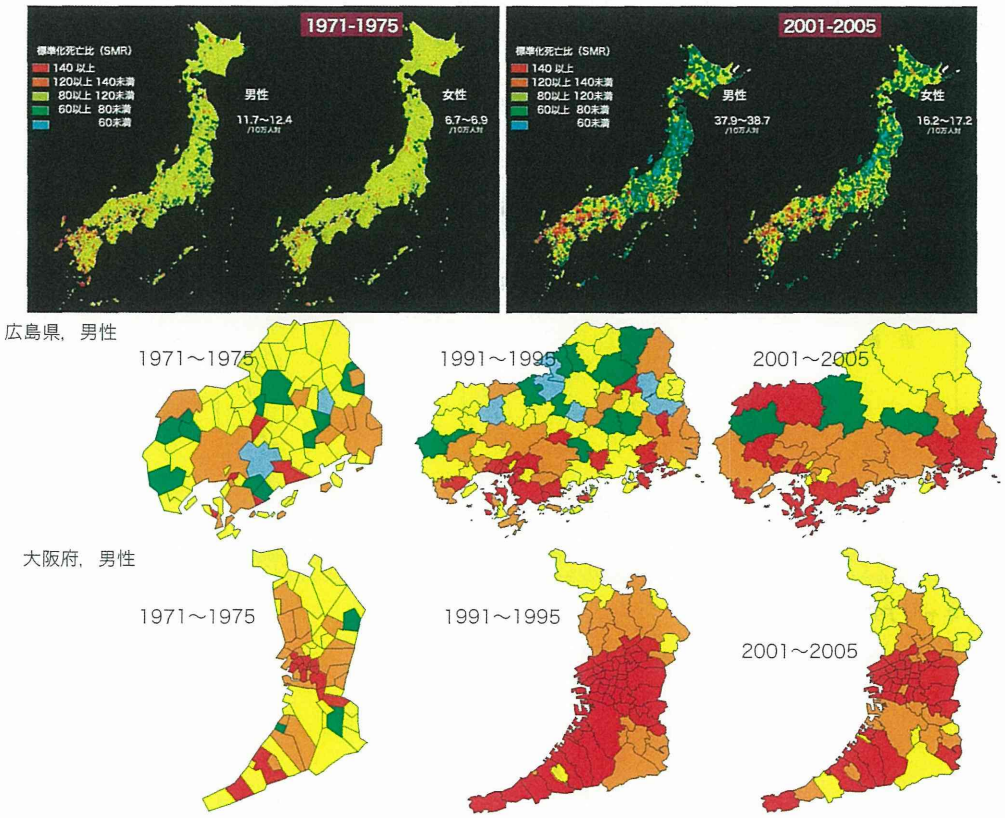


**Fig. 3. 市町村別にみた肝癌標準化死亡比（Bayesian method）の経年推移（1971～2005 年）**
［厚生労働省：肝炎ウイルス感染状況・長期経過と予後調査および治療導入対策に関する研究班 2010 年報告より］

間の「初回供血者」3,748,422 人の資料から，日本赤十字社の協力の元に厚生労働省疫学班研究として算出した，20～39 歳（2005 年時点の年齢換算）の 5 歳刻みの年齢階級別 HBs 抗原陽性率および HCV 抗体陽性率の成績である．

もう一つは，2002 年度から 5ヵ年計画で実施された「肝炎ウイルス検診」の成績[3]のうち，「節目検診」（40～70 歳の 5 歳刻みの節目の年齢にあたる人を対象とした検診）から得た年齢階級別 HBs 抗原陽性率および HCV キャリア率である．

HBs 抗原陽性率（HBV キャリア率）をみると，8 地域ともに団塊の世代と考えられる 2005 年時点の年齢換算で 60 歳前後の年齢層で緩やかな一峰性を示し，北海道九州地域で全国平均（60 歳前後，1.4%）よりも高い値が認められる．20 歳以下の若い集団ではいずれの地域も 0.1% 以下の低い値を示している．一方，HCV キャリア率（初回供血者集団における HCV 抗体陽性率に 70% を乗じた値を HCV キャリア率と読み替えている）は，20 歳以下ではいずれの地域も 0.1% 以下のきわめて低い値を，また肝発癌年齢と考えられる 60 歳以上の高年齢集団では，関東以西の地域でとくに高い値を示す傾向がある．年齢階級とキャリア率の関係は，地域により若干のキャリア率の高低差が認められるものの，その傾向は全国と同様であることが明らかとなっている．

