the amplification reaction confirmed by sequencing of the amplicons. Using this strict criterion, occult HBV infection was found in 9 (11.0%) of 82 Japanese patients with non-B, non-C cirrhosis. Background features of the nine patients with serum HBV DNA showed a slightly younger age, a lower ALT, a slightly lower bilirubin, and a slightly higher platelet count (Table 1). Although all these demographic and laboratory findings were considered to favour low carcinogenetic risk, the patients with cryptic HBV DNA infection developed HCC more frequently. After adjustment of these background covariates in the multivariate analysis, positivity of serum HBV DNA proved to be an independent risk factor for hepatocarcinogenesis (Table 2).

As this retrospective cohort consisted of only cirrhosis as an advanced liver disease, and as it included both alcoholic and non-alcoholic cirrhosis, the hazard ratio of 8.25 could not be applied for varied stages and varied aetiologies of liver disease. In order to elucidate the impact of occult HBV infection on carcinogenesis, future studies should be performed also in the other cohort of chronic liver disease, such as HCV-related disease. Although anti-HBc and anti-HBs antibody were measured in a small numbers of the patients, an exact relationship between serum HBV DNA and serum positivity of anti-HBc antibody was not analysed in this study. When we tested anti-HBc antibody in a small part of subjects, 3 of 6 patients (50.0%) with positive HBV DNA had serum anti-HBc antibody and 7 of 19 patients (36.8%) without HBV DNA had anti-HBc (Fisher's exact test, $P = 0.69$). For the convenience of clinical circumstance and practical usefulness, significance of positive anti-HBc on carcinogenesis risk should be elucidated through a large-scale cohort study with an identical assay for anti-HBc antibody.

Although a lot of epidemiological and clinicopathological evidence of the relationship has been published, precise role of occult HBV in this setting has been still unclear. Patients with occult hepatitis B overlap with those who previously have been classified as having recovered [44]. In fact, the distinction between recovery and occult hepatitis B is likely to be somewhat arbitrary, as recovery does not necessarily imply eradication of infection in all cases [30], but includes the possibility of complete suppression in some cases by a broad and vigorous immune response [44]. One of the most important clinical questions is whether occult hepatitis B merely represents a marker of past infection, or whether HBV genome persistence contributes to liver disease. It is very likely that occult HBV is a cofactor in the development of HCC. Several studies found that patients co-infected with HBV and HCV have increased risks of HCC compared with those with mono-infection. Our cohort studies [45] also showed that a risk factor of a history of heavy drinking interacted with HBV or HCV subtypes in a characteristic manner from the viewpoint of carcinogenesis in cirrhosis. The other important problem is whether occult HBV infection alone causes HCC. To address this question, studies on occult HBV infection in patients with HCC might provide details on other causes of chronic liver disease including nonalcoholic fatty liver disease, which may masquerade as cryptogenic cirrhosis, hemochromatosis, alfa-1-antitrypsin deficiency, and autoimmune liver disease [46]. Recently,

Castillo *et al.* [47] reported a clinical state of occult HCV infection, which shows negative serum anti-HCV, negative serum HCV RNA, and positive HCV RNA in liver biopsy specimen. Although we did not tested the possibility of occult HCV infection in this study, future studies should be also aimed at the influence of latent HCV infection on hepatocarcinogenesis.

In conclusion, occult HBV infection significantly increased the incidence of hepatocellular carcinogenesis in patients with non-B, non-C cirrhosis. Although non-B, non-C cirrhosis seemed to include varied aetiology of liver disease, cryptic HBV infection should be taken account in the prediction of future HCC development.

## ACKNOWLEDGEMENT

This study was supported in part by a research grant of Ministry of Health, Labor and Welfare, Japan.

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*Hepatology Research* 2009; 39: 346–354 doi: 10.1111/j.1872-034X.2008.00466.x

***Original Article***

# Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin

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*Aim:* Hepatocellular carcinoma (HCC) often shows resistance to transcatheter arterial chemoembolization (TACE). Such patients often have a poor prognosis and are unresponsive to other forms of therapy. The aim of this retrospective study was to determine the response to TACE using platinum analogues in patients deemed resistant to TACE using epirubicin.

*Methods:* We studied 152 consecutive patients with advanced HCC resistant to TACE using epirubicin. All cases were treated with platinum analogue using transcatheter arterial chemotherapy with or without embolization.

