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胸水・腹水注入下治療の実際

1 summary

- ① 腹部超音波検査で描出できない(ドーム直下の)腫瘍を治療するために欠くことができない手技である
- ② 近接臓器の損傷(熱傷)を防ぐのに欠かせない手技である
- ③ 自然な穿刺ラインを確保するために必要である
- ④ 腫瘍に対し最短距離の穿刺が可能になる
- ⑤ 気腹針を用いた方法が簡便である

2 commentary

a) 治療対象拡大に向けて

肝癌の局所治療の進歩により、内科的治療でも長期経過が得られるようになった。そして再発、再々発や複数個の肝癌を治療する機会が増えてきた。肝癌は肝のどの部位にも生じるため、長期の良好な治療成績を残すためには、様々な肝癌に対応できる治療環境が必要である。

人工胸水法や人工腹水法は治療対象の拡大と安全性向上の点で、経皮的治療に必要不可欠で獲

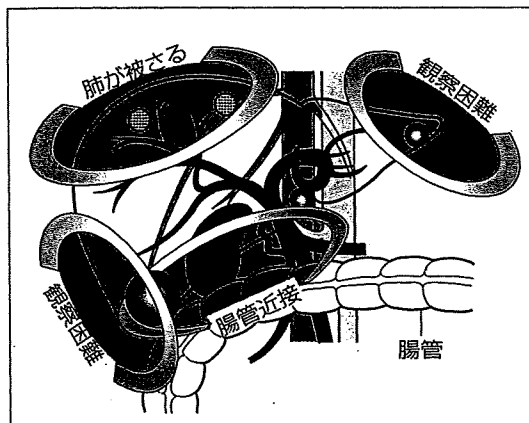


図1 死角および腸管近接部(人工胸水・腹水が有用な場合)

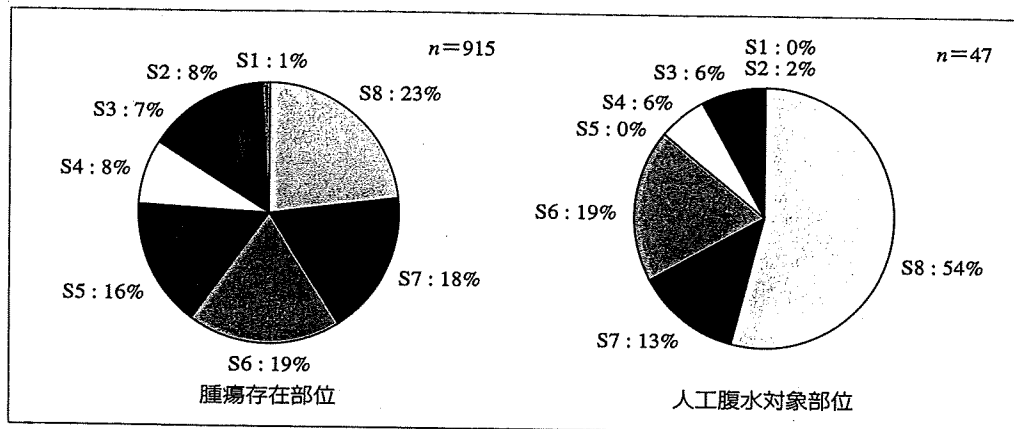


図2 腫瘍存在部位と人工腹水対象部位

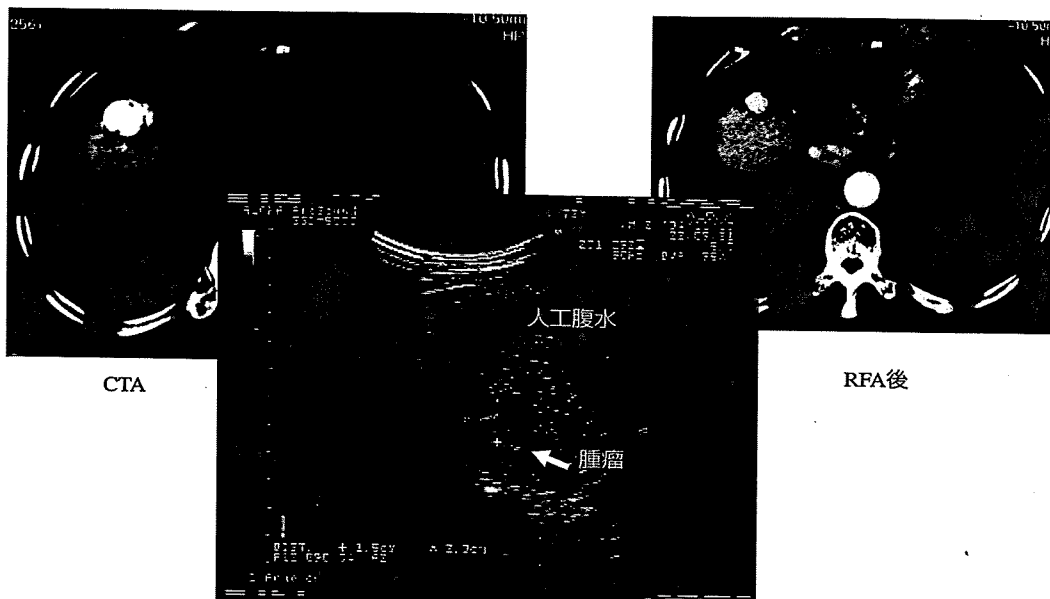


図3 S8腹側のHCC

得すべき技術である。肝臓を通常超音波検査で観察すると観察不能な死角が数か所存在する(図1)。特に横隔膜直下で腹側に存在する部位は肺が覆いかぶさり、通常の超音波操作では観察が不可能である。また、この部位に存在する腫瘍は多い。大分大学医学部消化器内科(以下、当科)でのRFA治療の対象となった腫瘍と人工腹水症例の腫瘍の存在部位の比率を示す(図2)。S8には23%腫瘍が存在し、死角に位置することも多い。人工腹水症例の治療対象部位はS8、S6、S7の順に多い。そのため、人工腹水法を身につければ、経皮的な治療対象は拡大される。また、人工胸水法や人工腹水法を用いると、肝全体の描出が容易になるだけでなく、見上げでの無理な穿刺による肋間動脈や静脈の損傷のリスクが少なくなる。さらに近接臓器の損傷を防ぐという点で安全性は向上する。

清家正隆

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部分的脾動脈塞栓療法併用 RFA 治療

1 summary

- ① 血小板低下(脾腫)を伴っている肝細胞癌の RFA を行う場合に有用である
- ② 部分的脾動脈塞栓療法(PSE)の塞栓方法としてはコイル法が有用である
- ③ 施行後2週間程度で RFA は可能となる
- ④ 発熱、腹痛など合併症は必発であるが2週間程度で改善する
- ⑤ 肝機能の改善も期待できる

2 commentary

a) PSE の位置づけ

肝癌の多くは肝硬変症を背景に生じるため、門脈圧亢進症に伴う脾機能亢進を合併していることが多い。脾機能亢進症、特に血小板低下があれば、局所療法においても、化学療法を必要とする進行肝癌においても治療は制限される。さらに背景肝の治療としてのウイルス排除を目的としたインターフェロン(IFN)を中心とした抗ウイルス療法は制限される。

部分的脾動脈塞栓療法(PSE)は門脈圧亢進症に伴う合併症に対して行われてきた¹⁾。しかし、肝癌患者の増加や肝癌に対する局所療法の新たな展開を背景に、日常診療における肝癌治療遂行のための補助療法としての位置づけが鮮明になった。この項では RFA 施行時における PSE の意義について主に述べる。

RFA の適応条件は一般的に(1)血小板5万以上、(2)PT50%以上、(3)腹水がコントロール可能であることなどが条件である。大分大学医学部消化器内科(以下、当科)において、初回 RFA 治療における血小板5万以下の症例は約8%であり、血小板の輸血後に RFA を行う必要がある。しかし、複数個ある場合や、年間20%の異所性再発をきたす症例に対し、その都度血小板輸血を行うのは非効率である。そこで、PSE が有用となる。

当科では IFN 導入の目的や進行肝癌に対するリザーバー動注療法(Low-doseFP)の治療継続の目的においても、PSE を併用しており、RFA 併用と合わせ30例の PSE が行われた。30例の血小板の推移を図1に示す。PSE 後2~4週間で前値の2倍程度に血小板は上昇する。RFA 施行前に PSE を行う場合は、血小板5万以下の症例が多い。また、IFN によるウイルス排除を目指す場合の PSE では導入前の血小板は5~8万程度の症例が多い。PSE 後血小板上昇は1年経過しても効果は持続しており、1度行くと、しばらく有効である。また肝機能の改善も得られるため²⁾、高齢で進行した肝硬変症を背景に発癌してくる症例に、安全で効果的な PSE は今後ますます重要性を増し