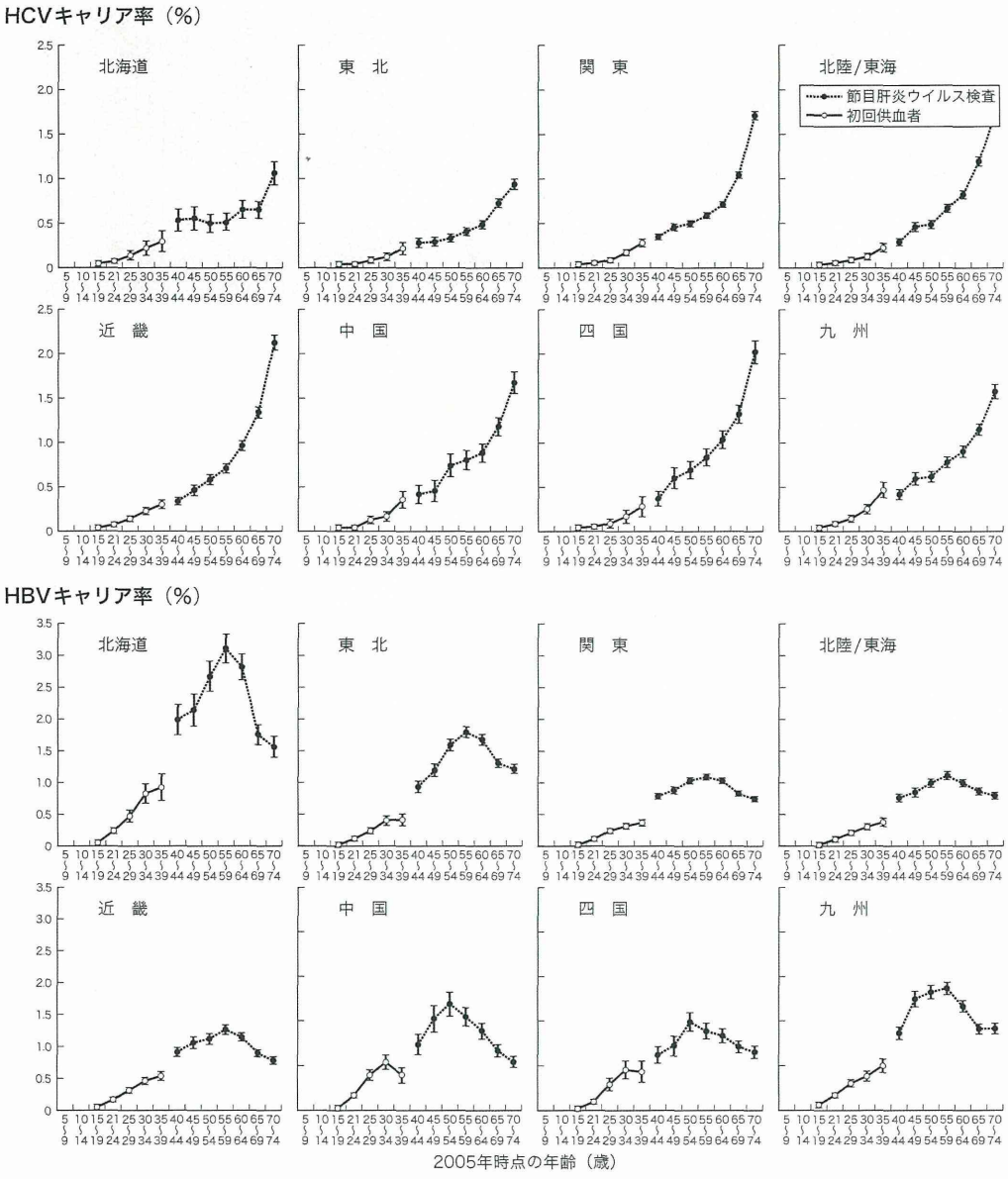


**Fig. 4.　地域別年齢階級別にみた HCV・HBV キャリア率**

Table 1. 肝炎ウイルスキャリア対策

| | |
|---|---|
| a．(感染を知らないまま)潜在しているキャリア | |
| ・肝炎ウイルス検査 | 検査の必要性<br>検査の機会の拡大(無料検査・出前検査)<br>対象者の拡大 |
| b．患者としてすでに通院・入院しているキャリア | |
| ・治療<br>・治療効果などの情報提供<br>・治療連携 | 医療費補助の運用<br>適切な治療への導入<br>専門医への受診<br>肝癌早期発見・治療プロトコール |
| c．(感染を知ったが)継続的な受診をしないままでいるキャリア | |
| ・受診への動機づけ<br>・公費助成により見出されたキャリアの健康管理 | 現状把握と要因分析<br>医療機関受診率の把握<br>肝炎診療ネットワークへの連携 |
| d．新規感染によるキャリア | |
| | 感染予防対策<br>ワクチン<br>キャリアの新規発生状況の把握と対策 |

## 感染を知らないまま潜在しているキャリア数の把握と肝炎ウイルスキャリア対策

前項に示した2つの大規模集団を元に得た年齢階級別HBV・HCVキャリア率と国勢調査人口を用いて，わが国におけるキャリア数の推計を行ったところ，2005年時点ではHBVキャリア推計数は，903,145人(95% CI：83.7-97.0万人)，HCVキャリア推計数は，807,903人(95% CI：68.0-97.4万人)となった[4]．この推計値，HBVキャリア数約90万人，HCVキャリア数約81万人は，検査前には自身が感染を知らなかった献血集団や肝炎ウイルス検診受検者集団におけるキャリア率を元に算出された数値であることから考えると，「感染を知らないまま潜在しているキャリア」の推計数に相当する．

社会における存在状態により肝炎ウイルスキャリア(肝炎ウイルスの持続感染状態にある人)を分類すると，「a．感染を知らないまま潜在しているキャリア」，「b．患者としてすでに通院・入院しているキャリア」，「c．感染を知ったが受診しないでいる，あるいは継続受診にいたっていないキャリア」，「d．新規感染によるキャリア」と大きく4分類される(Table 1)．わが国の肝炎ウイルスに持続感染しているキャリア数の全体を把握するためには，さらに「b」，「c」，「d」それぞれの数の把握(burden)が必要である．その大きさと社会における存在状態に応じて具体的なキャリア対策を講じることが効果的と考えられ，把握するための大規模調査や研究が行われているところである．

今後の肝炎ウイルスキャリア対策，ひいては肝癌対策として，「d．新規感染によるキャリア」に対しては，肝炎ウイルスの新規感染の動向調査・従来の感染防止対策を継続すること，「a．感染を知らないまま潜在しているキャリア」に対しては，肝炎ウイルス検査の必要性を周知し，家族を含んだ職域集団などの対象者の拡大を図り，対象集団ごとの検査機会の利便性を促進すること，「b．患者としてすでに通院・入院しているキャリア」に対しては，肝炎治療に適した医療へのアクセス状況，最新の抗ウイルス療法の治療効果や肝癌早期発見のための検査プロトコールなどの情報提供の現

状，医療費補助の運用と効果の把握をすること，さらに「c．感染を知ったが受診しないままでいるキャリア」に対しては，その現状把握と要因分析を行うために，公費助成により見出されたキャリアの健康管理や医療機関受診状況の追跡調査を行うこと，が重要と考えられる[5]．

## おわりに

わが国の社会生活全般における肝炎ウイルス感染の発生要因が徐々に減少し，若い世代におけるHBV キャリア率や HCV キャリア率は低い値を示すにいたっている．肝炎対策基本法(2009 年 12 月)を基盤として，すでに感染しているキャリアへの対策，具体的には，肝炎ウイルス検査の推進，肝疾患診療ネットワークの構築，新規治療法の開発などが積極的に進められている．