*Results:* Computed tomography at 3 months after therapy showed complete response (CR) in 6 patients (4.0%), partial response (PR) in 28 (18%), stable disease (SD) in 35 (23%), and progressive disease (PD) in 83 (55%). The cumulative survival rates for PR/CR patients who received platinum analogue-transcatheter arterial chemotherapy with or without embolization (81.8% at first year, 53.9% at second year, and 33.1% at third year) were significantly higher than those of SD/PD patients (36.6%, 17.5% and 7.4%, respectively) ($P < 0.001$). The 50% survival period was extended almost 1.4 year in PR/CR patients who received platinum analogue-transcatheter arterial chemotherapy with or without embolization.

*Conclusion:* Our retrospective study is the first to report the efficacy of platinum analogues for advanced HCC unresponsive to TACE using epirubicin.

**Key words:** hepatocellular carcinoma, platinum analogue, transcatheter arterial chemoembolization, unresponsive

## INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common neoplasms in Africa and Asia including Japan. Since it is a well known that more than 80% of the cases with HCC are associated with liver cirrhosis, a routine check-up for cirrhotic patients with ultrasound (US) could potentially lead to the detection of small HCC. However, because of the association of cirrhosis and tumor multiplicity, surgical resection is performed only in 20% of the cases or less.[1,2] Transcatheter arterial chemoembolization (TACE) has been reported to be an effective palliative treatment for patients with unresectable HCC, and many chemotherapeutic agents such as doxorubicin, epirubicin, mitomycin were used with lipiodol in Japan.[3–10] Although repeated TACE is one of the most potent therapies for unresectable HCC, resistance to the therapy often ensues after therapy repetition, and long-term survival rates after 3 years are not sufficiently high at present.

Platinum analogues are effective against many malignant tumors, and in recent years, they have been used in the treatment of HCC. It has been reported that carboplatin-lipiodol treatment improved 1-year survival rate compared with doxorubicin-lipiodol treatment in patients with advanced HCC.[11] As for cisplatin, several studies reported its effectiveness for advanced HCC. Furthermore, the efficacy of cisplatin and lipiodol combination therapy has been reported by several investigators.[12–19] To our knowledge, however, there is no information on the efficacy of platinum analogues in TACE- epirubicin resistant HCC patients.

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*Received 29 August 2008; revised 6 October 2008; accepted 6 October 2008.*

The purpose of this retrospective study was to examine the efficacy of platinum analogues (carboplatin and cisplatin) for advanced HCC unresponsive to TACE using epirubicin.

## PATIENTS AND METHODS

### Study population

FROM 1980 TO 2006, 1,250 patients were diagnosed with HCC at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these, 565 patients underwent TACE treatment for HCC. Among the 565 patients, 184 patients were judged by two hepatologists as embolization-resistant HCC and they received a platinum analogue. All 184 patients had been considered to have unresectable HCC at the time of diagnosis of HCC, and had undergone TACE therapy at least twice until being considered TACE-resistant. Embolization-resistant HCC was defined as an HCC whose number and/or size had increased in the treated segment and/or extended other segments despite repetitive course of TACE using epirubicin, lipiodol and gelatin sponge. Thus, this retrospective cohort study was based on 184 consecutive patients with TACE-resistant HCC.

Before treatment with carboplatin or cisplatin, all the patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver imaging studies [computerized tomography (CT), ultrasonography (US), digital subtraction angiography (DSA) ], complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of US, CT, and DSA.

Of the 184 patients, 32 were excluded because they did not meet the following inclusion criteria: (i) typical hypervascular HCC by all imaging modalities; (ii) no history of other malignancies; (iii) no evidence of extrahepatic metastasis of HCC; (iv) performance status of 0–1; (v) adequate liver function with bilirubin value of 5 mg/dL or less; (vi) sufficient hematopoietic function with a platelet count of more than 25,000/mm$^3$ and leukocyte count of more than 2,000/mm$^3$; and (vii) an expected survival time of at least 3 months. All patients gave informed consent to the treatment. Accordingly, 152 patients with TACE-resistant HCC were retrospectively evaluated for efficacy of platinum analogue for advanced HCC unresponsive to TACE-epirubicin. The observation starting point was the time of first therapy using platinum analogue at our hospital.

### Serologic markers for HCV and HBV

The diagnosis of HCV infection was based on detection of serum HCV antibody with RNA positivity. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc., NJ). Hepatitis B surface antigen (HBs-Ag) was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). Serum HBV-DNA level was determined independently, using the nested PCR, by an experienced technician (M.K), who was blinded to the clinical information. The used serum samples were stored −80°C at first consultation.

