Table 5. Decrease of undiagnosed HCV and HBV carriers in the 15- to 69-year-old population in Japan

	Survey in 2000[a]		Survey in 2005		Difference	
	number estimated	carrier rate in area[b]	number estimated	carrier rate in area[b]	number estimated	balance
Shift of HCV carriers during 5 years from 2000 to 2005						
Hokkaido	41,139	0.99%	17,658	0.44%	−23,481	−57.1%
Tohoku	61,658	0.71%	30,525	0.37%	−31,133	−50.5%
Kanto	277,644	0.90%	126,283	0.41%	−151,361	−54.5%
Hokuriku/Tokai	88,724	0.64%	48,360	0.35%	−40,364	−45.5%
Kinki	178,871	1.06%	70,526	0.43%	−108,345	−60.6%
Chugoku	72,431	1.32%	24,595	0.47%	−47,836	−66.0%
Shikoku	43,497	1.49%	16,504	0.59%	−26,993	−62.1%
Kyushu	120,989	1.16%	64,115	0.63%	−56,874	−47.0%
Total	884,954	0.95%	398,567	0.44%	−486,387	−55.0%
Shift of HBV carriers during 5 years from 2000 to 2005						
Hokkaido	106,896	2.56%	54,557	1.35%	−52,339	−49.0%
Tohoku	104,923	1.21%	48,490	0.58%	−56,433	−53.8%
Kanto	255,207	0.83%	132,414	0.43%	−122,793	−48.1%
Hokuriku/Tokai	78,481	0.56%	51,477	0.37%	−27,004	−34.4%
Kinki	165,915	0.98%	85,083	0.52%	−80,832	−48.7%
Chugoku	90,041	1.64%	37,706	0.71%	−52,335	−58.1%
Shikoku	38,411	1.32%	19,162	0.69%	−19,249	−50.1%
Kyushu	127,879	1.23%	77,941	0.77%	−49,938	−39.1%
Total	967,753	1.04%	506,830	0.55%	−460,923	−47.6%

[a] Data for the year 2000 were extracted from a previous survey of hepatitis virus infections in Japan [5].
[b] The carrier rate specific for respective jurisdiction area was applied.

Discussion

There are many constraints in estimating total HCV and HBV infections in a given nation. Since it is not feasible to test every member for serological markers of hepatitis virus infection, populations representative of the entire nation have served for the estimation. Volunteer blood donors are recruited, but they have a restricted age range (16–64 years in Japan). Students attending schools and universities can close the opening in younger generations, but infants younger than the school age are not enrolled. Moreover, there are no means of estimating carrier rates of hepatitis virus infections in the individuals aged beyond the eligibility of blood donation. In addition, blood donors are selected individuals who are leading healthy lives above the average. In the survey of inhabitants in sentinel counties of the USA [6], who represent the average Americans, patients with liver disease and persons with restricted activities, such as those incarcerated or institutionalized, are not included.

Patients with clinical liver disease, as well as individuals found with HCV or HBV infection by health check-ups, can receive the medical care. However, many blood donors found with viral infections have developed severe liver disease already, and therefore, cannot receive efficient medical interventions [7, 8]. Hence, it is necessary to detect undiagnosed HCV and HBV infections hidden in the society. For this purpose, periodical health check-ups for screening hepatitis virus markers were started in April 2002 on the individuals, who turned 40, 45, 50, 55, 60 and 70 years, by a 5-year national project in Japan. The target age range (40–70 years) was selected due to a high incidence of hepatocellular carcinoma [9]. Since by far the majority of the first-time blood donors were younger than 40 years, the prevalence of HCV or HBV beyond that age dispersed widely (fig. 1, 4). In this study, therefore, the coverage by the first-time blood donors was confined to 20–39 years of age, and it was taken place by examinees of health check-ups aged 40–74 years; they left age groups ≤15 and ≥75 years uncovered, however.

The national prevalence of hepatitis virus infections in individuals ≤19 years was presumed to be similar to that in the Iwate prefecture situated in northern Japan. Since the prevalence of HCV or HBV infection in them was extremely low and stayed between 0.01 and 0.02%, such an assumption would not have affected the overall results to any significant extent. The prevalence of HCV in age groups ≥75 was simulated by a premise that it would be an exponential function of the age. Consequently, the formula based on profiles in five age groups from 50 to 74 years (at a 5-year notch) was extrapolated to three age groups ≥75 years. The simulation matched closely with the prevalence determined in corresponding age groups, with R^2 values ranging from 0.83 to 0.99 ($p < 0.05$ and $p < 0.01$, respectively) throughout 8 jurisdiction areas in Japan (fig. 3).

Japan has an axis spanning 2,000 kilometers from the north-east towards the south-west over the four major islands (Hokkaido, Honshu, Shikoku and Kyushu). Within a rather small land, the prevalence of HCV or HBV is not uniform all over Japan. The prevalence of HCV had an increasing gradient from north to south, and was the highest in Kyushu (table 2), while that of HBV was the highest in Hokkaido, decreased in between and then increased towards Kyushu (table 4). Reflecting such local differences, age-specific prevalence of HCV or HBV differed widely among 8 jurisdiction areas (fig. 2, 5).

Based on the results obtained on the area- and age-specific prevalence of HCV or HBV, carriers of these hepatitis viruses in 8 jurisdiction areas were tabulated separately over age groups from 20 to 74 years. Those in age groups ≤19 years were represented by the Iwate prefecture. The prevalence of HCV in age groups ≥75 years was simulated by the formula, and that of HBV was represented by individuals aged 70–74 years. Japan was populated by 127,767,994 people in 2005. Of these, 807,903 (95% CI 679,886–974,292) were estimated to have undiagnosed HCV infection at an overall prevalence of 0.63%, and 903,145 (837,189–969,572) to possess undiagnosed HBV infection at that of 0.71%. These estimates are much less than publically inferred numbers of HCV and HBV carriers in Japan at 1.5–2.0 million each. Leaving aside HCV and HBV carriers who have developed liver disease and stayed outside the scope of the present study, our estimates based on reasonable scientific grounds are much smaller; they add up barely half of generally referred figures around 1.5–2.0 million in Japan.

Based on the sex- and age-specific prevalence of hepatitis virus markers in the 3,478,422 first-time blood donors during 2001–2006, with the same criteria used in the previous study [5], we have estimated the number of undiagnosed HCV carriers aged 15–69 years in the year 2005 to be 398,567 (95% CI 295,410–501,453) and that of undiagnosed HBV carriers to be 506,830 (95% CI 398,115–616,113). In the previous study [5], undiagnosed HCV and HBV carriers aged 15–69 years in the year 2000 were assessed to be 884,954 (95% CI 725,082–1,044,826) and those with HBV to be 967,753 (95% CI 806,760–1,128,745). They decreased by 55.0 and 47.6%, respectively, during 5 years (table 5). In support of this view, the incidence of HCV or HBV infection during 10 years (1994–2000) in Japan is very low and estimated at 1.86 (95% CI 1.06–3.01) or 2.78 (1.87–4.145) per 100,000 person-years [10]. Decreases in undiagnosed HCV and HBV carriers in Japan would have been attributed to increased chances of receiving tests for hepatitis virus infections at health checkups and medical institutions, as well as increased awareness due to educational programs or other healthcare campaigns or screening programs in high-risk individuals. Additionally, there would have been a cohort effect in individuals aged 15–69 years who have shifted by 5 years during the observation period.

The results of the Third National Health and Nutrition Survey (HANES III, 1988–1994) [11] and those of more recent HANES (2001–2002) [6] in the USA are essentially similar with respect to age-specific profiles of HCV infection, and shifted by 10 years. The incidence of de novo HCV and HBV infections may have decreased substantially both in the USA and Japan, driven partly by the introduction of the nucleic acid amplification test and a more stringent questionnaire on donors to exclude blood donations in the window period of infection [12–17]. The national burden of HCV infection has been reported in Great Britain [18], where the prevalence of anti-HCV in hospitalized patients was 3.4% and that in the first-time blood donors was 0.03% in the year 2008.