肝炎・肝癌対策をその病因論的また疫学的視点から捉えた場合，これまで行ってきた肝炎ウイルス感染の動向調査・感染防止対策を継続しつつ，社会における肝炎ウイルスキャリアの存在状態別にそれぞれの課題を掲げて具体的な対策を推進することが肝癌対策にとっても重要であるといえる．肝炎対策の先進国であるわが国は，肝癌対策の新たな局面を迎えていると考えられる．

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# Total Numbers of Undiagnosed Carriers of Hepatitis C and B Viruses in Japan Estimated by Age- and Area-Specific Prevalence on the National Scale

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**Key Words**
Hepatitis C virus · Hepatitis B virus · Blood donors · Liver cirrhosis · Hepatocellular carcinoma · Healthcare · Japan

**Abstract**
***Objective:*** To estimate total numbers of undiagnosed carriers of hepatitis C virus (HCV) and hepatitis B virus (HBV) in Japan. ***Methods:*** Area- and age-specific prevalence of HCV as well as HBV was determined in the first-time blood donors [20–39 years (n = 2,429,364)] and examinees of periodical health check-ups [40–74 years (6,204,968 for HCV and 6,228,967 for HBV)] in Japan. Prevalence in adolescents [5–19 years (79,256 for HCV and 68,792 for HBV)] was determined in a single prefecture, and that of HCV in the elderly (≥75 years) was estimated by the exponential model. HBV infection was determined by the detection of hepatitis B surface antigen, and HCV infection by either the algorithm or assuming persistent infection in 70% of the individuals with antibody to HCV. ***Results:*** Of the total population of 127,285,653 in 2005, 807,903 (95% CI 679,886–974,292) were estimated to be infected with HCV at a carrier rate of 0.63%, and 903,145 (837,189–969,572) with HBV at that of 0.71%. ***Conclusion:*** Accurate estimation of undiagnosed HCV and HBV carriers in the general population would help to predict the future burden of liver disease, and take appropriate measures for improving healthcare.


## Introduction

Hepatitis C virus (HCV) and hepatitis B virus (HBV) are estimated to infect 170 and 350 million people over the world, respectively [1, 2]. Most infections with HCV or HBV do not induce clinical liver disease, while ~30% of them develop severe liver disease such as cirrhosis and hepatocellular carcinoma [3, 4]. Hence, there is a pressing need to identify the individuals who have undiagnosed HCV or HBV infection, and take effective measures for terminating viral infections and preventing the progression of liver disease.

For management of persistent HCV and HBV infections in a given country, it is necessary to know their exact numbers for assessing medical and financial needs in the foreseeable future. Prevalence of undiagnosed HCV or HBV

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infection has been estimated by survey of blood donors in Japan [5] and a representative population in the USA [6].

In the present study, area- and age-specific prevalence of HCV or HBV infection was determined in 8 jurisdiction areas of the Japanese Red Cross Blood Center. Then, the total numbers of undiagnosed HBV and HCV infections were estimated by compiling the results in the first-time blood donors and examinees of the periodical health check-up program. Of the 127,285,653 Japanese registered in 2005, 807,903 (0.63%) were estimated to be infected with HCV and 903,145 (0.71%) with HBV.

## Materials and Methods

*Japanese Population*

Japan is divided into 8 areas, along its north-to-south axis, according to jurisdiction of the Japanese Red Cross Blood Center, into Hokkaido, Tohoku, Kanto, Hokuriku/Tokai, Kinki, Chugoku, Shikoku and Kyushu. Populations in 5-year age groups in each jurisdiction area were obtained from the registry at the National Census 2005.

*First-Time Blood Donors*

During 6 years from January 2001 to December 2006, 3,748,422 individuals (aged 16–64 years) donated whole blood or apheresis products for the first time, and their sera were tested for markers of HCV and HBV infections. Ongoing HCV infection was estimated by assuming the detection of HCV RNA in 70% of individuals with the antibody to HCV (anti-HCV), in accordance with a previous report [5].

*Examinees of Hepatitis Virus Infections*

Since the fiscal year 2002 in Japan, individuals who turned 40, 45, 50, 55, 60, 65 and 70 years were offered to take tests for hepatitis viruses at periodical health check-ups by a 5-year national project. During 5 years through 2006, 6,204,968 individuals received tests for HCV and 6,228,967 for HBV, corresponding to ~30% of the eligible Japanese, and their area- and age-specific prevalence of HCV or HBV infection was determined.

*School Children and Adolescents*

In the Iwate prefecture located in the north of Japan, biochemical markers of diseases dependent on the lifestyle were examined in children and adolescents at the entrance to schools. Their serum samples had been stored frozen, and were tested for markers of hepatitis virus infections. Carrier rates of HCV and HBV among them were calculated, with their ages adjusted to those in 2005; infants aged <5 were represented by the children aged from 5 to 9 years. Designs and procedures of this investigation were approved by the Ethics Committee of Hiroshima University.

*Simulation of HCV and HBV Infections in the Elderly*

By its age-specific profile, the prevalence of HCV was deduced to be an exponential function of the age. Accordingly, age-specific prevalence of HCV in the individuals aged ≥75 years was simulated by an exponential function model; it was constructed on the prevalence of HCV in each age group ≥50 years.

The formula was constructed as:

$$\log y(x) = a + bx$$

where $x$ is the 5-year age code, $y(x)$ is an estimator of HCV prevalence in $x$, and $a$ and $b$ are coefficients.

The equation is transformed into:

$$y(x) = e^a e^{bx}$$

in which $e^a$ represents the HCV prevalence when $x = 0$ (in the group aged 0–4 years), since $y(0)$ is equal to $e^a$. By replacing $x$ for $x + 1$ in the above equation, it is converted to $y(x + 1) = e^a e^{b(x+1)}$.

Then, the following equation can be constructed:

$$y(x + 1) = e^b y(x)$$

where $e^b$ is the slope of HCV prevalence increasing with age. Thus, the HCV prevalence is multiplied by a factor $e^b$ for an increment of the age code by 1.

The simulation model was applied to estimate age-specific prevalence of HCV in each of 8 areas in the individuals ≥75 years.

Prevalence of HBV in the individuals ≥75 years was represented by that in those aged 70–74 years, since it stayed constant from 65 through 75 years.