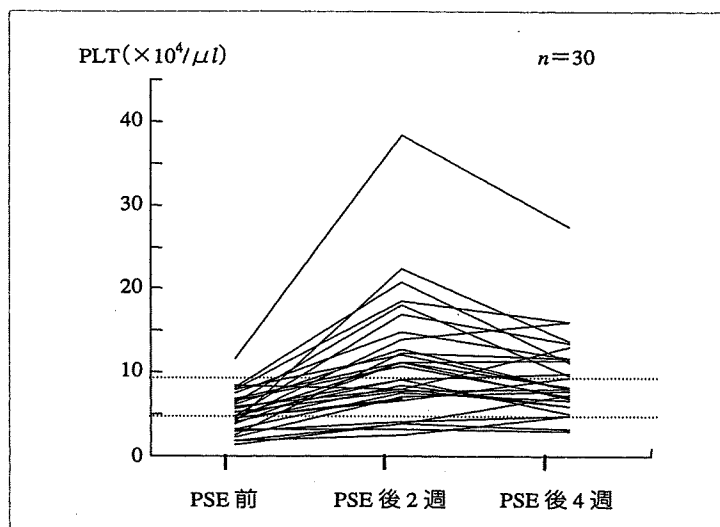


図1 PSE直後のPLTの推移



図2 PSE併用後、人工胸・腹水を行いRFAを行った肝細胞癌(62歳、男性)

てくると思われる。ただし、合併症には十分注意することが必要である。敗血症や腹膜炎で不幸な転帰をとった症例³⁾も報告されており、肝機能低下例では特に注意を必要とする。

b) 症 例

本症例は脾機能亢進に伴い、血小板は3.2万であった。腫瘍はS8腹側であり、人工腹水を必要としたため、PSEを行った。2週間後血小板は7.6万まで回復し、人工胸・腹水下のRFAを行った。施行前後の画像を示す(図2)。

Amino Acid Substitutions in the Hepatitis C Virus Core Region are the Important Predictor of Hepatocarcinogenesis

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We showed previously that amino acid (aa) substitutions in hepatitis C virus core region (HCV-CR) are negative predictors of virologic response to pegylated interferon (IFN) plus ribavirin therapy. HCV-CR induces hepatocellular carcinoma in transgenic mice, but the clinical impact is still unclear. To evaluate the impact of aa substitutions in HCV-CR on hepatocarcinogenesis, we performed a follow-up study on 313 noncirrhotic consecutive naïve patients infected with HCV genotype 1b who received IFN monotherapy. The median follow-up was 14.7 years. A sustained virologic response (SVR) after the first IFN was achieved by 65 patients (20.8%) (group A). Of 248 patients (79.2%) of non-SVR after first IFN, 112 (35.8%) did not receive additional IFN (group B), and the remaining 136 (43.5%) received multicourse IFN monotherapy (group C). As a whole, cumulative hepatocarcinogenesis rates in double wild-type (arginine at aa 70/leucine at aa 91) of HCV-CR were significantly lower than those in nondouble wild-type. Multivariate analyses identified 3 parameters (fibrosis stage 3, nondouble wild-type of HCV-CR, and group B) that tended to or significantly influenced hepatocarcinogenesis independently. With regard to hepatocarcinogenesis rates in group C according to HCV-CR and the mean alanine aminotransferase (ALT) during IFN-free period, significantly higher rates were noted in patients of nondouble wild-type with ALT levels of more than 1.5 times the upper limit of normal (25.7%) compared with the others (2.4%). **Conclusion:** Amino acid substitutions in the HCV-CR are the important predictor of hepatocarcinogenesis. In multicourse IFN therapy to nondouble wild-type, we emphasize the importance of reducing the risk of hepatocarcinogenesis by mean ALT during an IFN-free period below 1.5 times the upper limit of normal. (HEPATOLOGY 2007;46:1357-1364.)

Hepatitis C virus usually causes chronic infection, which can result in chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).¹⁻⁵ In patients with chronic HCV, treatment with IFN can induce viral clearance and marked biochemical and histological improvement.^{6,7}

For chronic HCV infection, peginterferon (PEG-IFN) plus ribavirin (RBV) combination therapy is an expensive treatment modality that is accompanied by severe side effects and high sustained virological response (SVR). Patients who do not achieve SVR need to be identified before the start of combination therapy to avoid unnecessary side effects and high costs. Thus, safer IFN monotherapy should be considered to reduce the risk of hepatocarcinogenesis in patients unsuitable for PEG-IFN plus RBV therapy. We studied previous determinants of response to PEG-IFN plus RBV in patients with high titers of HCV genotype 1b (≥ 100 KIU/mL), which is dominant in Japan. Our results identified substitution of amino acids (aa) 70 and/or 91 in the HCV core region (HCV-CR) as an independent and significant negative predictor associated with virological response.⁸⁻¹⁰ Furthermore, we reported that multicourse IFN monotherapy reduces the risk of hepatocarcinogenesis and increases survival even if patients fail to achieve SVR after a single-course IFN, and

Abbreviations: aa, amino acid(s); HCV-CR, hepatitis C virus core region; MU, million units; PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virologic response.

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that low ALT levels during an IFN-free period is associated with lower rates of hepatocarcinogenesis.¹¹ Hence, multicourse IFN monotherapy might be expected to reduce the risk of hepatocarcinogenesis in patients who have negative predictors for PEG-IFN plus RBV.

Despite numerous lines of epidemiological evidence connecting HCV infection and the development of HCC, it remains controversial whether HCV itself plays a direct or indirect role in the pathogenesis of HCC.¹² It has become evident that HCV-CR has oncogenic potential through the use of transgenic mice, but the clinical impact of HCV-CR on hepatocarcinogenesis is still unclear.¹³ Whether substitution of aa 70 and/or 91 in HCV-CR as a predictor of virological response for PEG-IFN plus RBV therapy also affects hepatocarcinogenesis awaits further investigation.

The present study included 313 consecutive naïve cases infected with HCV genotype 1b in whom 15 years had elapsed since induction of IFN monotherapy. The aims of the study were: (1) to evaluate the clinical impact of aa substitutions in the HCV-CR on hepatocarcinogenesis; (2) to analyze the predictive factors associated with hepatocarcinogenesis in patients who received IFN monotherapy; and (3) to evaluate the long-term efficacy of multicourse IFN monotherapy on hepatocarcinogenesis as examined through analysis of the outcomes of single and multicourses of IFN.

Patients and Methods

Patients. Among 573 consecutive HCV-infected patients in whom IFN monotherapy was induced between February 1987 and August 1992 at Toranomon Hospital, 313 were selected in the present study based on the following criteria: (1) patients naïve to IFN monotherapy; (2) patients infected with HCV genotype 1b alone; (3) patients with chronic hepatitis, without cirrhosis or HCC, as confirmed via biopsy examination within 6 months of enrollment; (4) patients not treated with IFN plus RBV combination therapy during follow-up time; (5) patients negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emeryville, CA), and positive for HCV RNA qualitative analysis with PCR (nested polymerase chain reaction or Amplicor, Roche Diagnostic Systems, CA); (6) patients free of coinfection with human immunodeficiency virus; (7) patients not treated with antiviral or immunosuppressive agents within 6 months of enrollment; (8) lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake); (9) patients free of other types of hepatitis, including hemochromatosis, Wilson's dis-

ease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease; (10) patients without or with well-controlled diabetes; and (11) patients who consented to the study.

With regard to the clinical features of 313 patients at the start of the first course of IFN monotherapy, there were 223 men and 90 women aged 15-66 years with a median age of 47 years. The numbers of patients with fibrosis stages 1, 2, and 3 were 179, 107, and 27, respectively. The median ALT level was 138 IU/L (range, 24-636 IU/L), and the median platelet count was $17.4 \times 10^4/\mu\text{L}$ (range, 8.9×10^4 - $39.2 \times 10^4/\mu\text{L}$). The median viremia level was 4.0 Meq/mL (range, <0.5-67.0 Meq/mL). The median follow-up time was 14.7 years (range, 0.1-20.1 years).

Furthermore, at the first course of IFN monotherapy, 222 patients (70.9%) received IFN- α alone; 83 patients (26.5%) received IFN- β alone; and the remaining 8 patients (2.6%) received a combination of IFN- α and IFN- β . A median IFN dose per day of 6 million units (MU) (range, 1-10 MU) was administered. As a whole, a median total dose of IFN of 525 MU (range, 22-3,696 MU) was administered during a median period of 23.9 weeks (range, 0.6 to 205.4 weeks). Patients mainly received IFN monotherapy, including initial aggressive induction therapy (every day within 8 weeks, followed by 3 times per week).