In spite of many improvements in the control of hepatitis virus infections, there are many HCV and HBV carriers buried in the society who need immediate identification for receiving timely and efficient medical interventions. Treatment of viral hepatitis keeps improving, especially for liver disease induced by HCV. The sustained virological response in the patients infected with HCV of genotype 1, who have received triple therapy with pegylated interferon, ribavirin and protease inhibitors, has increased to 70% or higher, from 50% with the state-of-care therapy with pegylated interferon and ribavirin [19, 20]. With the advent of new antiviral drugs that will enter the scene in the foreseeable future, the virological response is expected to increase further. There would be

nothing like early detection of HCV and HBV infections for appropriate and timely medical care to prevent the progression of liver disease. Such a rational strategy will benefit not only patients themselves, but also merit the society and government, which are going to be burdened with ever-increasing morbidity and mortality along with skyrocketing costs.

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Interferon Alone or Combined with Ribavirin for Acute Prolonged Infection with Hepatitis C Virus in Chimpanzees

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Key Words
Chimpanzee · Hepatitis C virus · Interferon · Ribavirin

Abstract
Infection with hepatitis C virus (HCV) persisted for longer than 29 weeks in 2 chimpanzees after they had been inoculated with it experimentally. One of them (C-210) received short-term subcutaneous interferon-α (IFN-α) 6 million units (MU) daily for 7 days at week 29. He cleared HCV RNA from the serum and remained negative for it during 25 weeks after the withdrawal of IFN. The other (C-224) did not respond to 2 courses of a short-term IFN monotherapy at weeks 20 and 23. Twelve weeks thereafter, he received IFN-α 3 MU daily for 2 weeks and then 3 times a week for 14 weeks combined with oral ribavirin 600 mg daily during 16 weeks. HCV RNA disappeared from the serum and stayed negative until the last follow-up 24 weeks after the completion of combination therapy.

Due to a very narrow species-specificity of hepatitis C virus (HCV), chimpanzees remain the only animal that can be infected with it. Once they served as the sole means of identifying the infection with HCV that had been referred to as non-A, non-B hepatitis virus until its discovery in 1989 [1]. HCV infection can persist in chimps at rates ranging from 30 to 60%, depending on the age and gender as well as viral strains in inocula they have received [2, 3]; the persistence rate is comparable to that of 55–85% in humans [4, 5]. The long-term outcome of chimpanzees infected with HCV is not known, nor have there been any attempts to treat them with either interferon (IFN) alone or IFN in combination with ribavirin.

Two chimps with acute prolonged HCV infection received antiviral treatment. They were chimps No. 210 (male, 14 years old and weighing 62.8 kg) and No. 224 (male, 14 years old and weighing 59.1 kg). Both of them were kept in individual cages and received humane care, in accordance with all relevant requirements for the use of primates in an approved facility. Chimp No. 210 participated in the experimental transmission study for determining the minimum infectious dose of HCV [6]. He received 1 ml of fresh-frozen plasma from a donor in the window period of HCV infection with mixed genotypes (1b plus 2a) containing 7.0×10^6 copies/ml of HCV RNA. Chimp No. 224 was inoculated with 1 ml of fresh-frozen plasma from another donor in the window period of HCV infection with genotype 1b containing 8.4×10^6

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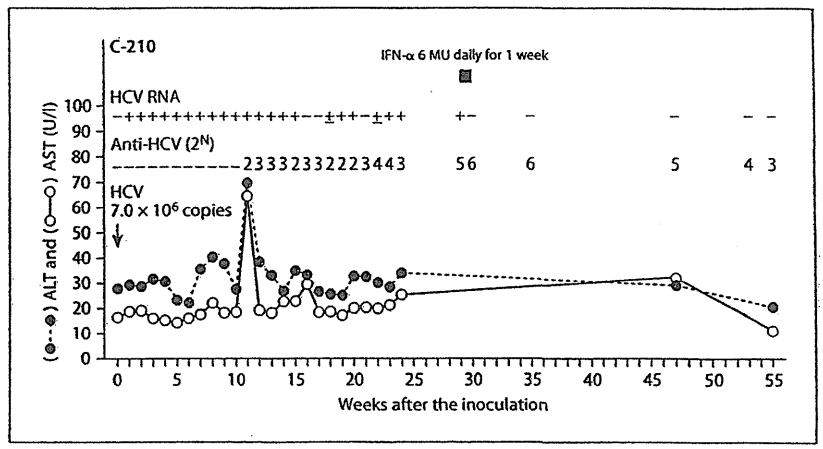

Fig. 1. Clinical course of chimpanzee No. 210 (C-210). The duration of IFN monotherapy is indicated at the top. HCV RNA was determined qualitatively by Amplinat MPX. Anti-HCV was determined by passive hemagglutination. Fluctuating levels of ALT and AST in the serum are shown below.

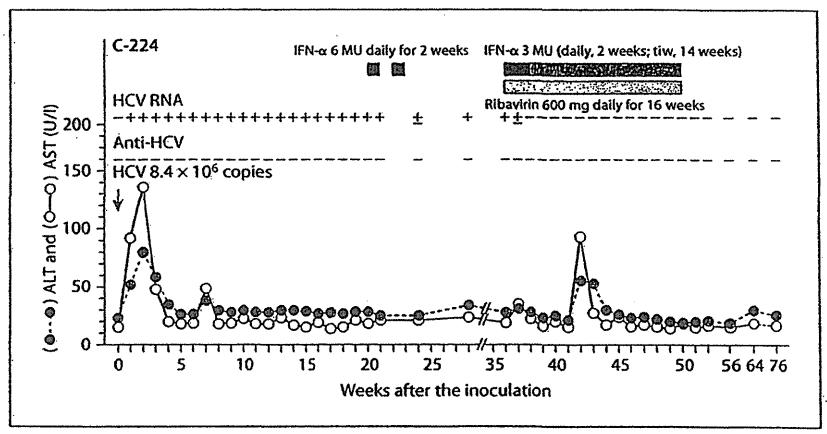

Fig. 2. Clinical course of chimpanzee No. 224 (C-224). The duration of 2 courses of IFN monotherapy (1 week each) as well as IFN (daily for 2 weeks and then 3 times a week for 14 weeks) combined with ribavirin (16 weeks) is indicated at the top.

copies/ml of HCV RNA; his preacute plasma has been included in the panel for standardization of polymerase chain reaction (PCR). They both received IFN therapy for evaluating the efficacy in treatment of acute prolonged HCV infection. The study design was approved by the Committee of Ethics for Handling of Primates in the institutions.

The 2 chimps were bled under anesthesia with ketamine hydrochloride weekly for the initial 21–24 weeks, and then at intervals until the completion of this study. HCV RNA was determined qualitatively by Amplinat MPX (Roche Diagnostics K.K., Tokyo, Japan). Antibody to HCV (anti-HCV) was determined semiquantitatively using a commercially available hemagglutination assay system (the second-generation HCV PHA, Abbott Japan, Co. Ltd., Tokyo, Japan), and the results were expressed by the highest twofold dilution of serum (2^N) that induced hemagglutination. They received IFN-α (Sumiferon®, Sumitomo Pharmaceutical Co. Ltd., Tokyo, Japan) alone or in combination with ribavirin.