*Markers of Hepatitis Virus Infections*

In blood donors, anti-HCV was determined by passive hemagglutination of the second generation with commercial assay kits (HCV PHA; Abbott Laboratories, North Chicago, Ill., USA) with a cutoff limit set at $2^5$, as well as by particle agglutination with commercial assay kits (HCV PA Test-II; Fujirebio, Inc., Tokyo, Japan). HBsAg was determined by reversed passive hemagglutination with reagents prepared by the Japanese Red Cross.

In examinees of periodical health check-ups, ongoing HCV infection was determined by the algorithm with anti-HCV and HCV RNA [7]. Anti-HCV was determined by passive hemagglutination of the second generation with commercial assay kits (HCV PHA; Abbott Laboratories), and since 2002, it was determined by enzyme immunoassay with commercial assay kits (AxSYM HCV Dinapack-III; Abbott Laboratories). Samples with high anti-HCV titers contain HCV RNA, and therefore, only those with low and middle titers were examined for HCV RNA. HBsAg was determined by reversed-passive hemagglutination with commercial assay kits (Institute of Immunology Co., Ltd, Tokyo, Japan).

*Statistical Analyses*

Statistical analyses for the evaluation of $R^2$ values were performed with JMP 8.0 (SAS Institute, Inc., Cary, N.C., USA) and DeltaGraph 5.5 (RedRock Software, Inc., Salt Lake City, Utah, USA). A p value > 0.05 was considered significant.

## Results

### *Age-Specific Prevalence of HCV in the First-Time Blood Donors and Examinees of Periodical Health Check-Ups*

Figure 1 illustrates age-specific prevalence of HCV in the first-time blood donors (aged 15–69 years in 2005) and examinees of periodical health check-ups (39–73 years in 2005); 70% of individuals with anti-HCV were considered

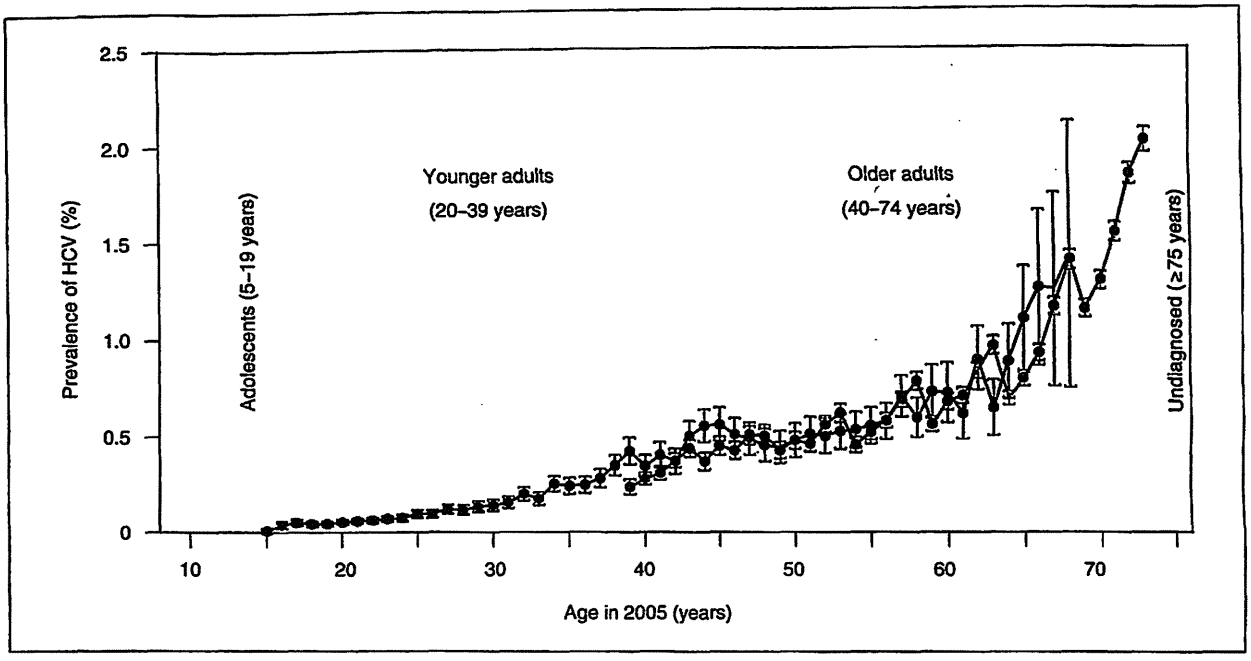


**Fig. 1.** Age-specific prevalence of HCV in Japan. The prevalence of HCV was determined in the first-time blood donors aged from 15 to 68 years (blue dots) and examinees of periodical health check-ups aged from 39 to 73 years (red dots). Their ages were adjusted to those in the year 2005. Bars indicate ranges of 95% CI.

to possess HCV RNA in serum [5]. Results of two distinct populations were well in accord. For the first-time blood donors, however, the variation (95% CI) widened increasingly with age. It would have reflected decreases in the first-time blood donors with age, since the majority of these (83.5%) were aged ≤39 years. As the prevalence of HCV in blood donors ≥40 years was unreliable in them, that in examinees of periodical check-ups was adopted for estimating the national prevalence of HCV.

*Area-Specific Prevalence of HCV in Eight Jurisdiction Areas*

In view of distinct geographic distribution of HCV, the prevalence of HCV in the general population would not be applicable to every area in Japan. Figure 2 compares results in the first-time blood donors and recipients of health check-ups among 8 jurisdiction areas spanning from north (Hokkaido) to south (Kyushu). They unfolded a wide variety in the age-specific prevalence of HCV. Although the prevalence of HCV increased with age in all areas, the slope of increase differed widely among them. Hence, it was necessary to employ a distinct age-specific prevalence in each of the 8 areas for estimating HCV carriers precisely.