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Methods. The primary measure of efficacy of treatment was sustained virological response (SVR), defined as negative HCV RNA via qualitative analysis with PCR at 24 weeks after cessation of IFN therapy. Patients who achieved SVR after the first course of IFN monotherapy were classified as group A. Patients who did not achieve SVR after the first course of IFN monotherapy were classified into 2 groups based on whether they received other courses of IFN monotherapy. Patients who did not receive further courses of IFN monotherapy based on concerns about adverse effects, lack of time for treatment, physician recommendation based on the appearance of depression, and cardiopulmonary disease during and after the first course of IFN, or the lower levels of ALT, were classified as group B. Patients who received 2 or more courses of IFN monotherapy were classified as group C.

Laboratory Investigations. Blood samples were frozen at -80°C within 4 hours of collection and were not thawed until used for testing. HCV genotype was determined via PCR using a mixed primer set derived from nucleotide sequences of the NS5 region.¹⁴ In all cases, HCV-RNA viremia level was measured by branched DNA assay version 2.0 (Chiron Corp.) at commence-

ment of therapy using frozen samples, and the results were expressed as 10^6 genomic equivalents per milliliter (Meq/mL). The lower limit of the assay was 0.5 Meq/mL. Samples with undetectable levels using this quantitative assay (<0.5 Meq/mL) were also evaluated via HCV-RNA qualitative analysis with PCR (nested PCR or Amplicor) during and after therapy especially, and the results were expressed as positive or negative. The lower limit of the assay was 100 copies/mL.

Detection of Amino Acid Substitutions in Core Region. We developed a simple and low-cost PCR method for detecting substitutions of aa 70 or aa 91 in HCV-CR of genotype 1b using mutation-specific primer as an alternative to the direct sequencing method. The major protein type was determined based on the relative intensity of the bands for wild (aa 70, arginine; aa 91, leucine) and mutant (aa 70, glutamine/histidine; aa 91, methionine) in agarose gel electrophoresis. If the intensities of the bands were similar, the case was regarded as competitive. The detection rate was 94.4%, the sensitivity was 10 KIU/mL using quantitative assay with PCR (Cobas Amplicor HCV monitor version 2.0 using the 10-fold dilution method), the reproducibility was high, and consistency with direct sequencing was 97.1% in positive cases.¹⁵ In this study, the pattern of arginine (wild) at aa 70 and leucine (wild) at aa 91 was evaluated as double wild-type, while the other patterns were nondouble wild-type. The mutation in this study refers to substitution from consensus sequence. In previous studies, HCV-J was considered as a prototype, and the aa substitution was evaluated by comparison with the consensus sequence prepared from 50 clinical trial samples.^{8,16} In this study, the PCR genotyping could be performed in 232 patients; the remaining 81 patients could not be analyzed due to the lack of adequate serum samples obtained before treatment.

Liver Histopathological Examination. Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens for examination contained 6 or more portal areas. Histopathological diagnosis was made by an experienced liver pathologist (H. K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histopathological assessment according to the scoring system of Desmet et al.¹⁷

Follow-Up. Clinical and laboratory assessments were performed at least once every month before, during, and

after treatment. Adverse effects were monitored clinically by careful interviews and medical examination at least once every month. Patient compliance with treatment was evaluated with a questionnaire. Blood samples were also obtained at least once every month before, during, and after treatment, and were also analyzed for ALT levels and HCV-RNA levels at various time points.

Follow-up time represented the time from the start of the first course of IFN treatment until death or until the last visit.

Diagnosis of HCC. Patients were examined for HCC via abdominal ultrasonography every 3-6 months. If HCC was suspected based on ultrasonographic results, additional procedures such as CT, magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy (if necessary), were used to confirm the diagnosis.

Statistical Analysis. The χ^2 test, Fisher exact probability test, and Mann-Whitney *U* test were used to compare background characteristics between groups. Multiple comparisons were examined by the Bonferroni test. Cumulative hepatocarcinogenesis were calculated using the Kaplan-Meier technique; differences between survival curves were tested using the log-rank test. Statistical analyses of hepatocarcinogenesis according to groups were calculated using the period from start of the first course of IFN monotherapy. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with hepatocarcinogenesis. We also calculated the OR and 95% CI. Potential predictive factors associated with hepatocarcinogenesis included the following 11 variables: age, sex, histological stage, viremia level, serum AST, serum ALT, platelet count, aa substitutions in HCV-CR, total IFN dose, total IFN duration, and group of treatment. Each variable was transformed into categorical data consisting of 2 simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were tested using the multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using the SPSS software (SPSS Inc., Chicago, IL). All *P*-values of less than 0.05 by the 2-tailed test were considered significant.

Results

Efficacy of IFN Monotherapy. 65 patients (20.8%) achieved SVR after the first course of IFN monotherapy (group A). Of 248 (79.2%) non-SVR patients after the first course of IFN, 112 (35.8%) did not receive a second course of IFN monotherapy (group B), while the remain-

Table 1. Patient Characteristics at Start of First Course of IFN Monotherapy

	Group A (n = 65)	Group B (n = 112)	Group C (n = 136)
Sex (male/female)	45/20	75/37	103/33
Age (years)*	44 (15-64)†	51 (23-66)	45 (22-63)‡
Viremia level (Meq/mL)*	0.6 (<0.5-45.0)	5.9 (<0.5-67.0)§	5.3 (<0.5-57.0)¶
Fibrosis stage (F1/F2/F3)	49/14/2	54/50/8 [¶]	76/43/17 [#]
AST (IU/L)*	83 (16-198)	74 (22-398)	75 (24-400)
ALT (IU/L)*	153 (24-416)	120 (38-636)	138 (50-594)
Platelet count ($\times 10^4/\mu\text{L}$)*	18.7 (9.7-31.0)	17.1 (9.7-39.2)	17.0 (8.9-31.2)
Core region (double wild/nondouble wild/ND)*	10/15/5	31/44/7	41/71/8

*Median † $P = 0.009$, ‡ $P = 0.007$ compared with group B via Bonferroni test. § $P < 0.0001$, ¶ $P < 0.0001$, [¶] $P = 0.006$, [#] $P = 0.009$, compared with group A via Bonferroni test.

** Amino acid substitutions were evaluated in pretreatment serum samples of 232 patients via PCR with mutation-specific primers. Two patterns of mutant and competitive were labeled as nonwild. Wild at aa 70 and wild at aa 91 were evaluated as double-wild-type, while the other patterns were considered nondouble wild-type. Abbreviation: ND, not determined.

ing 136 (43.5%) received 2 or more courses of IFN monotherapy (group C). Of 136 patients in group C, 80 patients received 2 courses of IFN (21 of whom achieved SVR), 44 patients received 3 courses (6 of whom achieved SVR), 11 patients received 4 courses (2 of whom achieved SVR), and 1 patient received 6 courses (and did not achieve SVR). Thus, 29 patients in group C achieved SVR after multiple courses of IFN monotherapy.

In groups A and B, the median total duration of IFN was 24.1 weeks (range, 4.0-205.4 weeks) and 23.7 weeks (range, 2.9-75.1 weeks). The median total dose of IFN was 528 MU (range, 43-3,696 MU) and 498 MU (range, 72-870 MU). In the first, second, third, fourth, fifth, and sixth courses of IFN monotherapy in group C, the median total durations of IFN were 23.9 weeks (range, 0.6-136.4 weeks), 24.0 weeks (range, 1.3-313.7 weeks), 25.3 weeks (range, 3.1-198.1 weeks), 40.4 weeks (range, 21.0-86.3 weeks), 23.6 weeks, and 67.9 weeks, respectively. In the first, second, third, fourth, fifth, and sixth courses of IFN monotherapy in group C, the median total doses of IFN were 525 MU (range, 22-2,312 MU), 558 MU (range, 57-4,005 MU), 522 MU (range, 28-3,477 MU), 565 MU (range, 363-1,080 MU), 708 MU, and 1,200 MU, respectively. The median cumulative total durations and cumulative total doses, which represented the cumulative total duration and total dose of every course of every patient of group C, were 65.6 weeks (range, 8.4-474.4 weeks) and 1,388 MU (range, 354-4,805 MU), respectively. The median periods free of IFN in group C were 3.6 years (range, 0.1-7.3 years). In conclusion, the median dose of IFN per week in group A, B, and C were 21.8 MU/week (range, 6.7-42.0 MU/week), 22.0 MU/week (range, 4.5-42.0 MU/week), and 21.9 MU/week (range, 3.7-43.9 MU/week), respectively.