Figure 1 illustrates the clinical course of chimp No. 210 who had been inoculated with 7.0×10^6 copies/ml of HCV of mixed genotypes (1b and 2a) and developed viremia during the first 24 weeks. He developed anti-HCV, 11 weeks after inoculation, along with sharp increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Anti-HCV increased, reached 2^6 hemagglutination titers and remained positive through the observation period of 55 weeks. Upon the confirmation of HCV RNA 29 weeks after inoculation, he received IFN-α 6 MU daily for 1 week. HCV RNA was not detectable by qualitative assay in his sera at the next week,

whereupon the IFN monotherapy was discontinued. He stayed negative for HCV RNA until the last observation 25 weeks after the withdrawal of IFN monotherapy.

Figure 2 depicts the clinical course of chimp No. 224 who was inoculated with 8.4×10^6 copies of HCV of genotype 1b. HCV RNA was detected in his serum at week 1. HCV RNA stayed positive through 20 weeks, and he was considered to have developed persistent infection. IFN-α 6 MU was given daily for 7 days since the 21st week. Because HCV RNA was positive at the next examination, IFN monotherapy was given again during the 23rd week.

However, HCV RNA did not disappear from the serum after 2 courses of IFN monotherapy. At 36 weeks when HCV RNA was confirmed to be present in the serum, he received a combination therapy with IFN-α 3 MU, daily for 2 weeks and then 3 times a week for 14 weeks, along with oral ribavirin 600 mg daily in 2 divided doses. HCV RNA decreased 1 week after the institution of combination therapy, and became undetectable the next week; the loss of HCV RNA continued throughout the following 15 weeks on treatment. He was confirmed negative for serum HCV RNA at tests performed 4, 12 and 24 weeks, respectively, after the completion of combined IFN and ribavirin. Transaminase levels increased moderately 6 weeks after the initiation of combination therapy, but thereafter they returned to normal through the observation till 24 weeks after the completion of therapy. Chimp No. 224 did not respond to HCV infection by raising anti-HCV, and remained seronegative throughout 76 weeks since he received inoculation.

The biggest problem with HCV infection in human beings is its strong propensity to persist in up to 85% of individuals who contract it, although chances of persistence depend on sex, age and route of transmission [4, 5]. We have reported that HCV replicates very rapidly in chimpanzees inoculated with it at a doubling time of 6.3–8.6 h [7]; it is much shorter than that of HBV estimated at 1.9–3.4 days [8]. Such a fast replication velocity of HCV might contribute toward a high persistence rate after the primary infection; cellular immune responses to clear HCV may not be able to catch up with exponentially increasing population and rapidly evolving HCV quasispecies.

The sustained virological response to pegyrated-IFN combined with ribavirin in patients with chronic hepatitis C remains insufficient; it is achieved in merely one half of the patients infected with HCV genotype 1 in a high viral load [9]. This stands in sharp contrast to the excellent efficacy of IFN on patients with acute prolonged hepatitis C [10]. Hence, we started treating 2 chimpanzees in whom acute infection with HCV had prolonged after they were experimentally transmitted with HCV [6, 7]. The preacute serum from one of them (chimp 210) served for illustrating the early dynamics of HCV infection, and provided blood centers with the standards of HCV RNA, containing defined copy numbers per milliliter, for calibrating nucleic acid amplification test (NAT).

Chimp 210 cleared HCV infection after he had received IFN-α 6 MU daily for 1 week (fig. 1). Chimp 224 failed to clear HCV after 2 courses of the IFN monotherapy. Thereafter, he responded to IFN 3 MU daily for 2 weeks followed by 3 times a week for 14 weeks in combination with oral ribavirin 600 mg daily. The virological response with loss of HCV RNA from the serum was achieved during treatment, and sustained 24 weeks after the completion of combination therapy (fig. 2). They both had kept HCV for 29 and 36 weeks before treatment, respectively, exceeding 6 months for the clinical definition of persistent infection. There remains a possibility, however, that chimp 210 may have been clearing HCV naturally without therapeutic intervention, in view of his remarkable response to a short-term IFN monotherapy. Chimp 210 was infected with HCV of genotype 1b and 2a, and chimp 224 with HCV of genotypes 1b. HCV of genotype 2a might have disappeared earlier than HCV of genotype 1b in chimp 210, in view of different sensitivity to IFN of these 2 HCV genotypes in clinical trials [11, 12].

We have shown that acute prolonged HCV infection can be cured in chimps if they receive IFN alone or combined with ribavirin soon enough after they have been infected, as in the treatment of acute hepatitis C in patients [10]. Hopefully, the efficacy of IFN with or without ribavirin would be extended in additional chimps with acute prolonged HCV infection after they have completed transmission studies. Furthermore, such treatments would need to be considered in many chimps who have acquired persistent HCV infection after experimental transmission during the long past.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Predictive value of tumor markers for hepatocarcinogenesis in patients with hepatitis C virus

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Abstract
Background Increases in tumor markers are sometimes seen in patients with chronic liver disease without hepatocellular carcinoma (HCC). The aim of this study was to determine the relationship between the levels of three tumor markers [alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des-γ-carboxy prothrombin (DCP)] and hepatic carcinogenesis to identify hepatitis C virus (HCV) carriers at high risk for cancer development.
Methods A total of 623 consecutive HCV carriers with follow-up periods of >3 years were included. The average integration values were calculated from biochemical tests, and tumor markers, including AFP, AFP-L3%, and DCP, and factors associated with the cumulative incidence of HCC were analyzed.
Results HCC developed in 120 (19.3%) of the 623 patients. Age >65 years [adjusted relative risk, 2.303 (95% confidence interval, 1.551–3.418), $P < 0.001$], low platelet count [3.086 (1.997–4.768), $P < 0.001$], high aspartate aminotransferase value [3.001 (1.373–6.562), $P < 0.001$], high AFP level [≥10, <20 ng/mL: 2.814 (1.686–4.697), $P < 0.001$; ≥20 ng/mL: 3.405 (2.087–5.557), $P < 0.001$] compared to <10 ng/mL, and high AFP-L3% level [≥5, <10%: 2.494 (1.291–4.816), $P = 0.007$; ≥10%: 3.555 (1.609–7.858), $P < 0.001$] compared to <5% were significantly associated with an increased incidence of HCC on multivariate analysis.
Conclusions Increased AFP or AFP-L3% levels were significantly associated with an increased incidence of HCC. Among HCV carriers, patients with ≥10 ng/mL AFP or patients with ≥5% AFP-L3% are at very high risk for the development of HCC even if AFP is less than 20 ng/mL or AFP-L3% is less than 10%, which are the most commonly reported cutoff values.

Keywords Alpha-fetoprotein (AFP) · *Lens culinaris* agglutinin-reactive fraction of AFP · Hepatic regeneration · Necroinflammatory activity · Hepatocarcinogenesis

Introduction

Serum alpha-fetoprotein (AFP) is a widely used marker for hepatocellular carcinoma (HCC) [1]. However, serum AFP levels are increased in patients with liver diseases other than HCC, including viral hepatitis [2–4], with a prevalence of 10–42% [2, 5–7]. Increases in AFP are a marker of hepatic regeneration following hepatocyte destruction in viral hepatitis [8]. However, the pathogenesis and clinical significance of this phenomenon remain unclear.

The *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%) and des-γ-carboxy prothrombin (DCP) are also markers for HCC [9–12]. Available data suggest that these tumor markers are more highly specific for HCC than AFP alone [9]. However, there are no reports examining the prognostic value of these markers in hepatocarcinogenesis.

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Results of biochemical tests, including tumor markers, can fluctuate for a given patient and can vary between different patients, and repeated measurements over time may provide a more accurate picture of disease development or progression. The arithmetic mean value is often used to assess biochemical parameters over time, but this value can be greatly affected by the interval between measurements such that a short period of very high values can inappropriately skew the mean. We have previously argued that the average integration value is more meaningful than the arithmetic mean value for the purposes of monitoring disease progression [13, 14].