**Table 1.** Age-specific prevalence of HCV in three different populations

| Age in 2005 | n | HCV-positive, n | Prevalence, % (95% CI) |
|---|---|---|---|
| School children | | | |
| 5–9 | 17,390 | 2 | 0.012 (0.000–0.027) |
| 10–14 | 29,817 | 3 | 0.010 (0.000–0.021) |
| 15–19 | 32,049 | 7 | 0.022 (0.006–0.038) |
| Blood donors | | | |
| 20–24 | 1,205,966 | 1,122 | 0.065 (0.061–0.070)[a] |
| 25–29 | 536,560 | 874 | 0.114 (0.105–0.123)[a] |
| 30–34 | 408,814 | 1,089 | 0.186 (0.173–0.200)[a] |
| 35–39 | 278,024 | 1,190 | 0.300 (0.279–0.320)[a] |
| HCV screening | | | |
| 40–44 | 611,146 | 2,127 | 0.348 (0.333–0.363) |
| 45–49 | 495,032 | 2,292 | 0.463 (0.444–0.482) |
| 50–54 | 675,350 | 3,485 | 0.516 (0.499–0.533) |
| 55–59 | 947,438 | 5,974 | 0.631 (0.615–0.646) |
| 60–64 | 1,081,854 | 8,423 | 0.779 (0.762–0.795) |
| 65–69 | 1,264,496 | 13,722 | 1.085 (1.067–1.103) |
| 70–74 | 1,054,472 | 17,649 | 1.674 (1.649–1.698) |

[a] The prevalence in blood donors was based on an assumption of HCV infection persisting in 70% of those with anti-HCV [5].

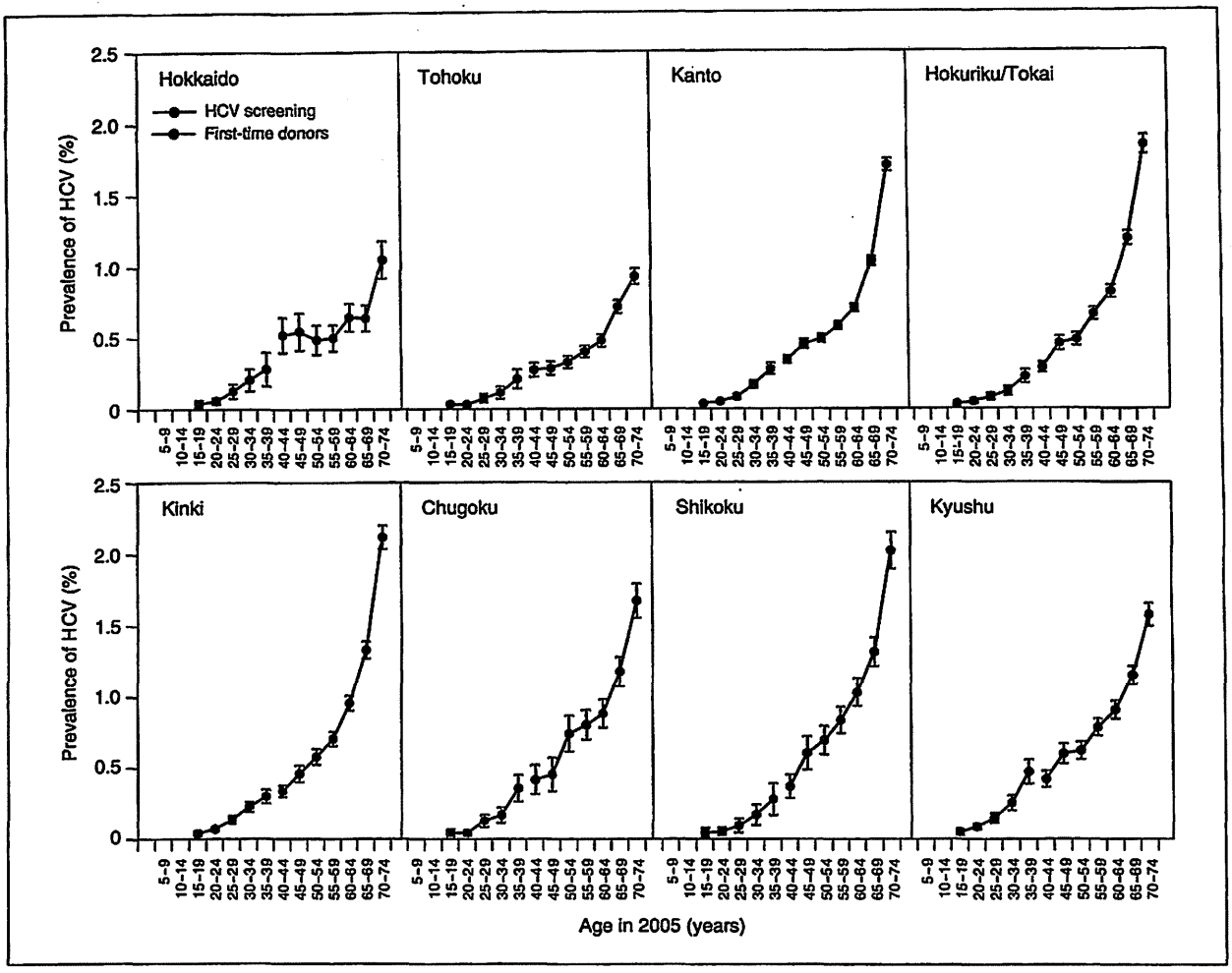


**Fig. 2.** Age-specific prevalence of HCV in 8 jurisdiction areas in Japan. The prevalence of HCV is calculated in each of twelve age groups notched by 5 years. The prevalence in five groups ≤39 years was represented by the first-time blood donors, and that in seven groups ≥40 years by recipients of HCV screening. Bars indicate ranges of 95% CI.

*Prevalence of HCV in Adolescents*

Since blood donors were restricted to 16–64 years of age, and health examinees were targeted on 40–70 years, they did not cover individuals aged ≤15 or ≥75 years in the year 2005. To fill in an opening on the younger side, the age-specific prevalence of HCV was determined in school children and adolescents in the Iwate prefecture (table 1). The prevalence in infants aged 0–4 years was assumed similar to that in the children aged 5–9 years; an extremely low prevalence of HCV (0.012%) would support such an assumption.