Clinical Features of Patients and Cumulative Hepatocarcinogenesis Rates According to Study Groups. The clinical features of patients in groups A, B,

and C, at the start of the first IFN monotherapy are summarized in Table 1. The age of patients of group B was significantly higher than those of group A ($P = 0.009$; Bonferroni test) and group C ($P = 0.007$; Bonferroni test). Viremia levels in group A were significantly lower than those in group B ($P < 0.001$; Bonferroni test) and group C ($P < 0.001$; Bonferroni test). Fibrosis stage of group A was significantly milder than those of group B ($P = 0.006$; Bonferroni test) and group C ($P = 0.009$; Bonferroni test). There were no other significant differences in clinical features at the start of IFN therapy among the 3 groups.

During follow-up, 1 (1.5%), 17 (15.2%), and 15 (11.0%) patients developed HCC in groups A, B, and C, respectively. In groups A, B, and C, the cumulative hepatocarcinogenesis rates were 2.3%, 11.5%, and 0.8%, respectively, at the end of 5 years; 2.3%, 25.3%, and 7.2%, respectively, at the end of 10 years; and 2.3%, 33.0%, and 25.6%, respectively, at the end of 15 years. The rates were significantly different among the 3 groups ($P < 0.001$; Log-rank test) (Figure 1). In particular, the rates in group B were significantly higher than in group C ($P < 0.001$; Log-rank test) and group A ($P < 0.001$; Log-rank test), and the rates in group C were significantly higher than group A ($P = 0.037$; Log-rank test).

Hepatocarcinogenesis Rates According to aa Substitutions of HCV-CR. During follow-up, 5 of 82 patients (6.1%) and 18 of 130 patients (13.8%) developed HCC in double wild-type and nondouble wild-type, respectively. In double wild-type and nondouble wild-type, the cumulative hepatocarcinogenesis rates were, respectively, 1.6% and 2.6% at the end of 5 years; 3.4% and 12.3% at the end of 10 years; and 11.3% and 23.5% at the end of 15 years. The rates in double wild-type of HCV-CR were significantly lower than those in nondouble wild-type ($P = 0.036$; log-rank test) (Fig. 2).

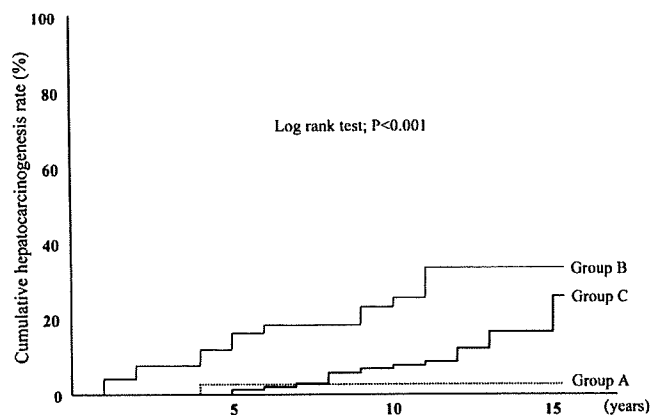


Fig. 1. Cumulative hepatocarcinogenesis rates were significantly different among the 3 study groups ($P < 0.001$; Log-rank test). In particular, the rates in group B were significantly higher than in group C ($P < 0.001$; Log-rank test) and group A ($P < 0.001$; log-rank test), and the rates in group C were significantly higher than in group A ($P = 0.037$; log-rank test).

Predictive Factors Associated with Hepatocarcinogenesis via Multivariate Analysis. We then analyzed the data for the whole population sample to determine those factors that could predict hepatocarcinogenesis. Univariate analysis identified 6 parameters that tended to or significantly correlated with carcinogenesis: age ($P < 0.001$), fibrosis stage ($P < 0.001$), platelet count ($P < 0.001$), group ($P < 0.001$), viremia level ($P = 0.018$), and aa substitution in HCV-CR ($P = 0.036$). These factors were entered into multivariate analysis, which identified 3 parameters that tended to or significantly influenced carcinogenesis independently: fibrosis stage ($P < 0.001$), aa substitutions in HCV-CR ($P = 0.008$), and group ($P = 0.056$) (Table 2).

We also analyzed the data for 219 patients, except for 94 patients who achieved SVR, to determine those factors

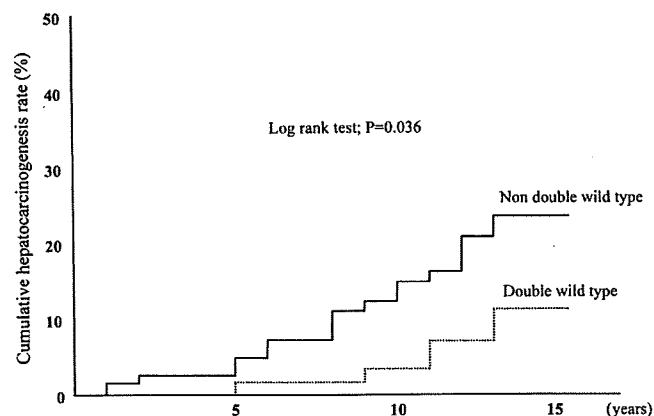


Fig. 2. Cumulative hepatocarcinogenesis rates according to aa substitutions of HCV-CR. The rates in double wild-type (arginine at aa 70/leucine at aa 91) of HCV-CR were significantly lower than those in nondouble wild-type ($P = 0.036$; log-rank test).

Table 2. Factors Associated With Hepatocarcinogenesis in 313 Patients Infected with HCV Genotype 1b, Identified via Multivariate Analysis

Factors	Category	Odds Ratio (95% CI)	P Value
Fibrosis stage	1: F1, F2	1	<0.001
	2: F3	10.2 (3.65-28.5)	
Amino acid substitutions in the core region	1: double-wild	1	0.008
	2: nondouble-wild	5.92 (1.58-22.2)	
Group	1: A, C	1	0.056
	2: B	2.75 (0.98-7.76)	

NOTE. Cox proportional hazard model.

that could predict hepatocarcinogenesis. Univariate analysis identified 5 parameters that tended to or significantly correlated with carcinogenesis: fibrosis stage ($P < 0.001$), platelet count ($P < 0.001$), age ($P = 0.001$), group ($P = 0.008$), and aa substitution in HCV-CR ($P = 0.028$). These factors were entered into multivariate analysis, which identified 2 parameters that significantly influenced carcinogenesis independently: fibrosis stage ($P < 0.001$) and aa substitution in HCV-CR ($P = 0.017$) (Table 3).

Hepatocarcinogenesis Rates in Group C According to HCV-CR and ALT Levels. In group C, the hepatocarcinogenesis rates were evaluated according to the ALT levels at the start of IFN. For this purpose, we selected 112 patients (82.4%) from group C in whom HCV-CR could be evaluated. In double wild-type, the hepatocarcinogenesis rates in patients with ALT levels below 1.5 (<75 IU/L) and above 1.5 (>75 IU/L) times the upper limit of normal (6-50 IU/L) were 0% (0/6 patients) and 8.6% (3/35 patients), respectively. In nondouble wild-type, the hepatocarcinogenesis rates in patients with ALT levels below 1.5 and above 1.5 times the upper limit of normal was 0% (0/7 patients), and 15.6% (10/64 patients), respectively (Table 4). In conclusion, regardless of whether aa substitutions in HCV-CR are present or not, lower hepatocarcinogenesis rates were noted in patients with ALT levels below 1.5 the upper limit of normal (0%) than in other patients (13.1%), but they did not achieve statistical significance on univariate analysis.

Table 3. Factors Associated with Hepatocarcinogenesis in 219 Patients of Non-SVR Infected with HCV Genotype 1b, Identified via Multivariate Analysis

Factors	Category	Odds Ratio (95% CI)	P Value
Fibrosis stage	1: F1, F2	1	<0.001
	2: F3	6.50 (2.39-17.6)	
Amino acid substitutions in the core region	1: double-wild type	1	0.017
	2: nondouble wild-type	4.65 (1.32-16.4)	

NOTE. Cox proportional hazard model.

Table 4. Hepatocarcinogenesis Rates in Group C According HCV Core Region and ALT Levels at the Start of IFN

	ALT Level (IU/L)*			
	<75	75-100	100-200	>200
Nondouble wild-type	0% (0/7)	14.3% (2/14)	13.3% (4/30)	20.0% (4/20)
Double wild-type	0% (0/6)	16.7% (1/6)	5.6% (1/18)	9.1% (1/11)

* Normal level of ALT: 6-50 IU/L.