### Treatment protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and a catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the platinum analogue with or without Lipiodol (Lipiodol Ultrafluide, Laboratoire Guerbet, Aulnay-sous-Bois, France) and small gelatin cubes (1 × 1 mm). The platinum analogue used was either carboplatin at 150 to 450 mg/body (63% of patients received 450 mg/body) or cisplatin at 40 to 100 mg/ body of (36% of patients received 100 mg/ body). Both analogues were administrated slowly under careful fluoroscopic guidance. When using Lipiodol, the platinum analogue and Lipiodol were divided into six to eight parts and mutually injected. In patients who received Lipiodol, the volume of injected Lipiodol ranged from 2.0 to 5.0 mL. The dose of Lipiodol was determined according to tumor size and the degree of liver dysfunction.

### Selection criteria of type of therapy

Patients were treated by three type of therapy, depending on the extent of their tumors and liver function; (i)hepatic arterial injection (HAI) were performed for those patients with a tumor thrombus into main portal trunk or with severe liver function, (ii)chemolipiodalizatio (CL) were performed for those patients with tumor thrombus in distal portal branch complicated with severe liver function, (iii)TACE were performed for those patients without main portal vein thrombus and severe liver function.

### Background and laboratory data

Table 1 summarizes the profiles and data of 152 patients who were treated with carboplatin or cisplatin. The

Table 1 Demographics and laboratory data of 152 patients with HCC who underwent transcatheter arterial chemotherapy using platinum analogue for advanced HCC unresponsive to TACE-epirubicin

| Parameter | ($n$ = 152) |
|---|---|
| Patient characteristics | |
| Sex (M : F) | 122:30 |
| Age (years) | 67 (38–85) |
| Back grounds of liver disease | |
| Hepatitis B surface antigen positive | 27 |
| HCV antibody positive | 123 |
| Both negative | 2 |
| Status of liver function | |
| Child–Pugh classification (A/B/C) | 98/51/3 |
| Laboratory data | |
| Platelet count (×10$^4$ /μL)* | 11.0 (3.4–35.5) |
| Albumin (g/dL)* | 3.0 (2.1–4.5) |
| Bilirubin (mg/dL)* | 1.0 (0.3–4.3) |
| AST (IU/L)* | 68 (21–488) |
| Prothrombin time (%)* | 84 (45.5–114) |
| ICG R15 (%)* | 38 (6.0–76) |
| AFP (μg/L)* | 236 (2.0–112,000) |
| DCP (AU/L)* | 153 (10–131,000) |

*Expressed as median (minimum, maximum).
AFP, alpha-fetoprotein; AST, aspartate aminotransferase; DCP, des-gamma carboxyprothrombin; HCC, hepatocellular carcinoma; ICG R15, indocyanine green retention rate at 15 minutes; TACE, transcatheter arterial chemoembolization.

patients consisted of 122 men and 30 women, and their age ranged from 38 to 85 years (median, 67 years). They included 27 (18%) HBs-Ag positive patients, 123 (81%) HCV antibody positive patients, and 2 (1%) negative for both. At the time of the first platinum analogue treatment, the median serum albumin concentration was 3.0 g/dL, total bilirubin 1.0 mg/dL, indocyanine green retention rate at 15 minutes (ICG R15) 38%, prothrombin activity 84%, alpha-fetoprotein (AFP) 236 μg/L, and des-gamma-carboxyprothrombin (DCP) was 153 AU/L. As for Child-Pugh classification, 98 (64%) were class A, 51 (34%) were class B, and 3 (2%) were class C patients.

## Characteristics of hepatocellular carcinoma

Table 2 summarizes the profiles of HCC that were treated with platinum analogue. The median tumor size was 40 mm. A solitary HCC was detected in 6 (4%) patients while multiple HCC were detected in 146 patients at the time of the first platinum analogue treatment. For the latter group, the tumors were localized to one segment in 10 (7%) patients, to one lobe in 32 (21%) patients, and in both lobes in 104 (68%) patients. Portal vein invasion was noted in 46 (30%) patients. The number of courses of TACE-epirubicin until judgment of embolization-resistance ranged from 2 to 16 with a median of 4 courses. The median interval between diagnosis of HCC and judgment of embolization-resistance was 30.1 months.

Table 2 Profile of HCC in 152 patients who underwent transcatheter arterial chemotherapy using a platinum analogue for advanced HCC unresponsive to TACE-epirubicin

| Profiles of liver cancer | |
|---|---|
| Tumor size (mm)* | 40 (8–180) |
| Intrahepatic multiplicity | |
| Solitary | 6 |
| Multiple, localized to one segment | 10 |
| Multiple, localized to one lobe | 32 |
| Multiple, extended to both lobes | 104 |
| Portal vein invasion (no/yes) | 106/46 |
| Embolization iteration until unresponsiveness | 4 (2–16) |
| The kind of used platinum analogue | |
| Carboplatin/Cisplatin | 105/47 |
| Treatment method | |
| HAI/CL/TACE | 73/20/59 |

*Expressed as median (minimum, maximum).
CL, chemolipiodalization; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

The type of platinum analogue used for treatment was carboplatin for 105 (69%) patients, and cisplatin for 47 (31%) patients. With regard to the method used for delivery of platinum analogue, hepatic arterial injection (HAI) was used in 73 (48%) patients, chemolipiodalization (CL) was used in 20 (13%) patients and TACE in 59 (39%) patients.