*Simulating Prevalence of HCV in the Elderly*

The prevalence of HCV appeared to be an exponential function of the age, according to its profiles in the first-time blood donors and examinees of health check-ups (fig. 1). Based on this assumption, a formula was constructed to simulate the prevalence of HCV in age groups ≥75 years for each of the 8 jurisdiction areas in Japan (see Materials and Methods).

Figure 3 compares actual (dots) and simulated data (red line) of five age groups from 50 to 74 years (corresponding to age codes 10–14) among the 8 areas. There was a high coefficient of determination between them,

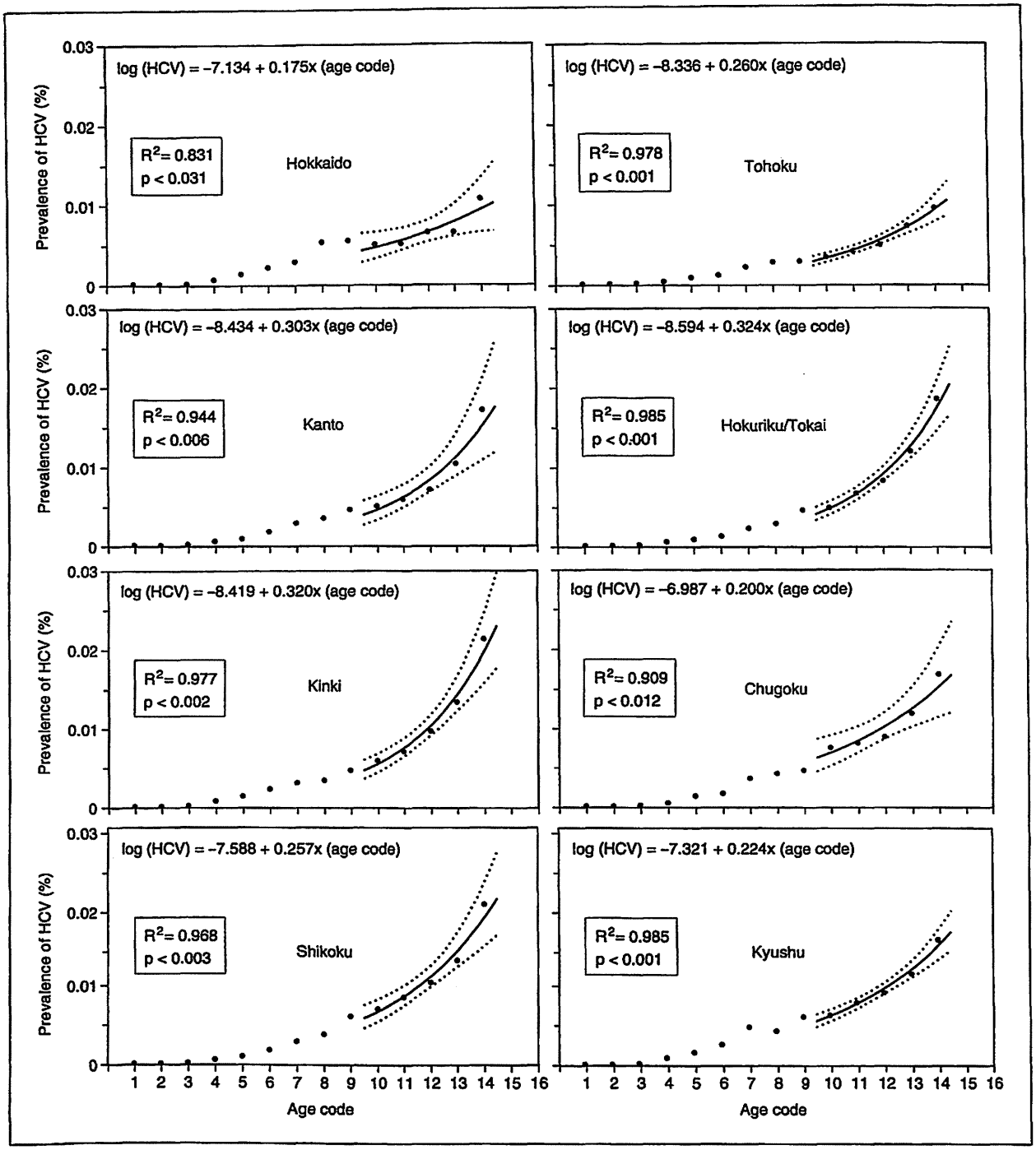


**Fig. 3.** Simulation of age-specific prevalence of HCV in the elderly. Prevalence of HCV in the first-time blood donors as well as examinees of periodical health check-ups (dots) and that simulated by formulation (red line with ranges of 95% CI in dotted line) are shown for 8 jurisdiction areas in Japan. Formula is shown at the top of each area. Age codes are: 1, 5–9 years; 2, 10–14 years; 3, 15–19 years; 4, 20–24 years; 5, 25–29 years; 6, 30–34 years; 7, 35–39 years; 8, 40–44 years; 9, 45–49 years; 10, 50–54 years; 11, 55–59 years; 12, 60–64 years; 13, 65–69 years; 14, 70–74 years, and 15, 75–79 years.

**Table 2.** Regional and total HCV carriers in Japan

| Areas | Population | HCV carriers (95% CI) | Carrier rate |
|---|---|---|---|
| Hokkaido | 5,620,813 | 26,097 (19,356–34,413) | 0.46% |
| Tohoku | 12,047,975 | 50,688 (42,754–59,953) | 0.40% |
| Kanto | 41,247,892 | 235,328 (195,408–293,611) | 0.57% |
| Hokuriku/Tokai | 19,294,443 | 132,434 (114,216–154,446) | 0.69% |
| Kinki | 22,657,542 | 173,808 (147,548–207,173) | 0.52% |
| Chugoku | 7,650,977 | 53,296 (42,299–67,698) | 0.70% |
| Shikoku | 4,083,698 | 35,159 (28,746–43,004) | 0.86% |
| Kyushu | 14,682,313 | 101,092 (89,379–113,993) | 0.80% |
| Total | 127,285,653 | 807,903 (679,886–974,292) | 0.63% |