In group C, the hepatocarcinogenesis rates were also evaluated according to the mean ALT levels at the IFN-free period. For this purpose, we selected 76 consecutive patients (55.9%) from group C in whom ALT levels were closely monitored. In double wild-type, the hepatocarcinogenesis rates in patients with ALT levels below 4 (<200 IU/L) and above 4 (>200 IU/L) times the upper limit of normal were 0% (0/26 patients) and 50% (1/2 patients), respectively. In nondouble wild-type, the hepatocarcinogenesis rates in patients with ALT levels below 1.5 (<75 IU/L), from 1.5 to 2 (75-100 IU/L), from 2 to 4 (100-200 IU/L), and above 4 (>200 IU/L) times the upper limit of normal were 0% (0/13 patients), 33.3% (3/9 patients), 22.7% (5/22 patients), and 25.0% (1/4 patients), respectively (Table 5). In conclusion, regardless of whether aa substitutions in HCV-CR are present or not, significantly lower hepatocarcinogenesis rates were noted in patients with ALT levels below 1.5 times the upper limit of normal (0%) than in other patients (18.9%) ($P = 0.027$). In particular, significantly higher hepatocarcinogenesis rates were noted in patients of nondouble-wild-type with ALT levels above 1.5 times the upper limit of normal (25.7%) than in other patients (2.4%) ($P = 0.004$).

Discussion

Despite numerous lines of epidemiological evidence connecting HCV infection and the development of HCC, it remains controversial whether HCV itself plays a direct or indirect role in the pathogenesis of HCC.¹² It is evident that the HCV-CR has oncogenic potential through the use of transgenic mice,¹³ but its clinical impact on hepatocarcinogenesis is still unclear. Our study identified that cumulative hepatocarcinogenesis rates of double wild-type HCV-CR, as a predictor of virological response for PEG-IFN plus RBV therapy, were significantly lower than those of nondouble wild-type. We spec-

ulate that the resistant cases for treatment might reasonably lead to HCC. To our knowledge, this is the first report to support the findings of oncogenic potential via HCV-CR from the clinical aspect. Previous reports identified PA28 γ -dependent pathway as one of the mechanisms of HCV-associated hepatocarcinogenesis. Morishi and colleagues showed that a knockout of the PA28 γ gene induces the accumulation of HCV core protein in the nucleus of hepatocytes of HCV core gene transgenic mice and disrupts development of both hepatic steatosis and HCC.^{18,19} Furthermore, HCV core protein also enhanced the binding of liver X receptor α /retinoid X receptor α to liver X receptor response element in the presence of PA28 γ .¹⁹ Thus, it is reported that PA28 γ plays a crucial role in the development of HCV-associated steatogenesis and hepatocarcinogenesis. Further studies should be performed to connect evidence from animal model studies and the clinical impact of aa substitution in HCV-CR on hepatocarcinogenesis.

Viral factors associated with hepatocarcinogenesis in patients infected with HCV are still incompletely investigated. Ogata et al. reported that HCV genotype 1b strains might be associated with HCC on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein.²⁰ Giménez-Barcons et al. reported that high aa variability within the NS5A of HCV might be associated with HCC in patients with HCV-1b-related cirrhosis.²¹ In the present study, we could not investigate the clinical impact of the other region on hepatocarcinogenesis, except for the HCV-CR. Further studies should be performed to investigate the clinical impact of the other region of HCV on hepatocarcinogenesis.

Patients who fail to achieve SVR after single-course IFN should receive multicourse IFN at the time of ALT relapse at certain intervals. Based on previous reports showing increased incidence of HCC in 5 years or more

Table 5. Hepatocarcinogenesis Rates in Group C According HCV Core Region and ALT Levels at the IFN-Free Period

	ALT level (IU/L)*			
	<75	75-100	100-200	>200
Nondouble wild-type	0% (0/13)	33.3% (3/9)	22.7% (5/22)	25.0% (1/4)
Double wild-type	0% (0/10)	0% (0/4)	0% (0/12)	50.0% (1/2)

* Normal level of ALT: 6-50 IU/L.

after IFN therapy in transient biochemical responders, it is important to normalize ALT levels via multicourse IFN monotherapy at certain intervals.^{11,22} We reported previously that results of multicourse IFN showed a 0% hepatocarcinogenesis rate in patients with ALT levels below 75 IU/L at the IFN-free periods, emphasizing the importance of keeping low ALT levels at such periods with respect to suppression of hepatocarcinogenesis.¹¹ Furthermore, hepatocarcinogenesis rates according to HCV-CR and ALT levels during the IFN-free period were also evaluated in this study. In double wild-type, the rates in patients with ALT levels below 200 IU/L and above 200 IU/L were 0% and 50%, respectively. In nondouble wild-type, the rates in patients with ALT levels below 75 IU/L and above 75 IU/L were 0% and 25.7%, respectively. Thus, significantly higher hepatocarcinogenesis rates were noted in patients of nondouble wild-type with ALT levels above 75 IU/L than in other patients. In particular, in multicourse IFN therapy in nondouble wild-type, we emphasize the importance of reducing the risk of hepatocarcinogenesis by the mean ALT during the IFN-free period below 1.5 times the upper limit of normal.

It is unclear whether ALT levels during the IFN-free period might be more important than those at the start of IFN. In the present study, at the start of IFN, lower hepatocarcinogenesis rates were noted in patients with ALT levels below 1.5 the upper limit of normal compared with other patients, but they did not achieve statistical significance on univariate analysis. During the IFN-free period, significantly lower hepatocarcinogenesis rates were noted in patients with mean ALT levels below 1.5 times the upper limit of normal compared with other patients. Thus, in multicourse IFN therapy, especially in nondouble wild-type, we emphasize the importance of reducing the risk of hepatocarcinogenesis via ALT levels below 1.5 times the upper limit of normal during the IFN-free period rather than at the start of IFN. Further studies should be conducted in the future to confirm this finding.

To our knowledge, our study is the first to report the hepatocarcinogenesis rates for a long-term follow-up period of 15 years in IFN monotherapy. Previous studies have shown that sex, age, fibrosis stage, and IFN regimen are important pretreatment predictors of hepatocarcinogenesis.^{11,23-25} In the present study, a more progressive fibrosis stage as host factor, nondouble wild-type of HCV-CR as viral factor, and group B (non-SVR after single-course IFN) as treatment-related factor were associated with higher hepatocarcinogenesis rates in the whole population sample. Even if we also analyzed non-SVR patients, multivariate analyses similarly identified more progressive fibrosis stage and nondouble wild-type of HCV-CR that significantly influenced hepatocarcino-

genesis independently. Hence, we assess that the risk of HCC is not necessarily secondary to the lack of response to IFN therapy rather than aa substitution. We conclude that hepatocarcinogenesis seems to be based on a dynamic tripartite interaction of virus, host, and treatment regimen. Further understanding of the complex interaction between these factors should facilitate the development of more effective therapeutic regimens. In Japan, only 5 years had elapsed since the induction of IFN- α 2b plus RBV combination therapy (especially, only 2 years in PEG-IFN- α 2b plus RBV) based on the Japanese Government Health Insurance system, so we could not exactly evaluate the long-term efficacy of combination therapy as a treatment-related factor of hepatocarcinogenesis in this study. Further studies that include patients treated not only with IFN monotherapy but also with RBV combination therapy should be performed in the future.

The relationship between the development of cirrhosis and HCC is still unclear. We investigated liver fibrosis stage of 13 patients who underwent partial hepatectomy for HCC in this study. Interestingly, 8 of 13 patients (61.5%) developed HCC in the absence of cirrhosis (5 patients of fibrosis stage 2, 3 patients of fibrosis stage 3). As a whole, it is regrettable that we could not exactly evaluate how frequently HCC occurs in the absence of cirrhosis. Further studies based on all patients, whether or not they develop HCC, should be performed to investigate the relationship between the development of cirrhosis and HCC.

In conclusion, aa substitutions in the HCV-CR are the primary predictor of hepatocarcinogenesis. In particular, in multicourse IFN therapy in nondouble wild-type as a pretreatment negative predictor of SVR for PEG-IFN plus RBV combination therapy, we emphasize the importance of reducing the risk of hepatocarcinogenesis via mean ALT levels below 1.5 times the upper limit of normal during the IFN-free period. Furthermore, IFN monotherapy should be recommended as a therapeutic regimen to reduce the risk of hepatocarcinogenesis in patients unsuitable for PEG-IFN plus RBV combination therapy. Large-scale prospective studies should be conducted in the future to confirm this finding.