## Assessment of therapeutic effects and follow-up

The effects of chemotherapy were evaluated by CT every three months after treatment. The presence of non-enhanced tumor areas reflects tissue necrosis, and according to the findings of this imaging technique, the response to treatment was defined according to the World Health Organization criteria:[20] complete response: no evidence of neoplastic disease; partial response: reduction in total tumor load of ≥ 50%; no change: reduction of < 50% or increase of < 25%; progressive disease: increase of ≥ 25%.

Patients were examined by physicians every 4 weeks including monitoring of AFP, DCP and other biochemical data after the diagnosis of embolization-resistance.

Table 3 Profile of 152 HCC patients who underwent transcatheter arterial chemotherapy using a platinum analogue for advanced HCC unresponsive to TACE-epirubicin, according to type of therapy

| | HAI ($n = 73$) | CL ($n = 20$) | TACE ($n = 59$) |
|---|---|---|---|
| Profile of liver cancer and tumor marker | | | |
| Tumor size (mm)* | 48 (8–180) | 40 (8–100) | 33 (12–180) |
| Intrahepatic multiplicity | | | |
| Solitary | 4 | 1 | 1 |
| Multiple, localized to one segment | 3 | 2 | 5 |
| Multiple, localized to one lobe | 14 | 7 | 11 |
| Multiple, extended to both lobes | 52 | 10 | 42 |
| Portal vein invasion (no/yes) | 45/28 | 12/8 | 49/10 |
| AFP (μg/L)* | 257 (3–112000) | 682 (17–65900) | 145 (2–103000) |
| DCP (AU/L)* | 400 (10–131000) | 68 (10–53900) | 40 (10–55420) |
| Status of liver function | | | |
| Child-Pugh classification (A/B/C) | 43/28/2 | 13/6/1 | 42/17/0 |

*Expressed as median (minimum, maximum).
AFP, alpha-fetoprotein; CL, chemolipiodalization; DCP, des-gamma carboxyprothrombin; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

Imaging studies, as required for measurement of tumor size, were performed at around 3 months after therapy. Some patients took oral or intravenous medicinal herbs or other palliative therapies during the follow-up period.

## Statistical analysis and ethical considerations

The factors that influenced the treatment outcome ((partial response (PR) or complete response (CR)) in this cohort were analyzed by the $\chi^2$ test, and the cumulative survival rate was analyzed by Kaplan-Meier method. The risk factors involved in survival were evaluated by univariate analysis with the log-rank test. The independent factors associated with the curative effect (PR or CR) and survival rate were identified using the stepwise Cox regression analysis. Potential risk factors assessed for curative outcome (PR and CR) and survival rate included the following 17 variables: age, sex, HBs-Ag, HCV-antibody, aspartate transaminase (AST), albumin, bilirubin, AFP, DCP, prothrombin activity, ICG-R15, tumor size, multiplicity, portal vein invasion of HCC, treatment methods (HAI/CL/TACE), the type of platinum analogue (carboplatin/cisplatin) and the dose of platinum analogue used for treatment. Several variables were transformed into categorical data consisting of two-three simple ordinal numbers for univariate and multivariate analyses. All factors found to be at least marginally associated with the curative effect and survival ($P < 0.10$) in univariate analysis were entered into a multivariate logistic regression and Cox proportional hazard models. Significant variables were selected by stepwise method in the procedure. Proportional hazard analysis was also employed in the identification of contributing factors to the curative effect and survival rate. A $P$-value of less than 0.05 in two-tailed test was considered significant. Data analysis was performed using SPSS software version 11.0 (SPSS Inc, Chicago, Ill).

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital. The physicians in charge explained the purpose and method of the clinical trial to each patient, who gave their informed consents for participation.

# RESULTS

## Efficacy of platinum analogue, according to type of therapy

TABLE 3 SUMMARIZES the profiles and data of 152 HCC patients who were treated with platinum analogue, according to type of therapy.