**Table 3.** Age-specific prevalence of HBV in three different populations

| Age in 2005 | n | HBV-positive, n | Prevalence, % (95% CI) |
|---|---|---|---|
| School children | | | |
| 5–9 | 17,363 | 3 | 0.017 (0.000–0.037) |
| 10–14 | 29,817 | 14 | 0.047 (0.022–0.072) |
| 15–19 | 32,049 | 12 | 0.037 (0.016–0.059) |
| Blood donors | | | |
| 20–24 | 1,205,966 | 1,826 | 0.151 (0.144–0.158) |
| 25–29 | 536,560 | 1,650 | 0.308 (0.293–0.322) |
| 30–34 | 408,814 | 1,759 | 0.430 (0.410–0.450) |
| 35–39 | 278,024 | 1,327 | 0.477 (0.452–0.503) |
| HBV screening | | | |
| 40–44 | 613,960 | 5,491 | 0.894 (0.871–0.918) |
| 45–49 | 497,589 | 5,373 | 1.080 (1.051–1.109) |
| 50–54 | 679,893 | 8,700 | 1.280 (1.253–1.306) |
| 55–59 | 950,508 | 12,891 | 1.356 (1.333–1.379) |
| 60–64 | 1,085,119 | 13,282 | 1.224 (1.203–1.245) |
| 65–69 | 1,268,304 | 12,406 | 0.978 (0.961–0.995) |
| 70–74 | 1,057,469 | 9,545 | 0.903 (0.885–0.921) |

with $R^2$ values ranging from 0.831 to 0.985 ($p < 0.031$ and $p < 0.001$, respectively), attesting to the validity of this simulation. Of note, the factor *b* in formula (by which age codes were multiplied) varied broadly among the 8 areas. Thus, it was the highest in Hokuriku/Tokai at 0.324 and lowest in Hokkaido at 0.175, with close to twofold differences between them.

*Estimation of Undiagnosed HCV Carriers in Eight Areas and the Entire Nation*

Based on age- and area-specific prevalence of HCV, numbers of undiagnosed HCV carriers were calculated for 8 jurisdiction areas, and they were compiled in the entire nation (table 2). The prevalence of HCV in each of three age groups (75–79, 80–84 and ≥85 years) was simulated by the formula, while that of HBV was represented by the prevalence in the group of 70–74 years. As of the year 2005, 127,285,653 were registered in the national census of Japan, and 807,903 of these are estimated to have undiagnosed HCV infection at an overall carrier rate of 0.63%. There was an increasing gradient in the prevalence of HCV along the north-to-south axis of Japan.

*Age-Specific Prevalence of HBV*

Figure 4 depicts age-specific prevalence of HBV in 2005. It was deduced from HBsAg in the first-time blood donors (15–69 years) and examinees of periodical health check-ups (39–73 years). Since the prevalence of HBV in the elderly did not increase with age so sharply as that of HCV (fig. 1), it was presumed not to increase further and stay around 1% in the individuals ≥75 years. The age-specific prevalence of HBV tabulated in three different

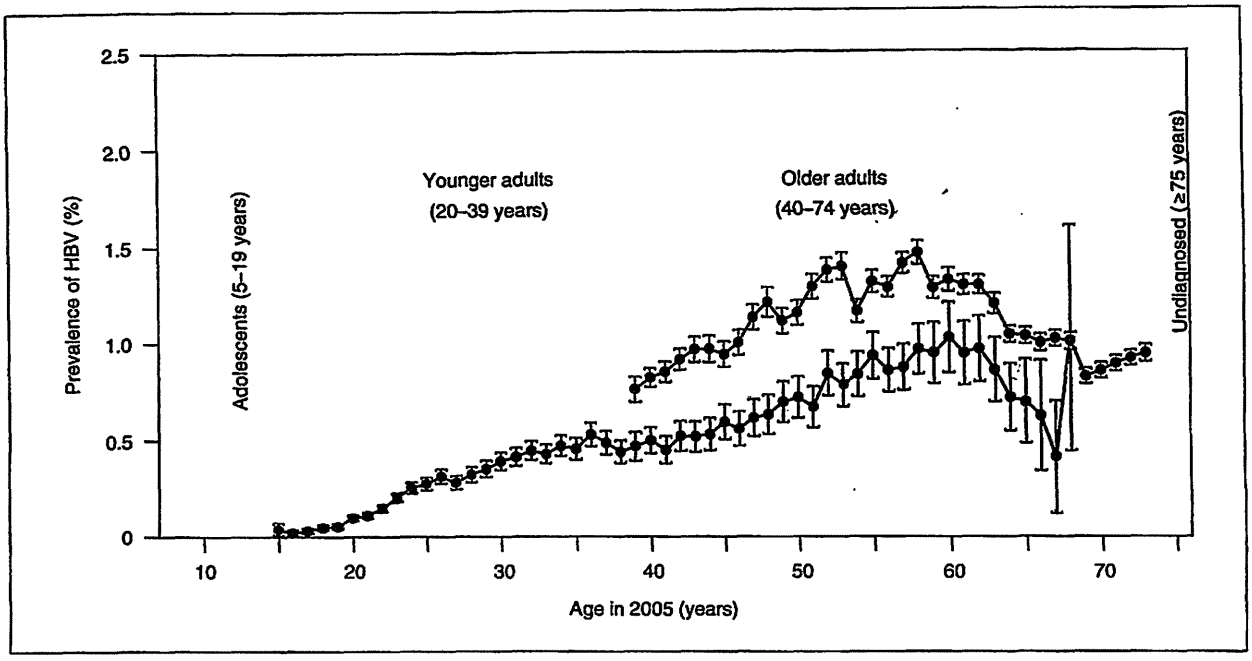


**Fig. 4.** Age-specific prevalence of HBV in Japan during 2002–2006. The prevalence of HBV was determined in the first-time blood donors aged from 15 to 68 years (blue dots) in the year 2005 and examinees of periodical health check-ups aged from 39 to 73 years (red dots) in the year 2005. Bars indicate ranges of 95% CI.

populations is listed in table 3. There was a constant decline with decreasing age in the frequency of HBV in individuals ≤39 years, and it was particularly low in children ≤9 years (0.017%).