The aim of this study was to determine the relationship between three tumor markers (AFP, AFP-L3%, and DCP) to better identify hepatitis C virus (HCV) carriers at high risk for the development of HCC. Of note, we used the average integration values of these parameters in our analysis.

Patients, materials, and methods

Patient selection

A total of 1623 consecutive patients positive for anti-HCV antibody visiting the Department of Gastroenterology at Ogaki Municipal Hospital during the period January 1995 to December 1997 were considered for enrollment. The present study cohort included the following criteria for enrollment: (1) positive for anti-HCV antibody by second- or third-generation enzyme-linked immunosorbent assay and detectable HCV RNA for at least 6 months; (2) no evidence of positivity for hepatitis B surface antigen; (3) exclusion of other causes of chronic liver disease (i.e., alcohol consumption lower than 80 g/day, no history of hepatotoxic drug use, and negative tests for autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); (4) follow-up period greater than 3 years; (5) measurement of AFP, AFP-L3%, and DCP at least every 6 months; (6) no evidence of HCC for at least 3 years from the start of the observation periods; and (7) interferon (IFN) therapy completed greater than 3 years before the detection of HCC in patients who received IFN therapy. A total of 623 patients fulfilled these criteria (Fig. 1).

Fibrosis was histologically evaluated in 187 of the 623 patients and staged according to Desmet et al. [15] as follows: F0, no fibrosis; F1, mild fibrosis; F2, moderate fibrosis; F3, severe fibrosis; and F4, cirrhosis. The remaining 436 patients were evaluated by ultrasound (US) findings and biochemical tests. The diagnosis of cirrhosis was made according to typical US findings, e.g., superficial nodularity, a coarse parenchymal echo pattern, and signs of portal hypertension (splenomegaly >120 mm, dilated portal vein diameter >12 mm, patent collateral veins, or ascites) [16–18]. In this study patients who did not satisfy these criteria were classified as having chronic hepatitis. Four hundred and sixty-three patients were diagnosed with chronic hepatitis and 160 patients with cirrhosis.

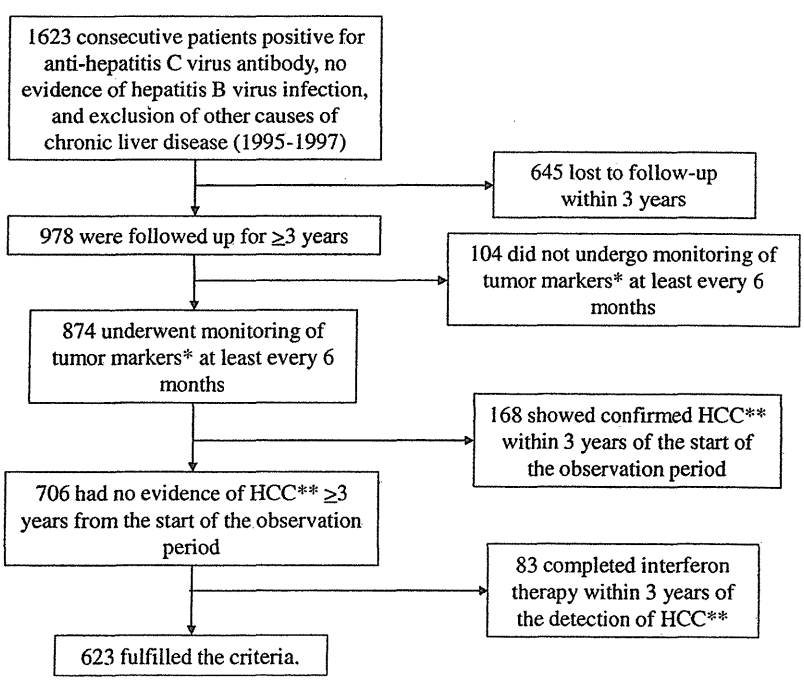

Fig. 1 Schematic flowchart of enrolled patients. *Serum alpha-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%), and des-γ-carboxy prothrombin (DCP). **Hepatocellular carcinoma (*HCC*)

All patients were followed up at our hospital at least twice a year. During each follow-up examination, platelet count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (γ-GTP), total bilirubin, cholinesterase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin, total cholesterol, AFP, AFP-L3%, and DCP were measured. Platelet count and ALT, AST, γ-GTP, total bilirubin, cholinesterase, ALP, LDH, albumin, total cholesterol, AFP, AFP-L3%, and DCP values were expressed as average integration values [13, 14]. Briefly, using ALT as an example, the area of a trapezoid is calculated by multiplying the sum of two ALT values by one-half of the interval between the measurements. This value is then divided by the observation period to obtain the average integration value, and this technique provides a better representation of values over time when there are extremes of high and low values [14, 16]. In patients who developed HCC during the observation period, AFP, AFP-L3%, and DCP values obtained at least 1 year before the diagnosis of HCC were assessed. Serum AFP concentration was determined with a commercially available kit. AFP-L3% was measured by lectin-affinity electrophoresis and antibody-affinity blotting with the AFP Differentiation Kit L (Wako Pure Chemical Industries, Osaka, Japan) [10]. DCP was measured with a DCP reagent (Picolumi PIVKA-II; Eizai, Tokyo, Japan) [11]. Cutoff levels for AFP, AFP-L3%, and DCP were set at 20 ng/mL, 10%, and 40 mAU/mL, respectively, according to previous reports [10–12]. HCV genotype and quantification of HCV RNA (Amplicor 2; Roche Diagnostics, Tokyo, Japan) were determined in 513 cases. All patients underwent imaging modalities (US, computed tomography [CT], or magnetic resonance imaging [MRI]), every 3 months in patients with cirrhosis and every 6 months in patients with chronic hepatitis.

The diagnoses of HCC were confirmed by histologic examination of resected hepatic tumors or US-guided needle biopsy specimens. When biopsy of the tumor was contraindicated, the HCC diagnosis was made using clinical criteria and imaging findings obtained from B-mode US, CT angiography, or MRI [19, 20]. HCC was histologically diagnosed in 46 patients, and in the remaining 74 patients, the diagnosis was made based on clinical criteria [19, 20]. All tumors were 3 cm or less in maximum diameter, and there were 3 nodules or less on diagnosis.

One hundred eighty-nine patients received IFN therapy. Patients were classified into three groups according to the type of response to IFN therapy: sustained virologic response (SVR), defined as the absence of serum HCV RNA at 6 months after IFN therapy; the non-SVR group, defined as the presence of serum HCV RNA at 6 months after IFN therapy; and the no IFN therapy group.

Patients were classified into three groups for each of the tumor markers according to the average integration values of AFP, AFP-L3%, and DCP: A1, <10 ng/mL ($n = 452$); A2, ≥10, <20 ng/mL ($n = 80$); and A3, ≥20 ng/L ($n = 91$); L1, <5% ($n = 588$); L2, ≥5, <10% ($n = 18$); and L3, ≥10% ($n = 17$); and D1, <20 mAU/mL ($n = 379$); D2, ≥20, <40 mAU/mL ($n = 170$); and D3, ≥40 mAU/mL ($n = 51$), respectively.

The present study ended on 31 December 2008 or the date of identification of HCC occurrence. The median follow-up period was 9.0 years (range 3.0–13.0 years). The total number of blood examinations was 25.721, and the median number of blood examinations was 23 (range 6–105) per subject.