In these patients, 6 of 152 (4%) patients showed CR, 28 (18%) patients showed PR, 35 (23%) patients showed stable disease (SD), and 83 (55%) patients showed progressive disease (PD). Analysis according to type of therapy showed 73 of 152 (48%) patients received HAI, 20 (13%) received CL, and 59 (39%) received TACE. The efficacy of transcatheter arterial chemotherapy using platinum analogue according to the type of therapy was as follow; in HAI group: 1 of 73

Table 4 Efficacy of transcatheter arterial chemotherapy using platinum analogue for advanced HCC in 152 patients unresponsive to TACE-epirubicin, according to type of therapy

| | CR (%) | PR (%) | SD (%) | PD (%) |
|---|---|---|---|---|
| Total ($n = 152$) | 6 (4%) | 28 (18%) | 35 (23%) | 83 (55%) |
| Type of therapy | | | | |
| HAI ($n = 73$) | 1 (1%) | 9 (12%) | 16 (22%) | 47 (65%) |
| CL ($n = 20$) | 1 (5%) | 3 (15%) | 7 (35%) | 9 (45%) |
| TACE ($n = 59$) | 4 (7%) | 16 (27%) | 12 (20%) | 27 (46%) |

CL, chemolipiodalization; CR, complete remission; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; PD, progressive disease; PR, partial response; SD, stable disease; TACE, transcatheter arterial chemoembolization.

(1%) patients showed CR, 9 of 73 (12%) patients showed PR, 16 of 73 (22%) patients showed SD, and 47 of 73 (65%) patients showed PD; in CL group: 1 of 20 (5%) patients showed CR, 3 of 20 (15%) patients showed PR, 7 of 20 (35%) patients showed SD, and 9 of 20 (45%) patients showed PD; in TACE group: 4 of 59 (7%) patients showed CR, 16 of 59 (27%) patients showed PR, 12 of 59 (20%) patients showed SD, and 27 of 59 (46%) patients showed PD (Table 4).

## Factor influencing curative effect (PR or CR)

We then investigated the factors associated with curative effect (PR or CR) after treatment using platinum analogue. Univariate analysis identified the following 11 factors that influenced the rate of curative effect (PR or CR): serum DCP (< 100 IU/L/≥ 100 IU/L, $P = 0.001$), serum AFP (< 200 μg/L/≥ 200 μg/L, $P = 0.005$), ICG-R15 (< 30%/≥ 30%, $P = 0.005$), tumor size (< 20 mm/≥ 20 mm, $P = 0.011$), portal vein invasion (yes/no, $P = 0.014$), total bilirubin (< 1.5 mg/dL/≥ 1.5 mg/dL, $P = 0.018$), treatment method (HAI/CL/TACE, $P = 0.021$), type of platinum analogue (carboplatin/cisplatin, $P = 0.021$), intrahepatic multiplicity that extended to both lobes (yes/no, $P = 0.028$), age (< 60/≥ 60, $P = 0.057$), and serum AST (< 50 IU/L/dL/≥ 50 IU/L, $P = 0.057$). These parameters were entered into multivariate logistic regression analysis. The curative effect (PR or CR) was significantly higher for elderly patients (aged ≥ 60, risk ratio: 7.75; 95% CI: 1.80–33.40), small size HCC (< 20 mm, risk ratio: 4.88; 95% CI: 1.62–14.71), TACE-platinum analogue treatment (yes, risk ratio: 3.91; 95% CI: 1.34–11.38), lower serum total bilirubin level (< 1.5 mg/dL, risk ratio: 3.44; 95% CI: 1.22–9.71), and Tumor multiplicity, extended to both lobes (no, risk ratio: 2.30 (1.03–7.09) (Table 5).

Table 5 Factors associated with curative effects in patients who underwent transcatheter arterial platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin

| Factors | Category | Risk Ratio (95% confidence interval) | *P* |
|---|---|---|---|
| Age (year) | 1: < 60 | 1 | |
| | 2: ≥ 60 | 7.75 (1.80–33.40) | 0.006 |
| Tumor size (mm) | 1: ≥ 20 | 1 | |
| | 2: < 20 | 4.88 (1.62–14.71) | 0.005 |
| Tumor therapy | 1: HAI | 1 | |
| | 2: CL | 2.47 (0.52–11.69) | 0.256 |
| | 3: TACE | 3.91 (1.34–11.38) | 0.012 |
| Bilirubin (mg/dL) | 1: ≥ 1.5 | 1 | |
| | 2: < 1.5 | 3.44 (1.22–9.71) | 0.020 |
| Multiple HCC, extended to both lobes (yes/no) | 1: yes | 1 | |
| | 2: no | 2.30 (1.03–7.09) | 0.044 |

CL, chemolipiodalization; CR, complete remission; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; PR, partial response; TACE, transcatheter arterial chemoembolization.