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Long-Term Outcome after Interferon Therapy in Elderly Patients with Chronic Hepatitis C

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Key Words

Chronic hepatitis C · Elderly patients · Interferon · Hepatocellular carcinoma · IFN therapy in elderly patients, survival

Abstract

Objective: The purpose of this study was to elucidate the long-term outcome after interferon (IFN) therapy in chronic hepatitis C elderly patients. **Methods:** We studied the incidence of hepatocellular carcinoma (HCC) and survival probability after the initiation of IFN therapy in 500 Japanese chronic hepatitis C patients >60 years. The mean age of initiation of IFN was 63 years and the mean follow-up period was 7.4 years. Cox proportional hazard regression analysis was used to evaluate the long-term outcome after initiation of IFN therapy. Sustained virological response (SVR) was defined as negative HCV-RNA by RT-nested PCR 6 months after the completion of long-term IFN therapy. Non-response (NR) was applied to patients who did not show SVR. Hepatic fibrosis was defined as the fibrosis score (score 0–4) according to Knodell et al. **Results:** 140 patients (28%) had an SVR and 360 patients (72%) had an NR. 71 of 500 patients developed HCC during follow-up. The cumulative incidence of HCC was 9.6% at the 5th year, 17.4% at the

10th year, and 31.3% at the 15th year. HCC developed with significance when: (1) HCV was not cleared after IFN therapy ($p < 0.0001$), (2) sex was male ($p < 0.0001$), and (3) staging of liver fibrosis was >2 ($p = 0.008$). 53 of the patients died. The cumulative survival probability was 95.7% at the 5th year, 86.4% at the 10th year, and 78% at the 15th year. Patients achieved a long survival with significance when: (1) staging of liver fibrosis was 1 ($p < 0.0001$), (2) HCV was cleared after IFN therapy ($p = 0.034$), and (3) sex was female ($p = 0.015$). **Conclusion:** Chronic hepatitis C patients with clearance of HCV after IFN therapy had a significantly reduced risk of HCC appearance and achieved prolonged survival even if they are ≥ 60 years.

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Introduction

Hepatocellular carcinoma (HCC) often occurs in patients with hepatitis C virus (HCV)-RNA-positive chronic liver disease [1]. The majority of deaths due to HCC are ascribed to hepatitis viruses, of which 70–80% corresponding to approximately 30,000 per year is attributed to the persistent infection with HCV in Japan [2, 3]. It is important to eradicate HCV or decrease levels of

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alanine aminotransferase (ALT) for preventing HCC with interferon (IFN) therapy [4, 5].

Nowadays, patients with HCV in Japan tend to be aged. Also, HCV-related HCC patients have been shown to become old with a peak around the age of 70 [6]. When such aged chronic hepatitis C patients with abnormal ALT levels consult a doctor, the first problem is whether or not therapy should be used for chronic hepatitis C. Moreover, when treatment for chronic hepatitis C is decided in such aged patients, whether IFN therapy should be used or not is the second problem. However, a few studies have targeted IFN therapy and prolonged prognosis in elderly patients with chronic hepatitis C [7, 8]. Until now, IFN treatment for chronic hepatitis C has mainly been introduced when patients are less than 60–65 years of age because of IFN-related side effects and safety standards in Japan. Owing to IFN-related side effects or various complicated diseases, there is a tendency not to give IFN to aged patients. Thus, IFN therapy for chronic hepatitis C has been conventionally limited to patients aged less than 60–65 years. We therefore assessed the long-term efficacy of IFN therapy in elderly patients with chronic hepatitis C by a retrospective cohort study.

Patients and Methods

Patients

The number of chronic hepatitis C patients treated with IFN therapy in our hospital between 1989 and 2004 was 3,320. Of these, 500 patients had the following criteria: (1) ≥ 60 years of age; (2) ALT elevation greater than double the upper limits (ALT normal range 12–50 IU/l) within 6 months; (3) no corticosteroid immunosuppressive agents, or antiviral agents used within 6 months; (4) no hepatitis B surface antigens, antinuclear antibodies, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay; (5) leukocytes $>3,000/\text{mm}^3$, platelet count $>80,000/\text{mm}^3$, and bilirubin <2.0 mg/ml, and (6) IFN therapy >4 weeks. Next, we excluded those patients from the study with a history of alcohol abuse or advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. Our study was approved by the institutional Ethics Review Board of our hospital. The physician in charge explained the purpose and method of this clinical trial, as well as the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

IFN Therapy

For the first IFN treatment regimen, the IFN treatment consisted of 3–12 million units (MU) of IFN- α or IFN- β . For the IFN treatment regimen, one group of 245 patients was assigned to receive IFN intramuscularly every day for the first 2–8 weeks and then 2–3 times/week for the following 16–96 weeks. Another group of 116 cases was assigned to receive IFN 3 times/week for 24–104

weeks. A third group of 108 patients was assigned to be treated with IFN by intravenous injection daily for 4–8 weeks. The fourth group of 31 patients was given combination therapy of IFN and ribavirin. The median total dose was 624 MU (range 168–2,430) and median administration period was 165 days (range 28–730).

Definition of Response of IFN Efficacy

Patients treated with IFN were divided into the following two groups based on the serum HCV-RNA after the termination of IFN. Sustained virological response (SVR) was defined as negative HCV-RNA by RT-nested PCR 6 months after the completion of long-term IFN therapy. Non-response (NR) was applied to patients who did not show SVR.

Blood and Urine Tests

Blood samples were obtained just before and 6 months after IFN treatment. The samples were stored at -80° until analyzed. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) [9]. On the other hand, HCV-RNA 6 months after the termination of IFN therapy was analyzed by qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/ml [10]. HCV genotype was examined by PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [11].

Follow-Up Protocol

The start of the follow-up period was defined as the first day of IFN treatment. Clinical evaluation and biochemical and hematologic tests were performed at 1–3 monthly intervals. Thirty-four patients were lost to follow-up. Because the appearance of HCC and death was not identified in these 34 patients, they were considered as censored data in statistical analyses [12]. Moreover, patients retreated with IFN in order to eradicate HCV-RNA were regarded as withdrawals at the start of IFN retreatment.

Diagnosis of HCC was based on the presence of typical hypervascular characteristics on angiography, in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy material was performed in patients whose angiograms did not demonstrate a typical image of HCC. Histopathological confirmation using surgically resected specimens was made in 21 patients.

Cause of death was divided into liver-related and liver-unrelated. The former included HCC, liver failure, and esophagogastric variceal bleeding, and the latter included extrahepatic malignancies, heart disease, cerebrovascular accidents, pulmonary disease, and others.

Liver Histology before IFN Therapy

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were scored according to the system of Knodell et al. [13]. Histologic index score: 0–10 for periportal bridging necrosis and 0–4 for interlobular degeneration and focal necrosis, portal inflammation, and fibrosis.

Table 1. Clinical characteristics before IFN treatment according to efficacy of IFN therapy in chronic hepatitis C elderly patients

Characteristics	SVR (n = 140)	NR (n = 360)	p
Age, years*	63 ± 3.2	64 ± 3.2	0.070
Male/female	83/57	168/192	0.011
Liver histology (fibrosis: 1/2/3/4)**	59/47/6/12	120/109/29/58	0.009
Liver histology (activity)*, **	9.3 ± 3.4	9.8 ± 2.7	0.223
HCV genotype (1b/2a/2b/others)	47/74/12/17	255/48/38/23	<0.0001
HCV load, kIU/ml*	172 ± 204	661 ± 506	<0.0001
AST, IU/l*	83 ± 70	87 ± 51	0.143
ALT, IU/l*	113 ± 102	118 ± 82	0.200
Hb, g/dl	14.2 ± 1.4	14.1 ± 1.3	0.547
Platelets, × 10 ⁴ /mm ³ *	15.0 ± 4.6	14.9 ± 4.8	0.768
WBC, × 10 ³ /mm ³ *	4.6 ± 1.4	4.6 ± 1.3	0.751
Period of observation, years	7.0 ± 3.3	7.7 ± 3.6	0.011

Activity was defined as sum score of periportal bridging necrosis, interlobular degeneration and focal necrosis, and portal inflammation.

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; NR = non-response; SVR = sustained virological response; WBC = white blood cells.

* Data are number of patients or mean ± SD.

** Histologic index score: 0–10 for periportal bridging necrosis and 0–4 for interlobular degeneration and focal necrosis, portal inflammation, and fibrosis.

Activity was defined as sum score of periportal bridging necrosis (score 0–10), interlobular degeneration and focal necrosis (score 0–4), and portal inflammation (score 0–4). Fibrosis was defined as fibrosis score (score 0–4).

Statistical Analysis

Baseline characteristics and treatment differences among groups based on efficacy of IFN treatment were analyzed using Kruskal-Wallis test. HCC appearance rates were analyzed by the log-rank test. A Cox proportional hazards model was used to analyze the factors contributing to the HCC appearance rate and death: factors examined included age, gender, histologic findings, HCV genotype, HCV load, aspartate aminotransferase (AST), ALT, and efficacy of IFN administration. A p value <0.05 was considered statistically significant. The SPSS Software Package (SPSS Inc., Chicago, Ill., USA) was used for analyses.