Statistical analysis

Statistical analysis was performed with the Statistical Program for Social Science (SPSS ver.17.0 for Windows; SPSS Japan, Tokyo, Japan). Continuous variables are shown as medians (ranges). The Mann–Whitney *U*-test was used for continuous variables, and Fisher's exact test was used for categorical variables. Actuarial analysis of the cumulative incidence of hepatocarcinogenesis was performed by the Kaplan–Meier method, and differences were tested by the log-rank test. The Bonferroni correction was performed for multiple comparisons. The Cox proportional hazards model and forward selection method were used to estimate the relative risk of HCC development associated with age (≤65 or >65 years), sex (female or male), body mass index (BMI ≤25.0 or >25.0 kg/m^2), HCV genotype (type 1 or type 2), viral concentration (≤100 or >100 KIU/mL), platelet count (<12.0 × 10^4/mm^3 or ≥12.0 × 10^4/mm^3), ALT (≤35 or >35 IU/mL), AST (≤40 or >40 IU/mL), total bilirubin (≤1.2 or >1.2 mg/dL), γ-GTP (≤56 or >56 IU/mL), ALP (≤338 or >338 IU/mL), cholinesterase (<431 or ≥431 IU/mL), LDH (≤250 or >250 IU/mL), albumin (<3.5 or ≥3.5 g/dL), total cholesterol (<130 or ≥130 mg/dL), cirrhosis (presence or absence), and IFN treatment (no therapy, non-SVR, or SVR) for univariate and multivariate analyses. We used the lower or upper limit of the reference values at our institute as cutoff values for platelet count, ALT, AST, total bilirubin, γ-GTP, ALP, cholinesterase, LDH, albumin, and total cholesterol levels. Statistical significance was set at $P < 0.05$.

The study protocol was approved by the Ethics Committee at Ogaki Municipal Hospital in January 2009 and the study was performed in compliance with the Helsinki Declaration. Informed consent was obtained from each patient for analyzing patient records and images.

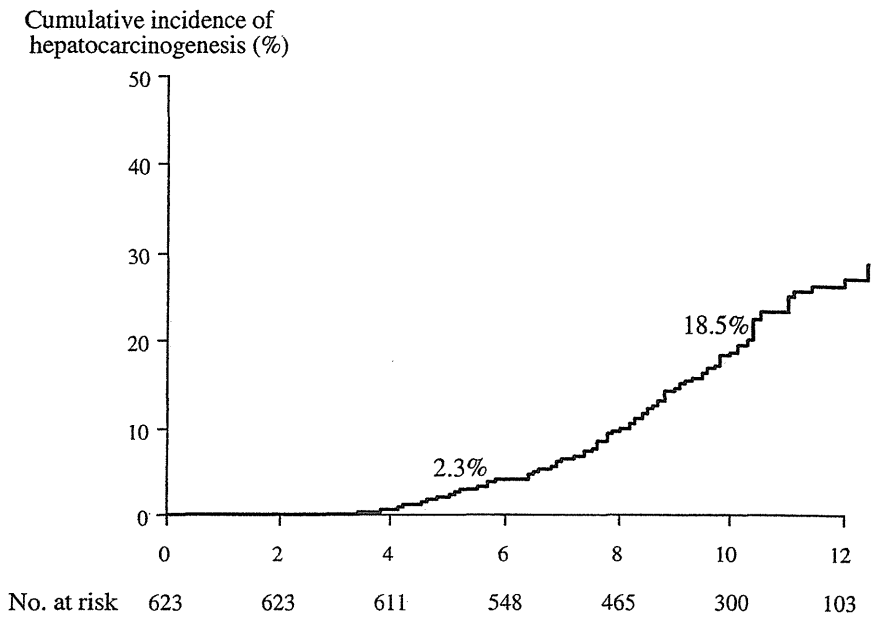

Fig. 2 Overall cumulative incidence rate of HCC

Table 1 Patient characteristics

Age (years)	61 (26–84)
Sex (F/M)	265/358
BMI (kg/m^2)	22.5 (12.0–34.9)
HCV genotype (type 1/type 2)	356/157
Viral concentration (KIU/mL)	270 (0.5–6300)
AFP (ng/mL)	4.8 (0.8–341.5)
AFP-L3 (%)	0.1 (0.0–32.5)
DCP (mAU/mL)	18.1 (8.5–99.6)
Platelets ($\times 10^4$/mm^3)	14.8 (3.0–33.9)
ALT (IU/L)	46.4 (10.1–340.4)
AST (IU/L)	48.5 (13.3–168.9)
γ-GTP (IU/L)	37.6 (9.9–2207)
Total bilirubin (mg/dL)	0.6 (0.2–2.7)
ALP (IU/L)	276.4 (86.8–845.5)
Cholinesterase (IU/L)	242.9 (38.8–545.30)
LDH (IU/L)	196.4 (118.4–650.1)
Albumin (g/dL)	4.0 (2.4–4.9)
Total cholesterol (mg/dL)	155.8 (77.9–264.1)
Fibrosis (F0/F1/F2/F3/F4)[a]	32/73/56/24/2
Cirrhosis (present/absent)	160/463
IFN therapy (none/non-SVR/SVR)	434/146/43

Continuous variables are quoted as medians (ranges)

BMI body mass index, *HCV* hepatitis C virus, *AFP* alpha-fetoprotein, *AFP-L3Lens culinaris* agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GTP* gamma glutamyl transpeptidase, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase, *IFN* interferon, *SVR* sustained virologic response

[a] Staging of chronic hepatitis according to Desmet et al. [15]

Results

HCC developed in 120 (19.3%) of the 623 patients. The 5- and 10-year cumulative incidences of HCC were 2.3 and 18.5%, respectively (Fig. 2). Demographic and medical data for the 623 patients are summarized in Table 1.

Factors associated with the incidence of hepatic carcinogenesis on univariate analysis

Factors associated with the incidence of HCC are listed in Table 2. Age ≥65 years, high AFP level, high AFP-L3% level, high DCP level, low platelet count, high ALT level, high AST level, high LDH level, high ALP level, low cholinesterase level, low albumin level, presence of cirrhosis, and response to IFN therapy were significantly associated with the development of HCC on univariate analysis.

The 5-, 7-, and 10-year cumulative incidences of HCC were 1.1, 2.1, and 7.5% in group A1; 2.6, 9.6, and 42.1% in group A2; and 6.6, 18.3, and 50.0% in group A3, respectively, and the cumulative incidence of HCC differed significantly between groups A1 and A2 and groups A1 and A3 (Fig. 3). The 5-, 7-, and 10-year cumulative incidences of HCC were 1.4, 4.6, and 15.6% in group L1; 19.6, 39.7, and 73.6% in group L2; and 12.5, 25.0, and 56.7% in group L3, respectively, and the cumulative incidence of HCC differed significantly between groups L1 and L2 and groups L1 and L3 (Fig. 4). The 5-, 7-, and 10-year cumulative incidences of HCC were 0.5, 4.6, and

Table 2 Factors associated with hepatocarcinogenesis (univariate analysis)

	Crude hazard ratio (95% CI)	*P*
Age (years)		
≤65	1	
>65	2.318 (1.580–3.400)	<0.001
AFP (ng/mL)		
A1; <10	1	
A2; ≥10, <20	6.061 (3.768–9.750)	<0.001
A3; ≥20	8.985 (5.874–13.744)	<0.001
AFP-L3 (%)		
L1; <5	1	
L2; ≥5, <10	8.032 (4.388–14.700)	<0.001
L3; ≥10	3.781 (1.838–7.778)	<0.001
DCP (mAU/mL)		
D1; <20	1	
D2; ≥20, <40	1.209 (0.788–1.855)	0.385
D3; ≥40	4.535 (2.840–7.241)	<0.001
Platelets ($\times 10^4$/mm^3)		
≥12.0	1	
<12.0	5.887 (3.982–8.702)	<0.001
ALT (IU/L)		
≤35	1	
>35	2.632 (1.574–4.400)	<0.001
AST (IU/L)		
≤40	1	
>40	8.120 (4.115–16.024)	<0.001
LDH (IU/L)		
≤250	1	
>250	1.970 (1.249–3.106)	<0.001
ALP (IU/L)		
≤338	1	
>338	2.509 (1.724–3.650)	<0.001
Cholinesterase (IU/L)		
>431	1	
≤431	3.288 (2.209–4.893)	<0.001
Albumin (g/dL)		
≥3.5	1	
<3.5	3.948 (2.635–5.917)	<0.001
Cirrhosis		
Absent	1	
Present	3.474 (2.413–5.002)	<0.001
IFN therapy		
No therapy	1	
Non-SVR	0.312 (0.180–0.539)	<0.001
SVR	0.215 (0.075–0.620)	0.004

Continuous variables are quoted as medians (ranges)

CI confidence interval, *AFP* alpha-fetoprotein, *AFP-L3Lens culinaris* agglutinin-reactive fraction of AFP, *DCP* des-γ-carboxy prothrombin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *LDH* lactate dehydrogenase, *ALP* alkaline phosphatase, *IFN* interferon, *SVR* sustained virologic response

14.8% in group D1; 1.8, 4.3, and 16.3% in group D2; and 10.0, 25.0, and 48.2% in group D3, respectively, and the cumulative incidence of HCC differed significantly between groups D1 and D3 and groups D2 and D3 (Fig. 5).

Factors associated with the incidence of hepatic carcinogenesis on multivariate analysis

Factors associated with the incidence of HCC as analyzed by the Cox proportional hazards model and the forward selection method are listed in Table 3. Age >65 years, low platelet count, high AST level, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC. Factors associated with the incidence of HCC were analyzed in patients with chronic hepatitis and cirrhosis (Table 4). High age, low platelet count, high AST level, and high AFP level were significantly associated with the incidence of HCC in chronic hepatitis, and male sex, high age, low platelet count, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC in cirrhosis. Factors associated with the incidence of HCC were analyzed in patients with and without IFN treatment (Table 5). Male sex, low platelet count, low cholinesterase level, and high AFP level were significantly associated with the incidence of HCC in patients with IFN therapy and male sex, high age, low platelet count, high AFP level, and high AFP-L3% level were significantly associated with the incidence of HCC in patients without IFN therapy.

Discussion

Advances in US, CT, and MRI have allowed for the more frequent and earlier detection of small HCC tumors less than 2 cm in diameter during the routine follow-up of patients with chronic liver disease [21–23]. However, the performance and resolution of the imaging device, the skills of individual operators, and the diagnostic acumen of the interpreting radiologist all affect the early detection of HCC. AFP, AFP-L3%, and DCP levels have been used as prognostic markers rather than diagnostic markers for HCC [9]. However, the detection rate of small HCC tumors with these markers is low; AFP-L3% and DCP have low sensitivity, and AFP has low specificity. Sassa et al. [12] reported detection rates of 22.6 and 48.4% for AFP-L3% and DCP, respectively, in patients with small HCC tumors. It is currently thought that serum markers are useful for follow-up after HCC therapy in patients with high tumor marker levels before treatment [24].

We have previously reported that the average integration value of ALT correlates with the cumulative incidence of hepatocarcinogenesis, even within the normal range [13, 14]. In the present study, the average integration value of AFP was not selected as a factor associated with the

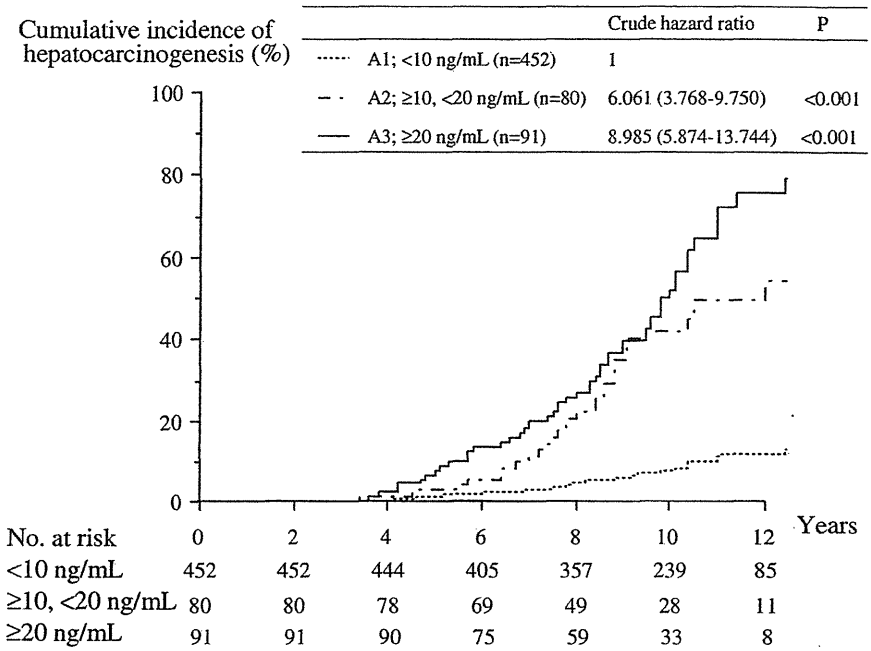

Fig. 3 Incidence of HCC according to the average integration value of AFP. The cumulative incidence of HCC differed significantly between groups A1 (<10 ng/mL) and A2 (≥10, <20 ng/mL) and groups A1 and A3 (≥20 ng/L)

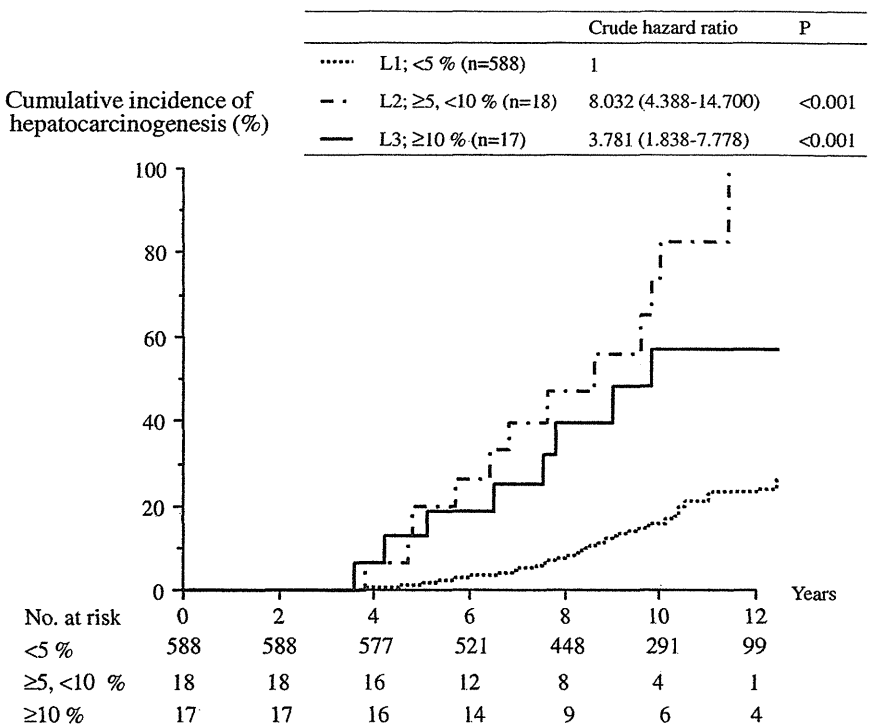

Fig. 4 Incidence of HCC according to the average integration value of AFP-L3%. The cumulative incidence of HCC differed significantly between groups L1 (<5%) and L2 (≥5, <10%) and groups L1 and L3 (≥10%)

incidence of HCC on multivariate analysis. AFP production is thought to be increased in response to injury, possibly due to increased hepatocyte turnover, in patients with HCV who do not have HCC [25]. In contrast, increased ALT levels are correlated with hepatocellular necrosis but not with hepatocyte proliferation. This difference may at least partially explain the absence of correlation between ALT and AFP levels.