Results

Characteristics of the Patients and the Efficacy of the IFN Therapy

500 patients were enrolled in the present study. 140 patients (28%) had a SVR and 360 patients (72%) had a NR. Table 1 shows the baseline characteristics of the patients based on the efficacy of IFN therapy. The frequency distributions of the HCV genotype, the stage of liver fibrosis and HCV load differed between the two groups.

Development of HCC and Risk Factors for Appearance of HCC

During follow-up, HCC developed in 71 patients. The cumulative incidence as shown in figure 1 was based on efficacy of IFN therapy. The cumulative incidence of HCC was 9.6% at the 5th year, 17.4% at 10th year, and 31.3% at 15th year.

Cox regression analysis was performed using nine variables, including age, sex, histopathological severity (staging), viral load, HCV genotype, serum AST, serum ALT, and efficacy of IFN therapy. Univariate analysis showed that the following five factors significantly affected the cumulative HCC appearance rate in all patients as shown in table 2. Because the variables were mutually correlated, multivariate Cox regression analysis was performed with the statistically significant variables in the model (table 3). HCC developed with significance when: (1) HCV was not cleared ($p < 0.0001$), (2) sex was male ($p < 0.0001$), and (3) staging of liver fibrosis was >2 ($p = 0.008$).

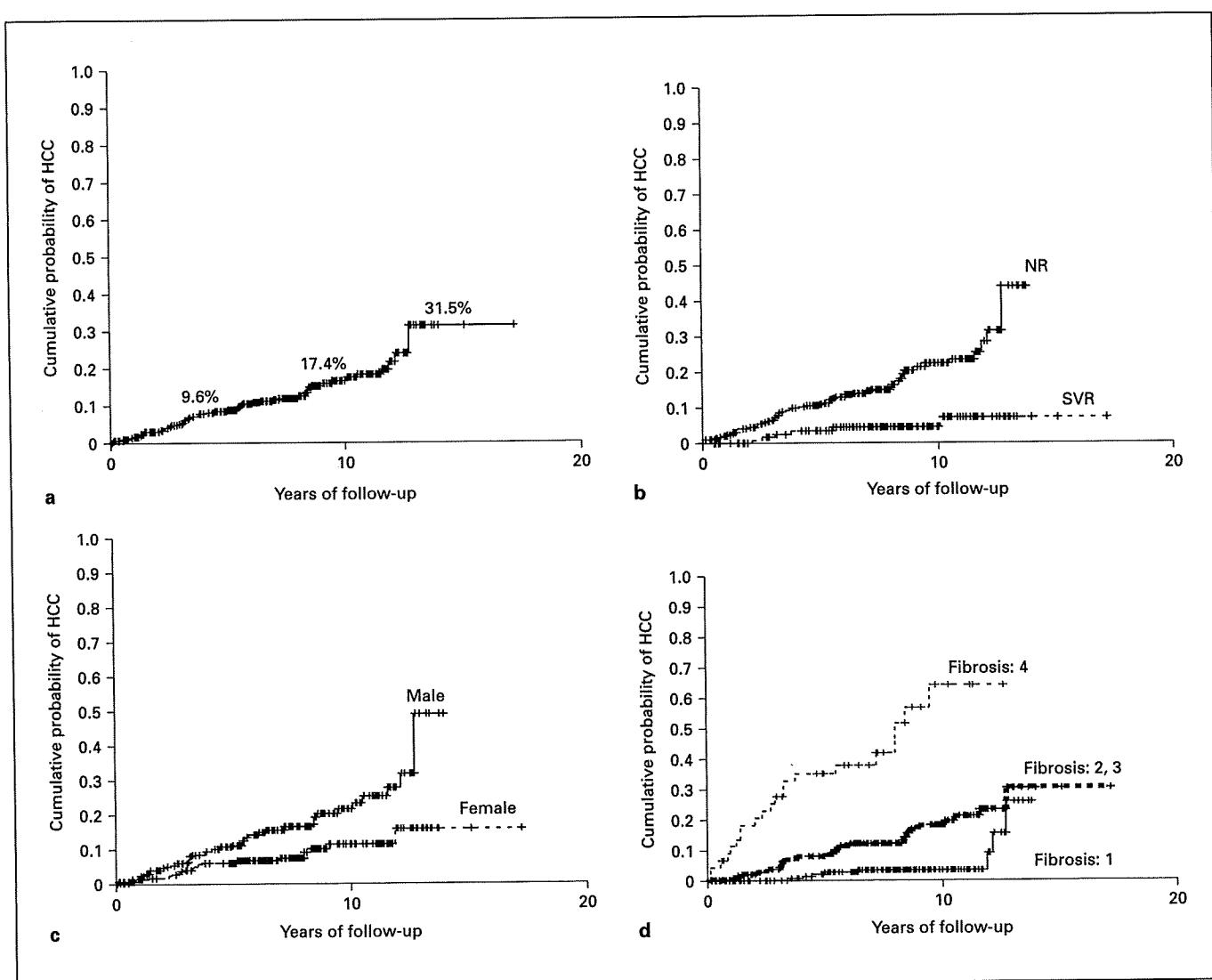
Fig. 1. Cumulative appearance probability of HCC. **a** In total patients; **b** based on difference of efficacy of IFN therapy; **c** based on difference of sex; **d** based on difference of histological fibrosis. SVR = Sustained virological response; NR = non-response.

Table 2. Predictive factors for hepatocellular carcinoma appearance after IFN therapy by Cox proportional hazards model (univariate analysis)

Factor	Category	Risk ratio*	95% CI	p
Liver histology (fibrosis)	1/2,3,4	1/4.22	2.81–6.34	<0.0001
Sex	Male/female	1/0.44	0.26–0.75	0.002
Age, years	<65/≥65	1/1.95	1.14–3.32	0.015
HCV genotype	1/2	1/0.55	0.31–0.97	0.046
AST, IU/l	<76/≥76	1/1.75	0.83–3.67	0.141
ALT, IU/l	<100/≥100	1/1.64	0.79–3.40	0.184
HCV-RNA, kIU/ml	<100/≥100	1/1.47	0.79–2.76	0.224
Liver histology (activity)	<10/≥10	1/1.55	0.79–3.07	0.206
Efficacy of IFN therapy	NR/SVR	1/0.22	0.096–0.52	<0.0001

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; NR = non-response; SVR = sustained virological response.

* Risk ratio for development of HCC (71 events among all 500 patients) were calculated by using Cox proportional hazards regression analysis.