The multivariate analysis in our series was carried out to minimize the influence of confounding factors, and 5 factors were selected by the forward selection method. Age >65 years, low platelet count, high AST value, high AFP

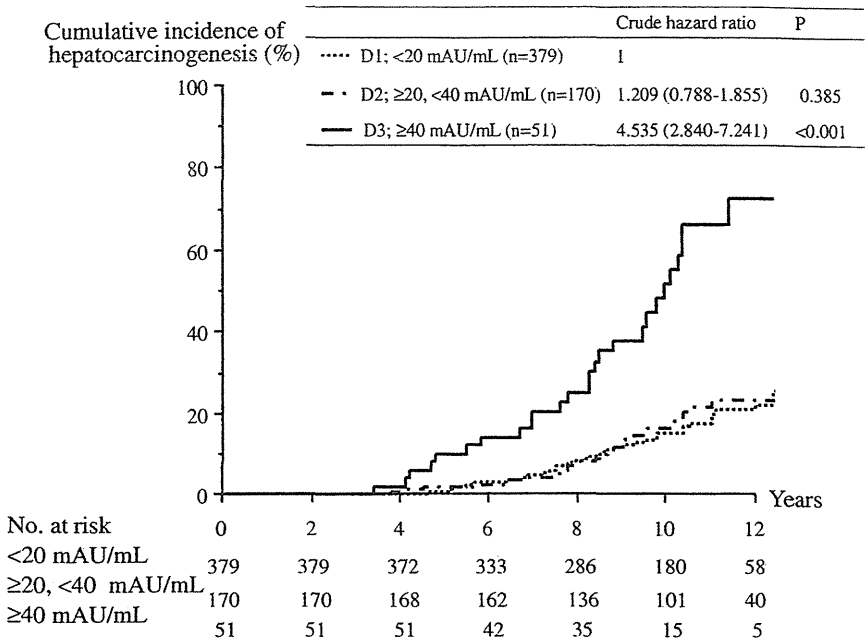

Fig. 5 Incidence of HCC according to the average integration value of DCP. The cumulative incidence of HCC differed significantly between groups D1 (<20 mAU/mL) and D3 (≥40 mAU/mL) and groups D2 (≥20, <40 mAU/mL) and D3

Table 3 Factors associated with hepatocarcinogenesis (multivariate analysis)

	Adjusted hazard ratio (95% CI)	*P*
Age (years)		
≤65	1	
>65	2.303 (1.551–3.418)	<0.001
Platelets ($\times 10^4$/mm^3)		
≥12.0	1	
<12.0	3.086 (1.997–4.768)	<0.001
AST (IU/L)		
≤40	1	
>40	3.001 (1.373–6.562)	0.006
AFP (ng/mL)		
A1; <10	1	
A2; ≥10, <20	2.814 (1.686–4.697)	<0.001
A3; ≥20	3.405 (2.087–5.557)	<0.001
AFP-L3 (%)		
L1; <5	1	
L2; ≥5, <10	2.494 (1.291–4.816)	0.007
L3; ≥10	3.555 (1.609–7.858)	0.002

AST aspartate aminotransferase, *AFP* alpha-fetoprotein, *AFP-L3 Lens culinaris* agglutinin-reactive fraction of AFP

level, and high AFP-L3% level were significantly associated with hepatic carcinogenesis in our multivariate analysis, but serum ALT level was not a risk factor for developing HCC. Ikeda et al. [26] reported that the cumulative incidence of HCC increased significantly in cirrhotic patients with an AFP level ≥10 ng/mL compared to those with an AFP level <10 ng/mL, and the adjusted risk ratio was 15.788 in HCV patients. They speculated that AFP is a marker of disease activity or severity and cellular regeneration, and it acts as a better predictor of HCC with viral etiology of cirrhosis. As an index of hepatic regeneration, the AFP level better represents the risk of hepatic carcinogenesis than an index of liver injury (e.g., ALT level). In addition to AFP, AFP-L3% was identified as a factor predicting the development of HCC, and this is a specific marker for the existence of HCC. Therefore, elevations in AFP-L3% may reflect an occult cancer that is undetectable with current imaging modalities. More intensive surveillance is needed for patients such as those who fulfill the criteria of groups L2 and L3 in our series, although these groups were very small in size. However, similar to other laboratory values, as high AFP-L3% values may be associated with severe liver damage, it is necessary to interpret these values carefully. DCP is well known to be also a specific marker of HCC. DCP is more closely related to tumor size than AFP and AFP-L3% [27]. Therefore, it is thought that these were the reasons that DCP was not selected as a predictive marker for HCC in our multivariate analysis.

Among the other risk factors we identified for the development of HCC, a low platelet count stands out. The platelet count is a useful marker for the diagnosis of cirrhosis [28], and cirrhosis is an established risk factor for HCC in HCV carriers [26, 28–30]. Taken together with our other findings, the low platelet count suggests that HCC develops in patients with progressive or advanced liver disease. We additionally used ultrasound (US) to distinguish cirrhotic patients from non-cirrhotic patients [16–18]. The presence of cirrhosis on US was strongly associated with an increased

Table 4 Factors associated with hepatocarcinogenesis on multivariate analysis in patients with chronic hepatitis and cirrhosis

	Chronic hepatitis ($n = 463$)	Cirrhosis ($n = 160$)
Age (years): ≤65 vs. >65	<0.001	0.008
Gender: female vs. male		<0.001
Platelets ($\times 10^4/mm^3$): ≥12.0 vs. <12	0.001	0.007
AST (IU/L): ≤40 vs. >40	0.043	
AFP (ng/mL): <10 vs. ≥10, <20 vs. ≥20	<0.001	0.003
AFP-L3 (%): <5 vs. ≥5, <10 vs. ≥10		0.017

AST aspartate aminotransferase, *AFP* alpha-fetoprotein, *AFP-L3 Lens culinaris* agglutinin-reactive fraction of AFP

Table 5 Factors associated with hepatocarcinogenesis on multivariate analysis in patients with and without IFN treatment

	With IFN ($n = 189$)	Without IFN ($n = 434$)
Age (years): ≤65 vs. >65		0.001
Gender: female vs. male	0.005	<0.001
Platelets ($\times 10^4/mm^3$): ≥12.0 vs. <12.0	0.047	<0.001
Cholinesterase (IU/L): ≥431 vs. <431	0.007	
AFP (ng/mL): <10 vs. ≥10, <20 vs. ≥20	<0.001	<0.001
AFP-L3 (%): <5 vs. ≥5, <10 vs. ≥10		<0.001

IFN interferon, *AFP* alpha-fetoprotein, *AFP-L3 Lens culinaris* agglutinin-reactive fraction of AFP

incidence of HCC on univariate analysis, but US-determined cirrhosis was not identified as a risk factor on multivariate analysis. Histologic assessment of fibrosis and cirrhosis was obtained in only 187 patients (30.0%), and patients with F4 fibrosis had a higher incidence of HCC in our univariate analysis. However, the population of patients with material available for histologic review was only one-third the size of the entire study population, and this small number may have negatively affected our ability to detect the predictive nature of fibrosis at all levels of severity. In contrast to serum ALT, serum AST levels were significantly associated with the incidence of HCC. AST levels are often abnormal in patients with cirrhosis when ALT values are in the normal range, and the AST/ALT ratio is frequently greater than 1 in cirrhotic patients [31]. Elevated AST activity is a surrogate marker for cirrhosis. Aging is associated with a number of events at the molecular, cellular, and physiological levels that influence carcinogenesis and subsequent cancer growth [32]. It has been hypothesized that an age-associated decrease in DNA repair [33] contributes to the development of HCC.

Recent reports have shown that AFP levels fall following the administration of IFN with or without ribavirin [34, 35]. IFN has been shown to have antiviral, anti-inflammatory, and anticancer activities [36]. One study demonstrated an anticancer effect of IFN when this agent was given following intrahepatic recurrence after HCC resection [37], and in our study, previous treatment with IFN was a factor associated with a reduced incidence of HCC on univariate analysis. The median ages of our patients with and without IFN treatment were 53 years (range 28–71) and 65 years (range 26–84), respectively; the age in those receiving IFN was significantly lower than the age in the group without IFN ($P < 0.0001$). It is thought that age and IFN therapy are confounding factors because IFN therapy has better results in younger patients. Although IFN was not identified as a predictive factor on multivariate analysis, the possibility cannot be denied that IFN may play an important role in modulating AFP levels prior to the onset of HCC.

In conclusion, increased AFP or AFP-L3% levels were significantly associated with an increased incidence of HCC. Among HCV carriers, patients with ≥10 ng/mL AFP or patients with ≥5% AFP-L3% are at very high risk for the development of HCC even if AFP is less than 20 ng/mL or AFP-L3% is less than 10%, which are the most commonly reported cutoff values. Intensive imaging modalities including US, CT, and MRI are recommended every 3–6 months for these patients.

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Conflict of interest There is no conflict of interest to disclose.

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プライマリケア・マスターコース

見逃さないで! あなたも診ている心の病気—PTSD

もう困らない! 患者指導—虚血性心疾患編

小児科診療のすすめ—新しい予防接種

質疑応答

C型肝炎ウイルスキャリアの慢性肝炎発症率

双極性障害のガイドラインと薬物療法

NEWS

INTERVIEW 医学部新設構想で目黒泰一郎氏に聞く

質疑応答

questions and answers

要項

- ●質問は「質疑応答係」宛に
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内科

Q C型肝炎ウイルスキャリアの慢性肝炎発症率

C型肝炎ウイルスの無症候性キャリアが慢性C型肝炎を発症する率は，どの程度か．

（京都府　N）

A 観察を続けると高率に慢性肝炎へ進展していく．定期的なフォローアップを心がける

肝臓は「沈黙の臓器」と言われ，肝炎ウイルスに感染していても自覚症状が乏しいため，感染していることが分かった時点ですでに肝疾患が進んだ状態であることも多い[1]．ASTやALTが正常でも，実際には肝組織での炎症がすでに起こっており，肝臓の線維化を認めることがある．そのため，一般的な血液検査が正常であるだけで簡単に無症候性キャリア（asymptomatic carrier；ASC）とすることは，実際の肝疾患の状態と異なり，予後の判断を誤ることがある．

献血を契機にC型肝炎ウイルスに感染していることが判明した供血者（自覚症状はない）を対象に行った前向き研究では，献血後の初診時にすでに52％が慢性肝炎の状態と診断された[1]．特に男性では，慢性肝炎を指摘された者が62.6％と女性に比べ有意に多く，性差が認められた．

ご質問の「無症候性キャリア」を，ここでは一般的に解釈して，血液検査や腹部超音波検査など侵襲性の少ない検査で特に異常が認められない者とする．

筆者らは，インターフェロン（IFN）治療を受けていないC型肝炎ウイルス持続感染者の1年ごとの病態推移を集計し，Markovモデルを用いて自然経過での肝疾患の進行を予測した（図1）[2]．各病態からの年間移行率を求めると，男性の40代ASCの14.3％が1年間で慢性肝炎に移行する．慢性肝炎は年率1.1％が肝硬変に移行する．すると表1に示すよう

表1　ASCからの肝疾患移行率

	年齢					
	40	41	45	50	60	70
男性						
ASC	100.00	85.71	46.27	21.41	7.13	2.62
CH	0.00	14.29	51.99	72.44	69.39	48.38
LC	0.00	0.00	1.31	4.62	12.94	14.62
HCC	0.00	0.00	0.44	1.54	10.55	34.38
女性						
ASC	100.00	83.61	41.35	17.96	6.22	1.85
CH	0.00	16.39	56.85	75.88	78.49	45.37
LC	0.00	0.00	1.80	6.16	10.84	32.79
HCC	0.00	0.00	0.00	0.00	4.45	20.00

*ASC：無症候性キャリア，CH：慢性肝炎，LC：肝硬変，HCC：肝がん

（文献[2]より）

に，40歳男性のASCは5年後に52%が慢性C型肝炎を発症，10年後までASCのままでいられるのは約21%で，慢性肝炎を発症しているのは約72%となる．この時さらに肝硬変への進行は4.6%，肝がんへの進行は1.5%となる．40歳女性では1年後の慢性肝炎の移行確率は男性より高いが，20～30年後の肝がんへの進展率は男性より低い．

以前はトランスアミナーゼの上昇を伴わないASCの場合，特に治療対象とみなされず，通院の必要性も重要視されていない時代があった．しかし，現在ではASCは経過観察中に高率にトランスアミナーゼが変動し始め，慢性肝炎へ移行することが指摘されているため，「通院の必要はありません」と説明できなくなっている．定期的な経過観察が重要であり，早期に治療を開始することも選択肢として考える必要がある．近年は，IFN治療効果が事前に予測できる遺伝子診断もあることから，抗ウイルス療法については専門医に相談し，連携をとりながら肝がんへの進展の阻止へ向けた治療を進めていただけると幸いである．

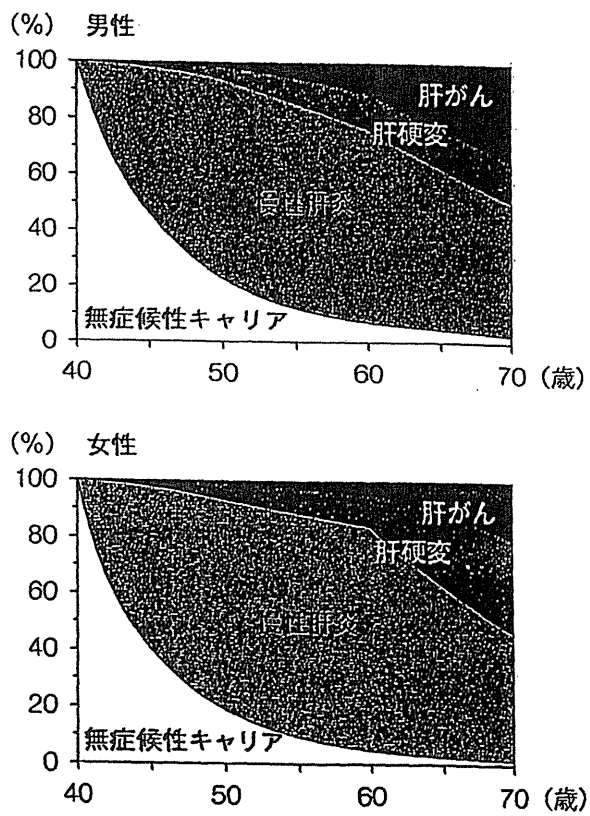

図1　ASCからの肝病態の推移
（無治療の場合，Markovモデルによる推計）

▶文　献

1) Mizui M, et al : Hepatol Res 37 : 994, 2007.
2) Tanaka J, et al : J Med Virol 70 : 378, 2003.

▶回　答

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Medical Practice

内科総合誌

8

ウイルス肝炎

最新の動向と最新の実地診療

● この症例から何を学ぶか

B型急性肝炎の1例
思わぬ合併症に注意

● One Point Advice

● 今月の話題

慢性疲労症候群の病因

● 知っておきたいこと ア・ラ・カルト

椎体圧迫骨折の治療
vertebroplastyとkyphoplasty

● 連載

Common diseaseから入る皮膚疾患

文光堂