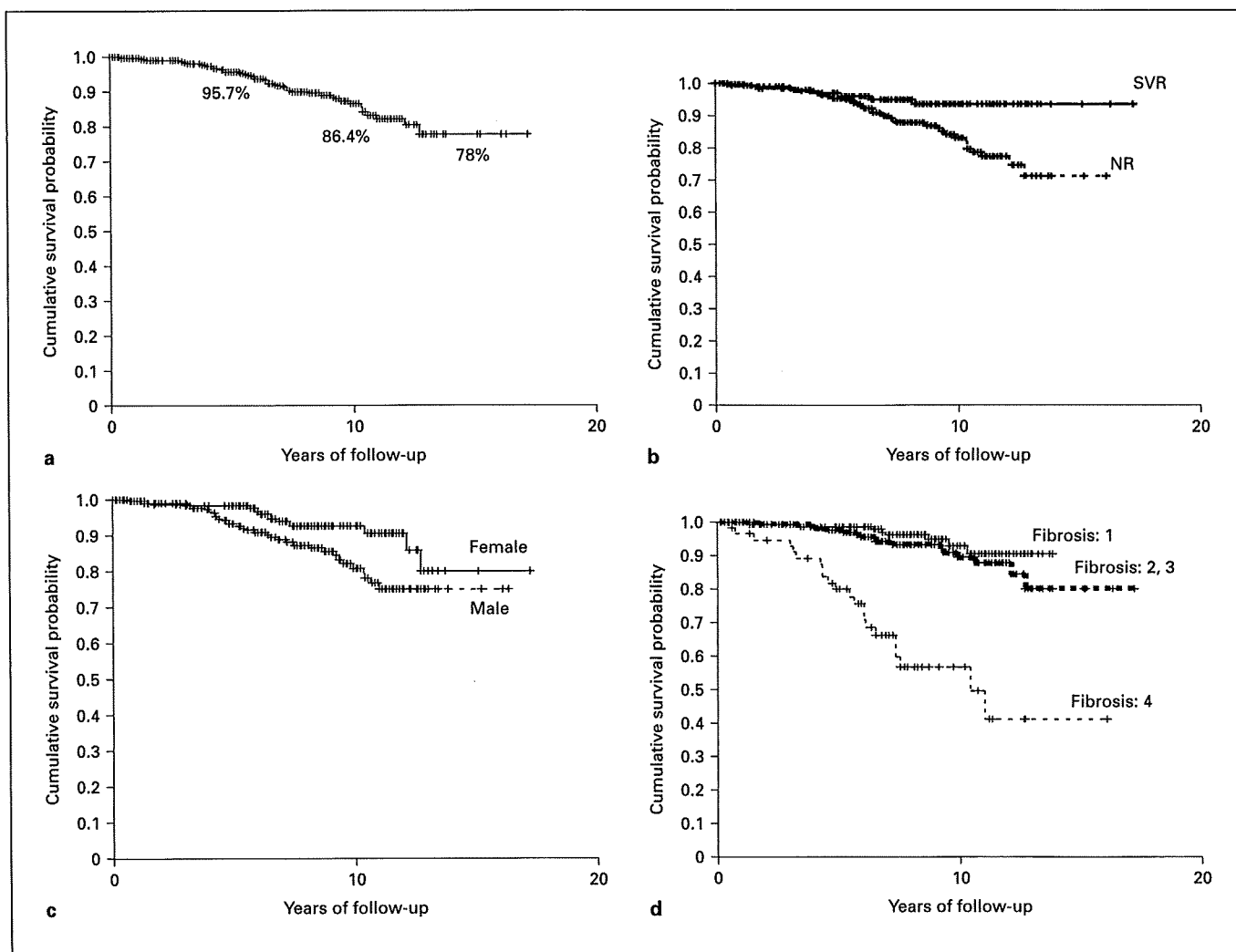


Fig. 2. Cumulative survival probability after IFN therapy. **a** In all patients; **b** based on difference of efficacy of IFN therapy; **c** based on difference of sex; **d** based on difference of efficacy of histological fibrosis. SVR = Sustained virological response; NR = non-response.

Table 3. Predictive factors for hepatocellular carcinoma appearance after IFN therapy by Cox proportional hazards model (multivariate analysis)

Factor	Category	Risk ratio	95% CI	p
Efficacy of IFN therapy	NR/SVR	1/0.193	0.083–0.45	<0.0001
Sex	Male/female	1/0.36	0.21–0.62	<0.0001
Liver histology (fibrosis)*	1/2,3,4	1/2.08	1.22–3.57	0.008

ALT = Alanine aminotransferase; CI = confidence interval; NR = non-response; SVR = sustained virological response.

* Histologic index score: 0–4 for fibrosis.

Cause of Death and Cumulative Survival Probability

Fifty-three of 500 patients died during an average follow-up of 7.4 years. The cumulative survival probability was 95.7% at the 5th year, 86.4% at the 10th year, and 78% at the 15th year (fig. 2). The number of liver-related and liver-unrelated deaths is shown in table 4. Liver-related death corresponded to 64.2% (34/53) of all deaths. HCC was the major cause of liver-related deaths. Univariate analysis showed that the following four factors significantly affected the cumulative survival probability in all patients as shown in table 5. Multivariate analysis revealed that patients achieved a significant survival when: (1) staging of liver fibrosis was 1 ($p < 0.0001$), (2) HCV was cleared ($p = 0.034$), and (3) sex was female ($p = 0.015$).

Discussion

In order to protect hepatocarcinogenesis, patients with chronic hepatitis C are often treated with antiviral drugs and/or anti-inflammatory drugs. Antiviral drugs, such as IFN, are given to eradicate HCV-RNA for chronic hepatitis C. Moreover, it has recently been reported that hepatitis C viral clearance or normalization of serum ALT after IFN therapy contribute to the notably suppressed incidence of HCC caused by chronic HCV infection [14–17]. Yoshida et al. [14] reported the efficacy of IFN for HCV-positive patients with chronic hepatitis or cirrhosis in a retrospective surveillance study in Japan, which focused on the HCC appearance rate and survival in a total of 2,890 patients, 2,400 treated with IFN and 490 not treated with IFN. Their conclusion was that IFN therapy improved survival of chronic hepatitis C patients by preventing liver-related deaths. Ikeda et al. [15] reported similar results from 1,643 patients of whom 1,191 had received IFN monotherapy. The incidence of HCC in treated patients was 7.6% after 10 years of follow-up evaluation, compared with 12.4% in untreated patients. Imazeki et al. [16] presented a follow-up study of 459 patients with chronic hepatitis C over 8.2 years to examine the survival rate relative to IFN therapy. Cirrhotic patients with a SVR showed a reduction in mortality (relative risk 0.219). Serfaty et al. [17] showed a beneficial effect of IFN on the development of HCC and on survival.

In the present study we found that the clearance of HCV after IFN therapy reduced HCC appearance and liver-related death in elderly patients (table 6). Horiike et al. [7] reported that elderly patients with a low HCV-RNA

Table 4. Cause of death

	Efficacy of IFN	
	SVR (n = 140)	NR (n = 360)
Deaths	9	44
Liver related	2 (22%)	32 (73%)
Hepatocellular carcinoma	2	26
Liver failure	0	5
Gastrointestinal bleeding	0	1
Liver unrelated	7 (78%)	12 (27%)
Malignancies	3	7
Heart disease	2	2
Cerebrovascular disease	2	1
Pulmonary disease	1	2

SVR = Sustained virological response; NR = non-response.

Table 5. Predictive factors for survival probability after IFN therapy by Cox proportional hazards model (univariate analysis)

Factor	Category	Risk ratio*	95% CI	p
Liver histology (fibrosis)	1/2,3/4	1/3.90	2.49–6.10	<0.0001
Sex	Male/female	1/0.47	0.26–0.86	0.014
Efficacy of IFN therapy	NR/SVR	1/0.37	0.17–0.83	0.015
Age, years	<65/≥65	1/2.08	1.14–3.78	0.016
HCV genotype	1/2	1/0.55	0.31–0.97	0.046
AST, IU/l	<76/≥76	1/0.80	0.37–1.74	0.571
ALT, IU/l	<100/≥100	1/0.81	0.37–1.76	0.588
HCV-RNA, kIU/ml	<100/≥100	1/0.92	0.49–1.71	0.781
Liver histology (activity)	<10/≥10	1/1.62	0.73–3.62	0.206

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; NR = non-response; SVR = sustained virological response.

* Risk ratio for development of HCC (71 events among all 500 patients) were calculated by using Cox proportional hazards regression analysis.

Table 6. Predictive factors for death after IFN therapy

Factors (category)	Overall deaths		Liver-related death		Liver-unrelated death	
	risk ratio* (95% CI)	p	risk ratio* (95% CI)	p	risk ratio* (95% CI)	p
Liver histology (fibrosis: 1/2,3,4)	1/3.55 (2.28–5.51)	<0.0001	1/6.18 (3.37–11.35)	<0.0001	1/1.10 (0.52–2.36)	0.799
Efficacy after IFN therapy (NR/SVR)	1/0.39 (0.16–0.93)	0.034	1/0.13 (0.03–0.59)	0.007	1/0.79 (0.27–2.32)	0.668
Male/female	1/0.46 (0.25–0.86)	0.015	1/0.40 (0.18–0.88)	0.022	1/0.71 (0.25–2.00)	0.799

CI = Confidence interval; IFN = interferon; NR = non-response; SVR = sustained virological response.

* Risk ratio for development of death (53 events among all 500 patients) were calculated by using Cox proportional hazards regression analysis.

level might benefit from IFN therapy, although they should decide the indications for IFN very carefully in this age group. Imai et al. [8] reported that some aged patients with chronic hepatitis C might be recommended IFN therapy.

Next, IFN therapy can be associated with various side effects and is costly, therefore the selection of aged patients for IFN therapy is extremely important. Factors predictive of a SVR to IFN have been extensively studied, i.e. short duration of disease, young age, absence of cirrhosis, low HCV-RNA levels and HCV genotype 2a [18]. Chronic hepatitis C patients with genotype 2a/2b or genotype 1b and lower virus load show a particularly good response to IFN therapy. Even if small amounts of IFN are given to patients with these factors, a biochemical response as well as a SVR can be expected. Therefore, when aged patients with chronic hepatitis C have various fac-

tors that would respond well to IFN treatment, IFN therapy can be recommended to aim for prolonged survival after screening for diseases other than chronic hepatitis C. On the other hand, when aged patients with chronic hepatitis C do not have factors that would respond well to IFN treatment, IFN therapy should not be recommended.

In conclusion, our results suggest that clearance of HCV after IFN therapy significantly reduces the risk of HCC appearance and death in aged chronic hepatitis C patients.

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