In examinees of periodical health check-ups, the age-specific prevalence of HBV did not diverge and stayed within a narrow 95% CI (fig. 4). By contrast, that in the first-time blood donors dispersed widely. Such a variation in the age-specific prevalence of HBV would have been ascribed to the first-time blood donors who clustered in age groups ≤40 years.

*Area-Specific Prevalence of HBV in Eight Jurisdiction Areas*

The age-specific prevalence of HBsAg varied widely among 8 jurisdiction areas (fig. 5). HBsAg was most frequent in the age group of 55–59 years in every area, and reached 3.1% in the northern-most Hokkaido. The peak frequency decreased in central Japan (1.1% in Kanto and Hokuriku/Tokai), and increased towards the southern end (1.9% in Kyushu). Thus, the prevalence of HBsAg was determined individually along the axis of Japan in estimating the total number of HBV carriers in Japan.

*Estimation of Undiagnosed HBV Carriers in Eight Areas and the Entire Nation*

Numbers of undiagnosed HBV carriers were complied by multiplying age-specific prevalence of HBsAg by corresponding subpopulations in 8 jurisdiction areas (table 4). In total, 903,145 of the 127,285,653 (0.71%) individuals are estimated to have undiagnosed HBV infection in Japan in 2005.

*Shift of Undiagnosed HCV and HBV Carriers during 5 Years (2000–2005) in Japan*

Table 5 compares numbers of HCV and HBV carriers aged 15–69 years between 2000 and 2005 for 8 jurisdiction areas in Japan. Data for the year 2000 were extracted from a previous survey [5]. Data for the year 2005 were obtained in the first-time blood donors during 2001–2006 in this study by the same method as in the previous survey [5]. Undiagnosed HCV and HBV carriers decreased during 5 years by 55 and 47.5%, respectively. The overall carrier rate of HCV declined sharply from 0.95 to 0.44%, and that of HBV from 1.04 to 0.55% in Japan.

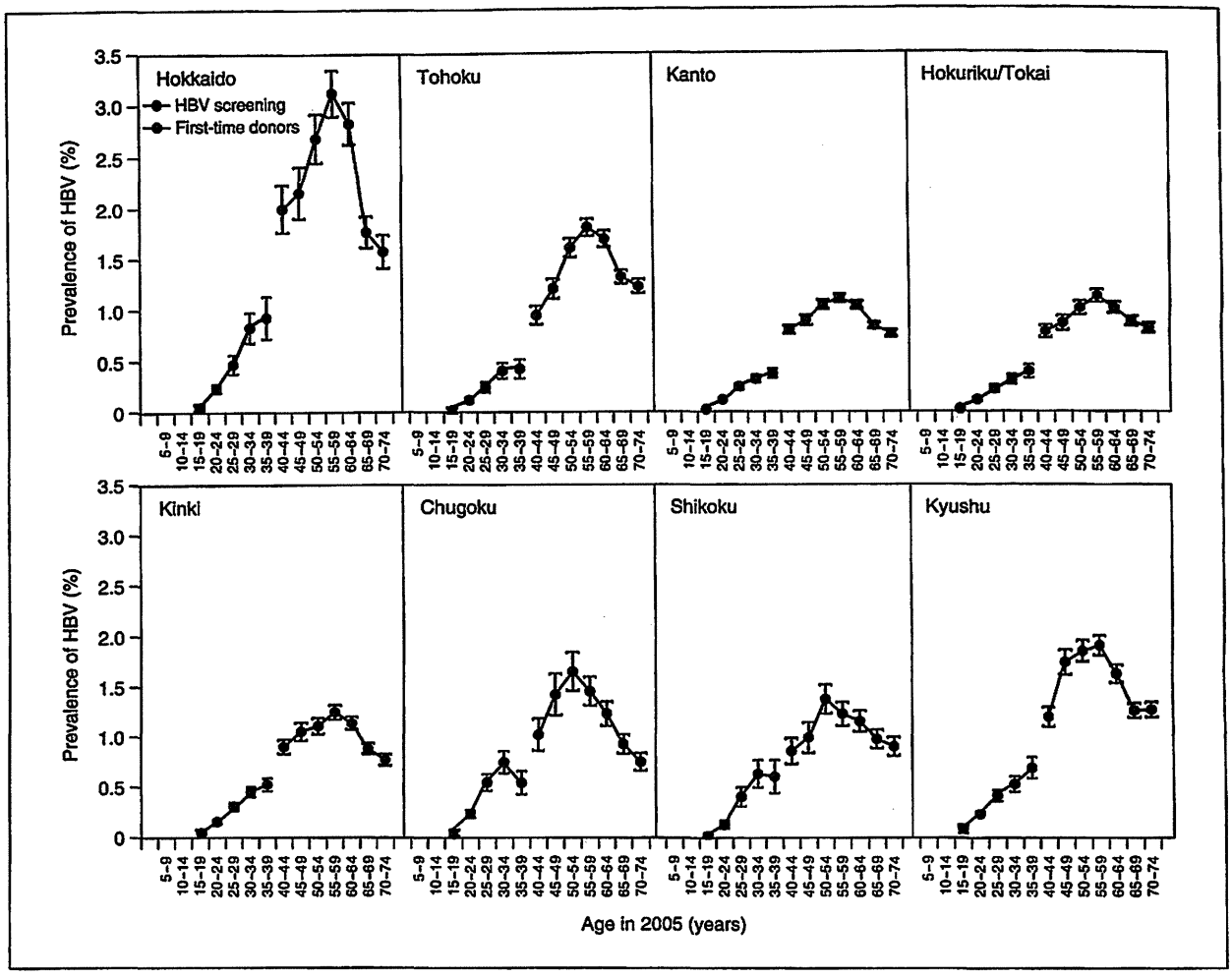


***Fig. 5.*** *Age-specific prevalence of HBV in 8 jurisdiction areas in Japan. The prevalence of HBV is calculated in each of twelve age groups notched by 5 years. The prevalence in five groups ≤39 years was represented by the first-time blood donors and that in seven groups ≥40 years by recipients of HCV screening. Bars indicate ranges of 95% CI.*

**Table 4.** Regional and total HBV carriers in Japan

| Areas | Population | HBV carriers (95% CI) | Carrier rate |
|---|---|---|---|
| Hokkaido | 5,620,813 | 80,573 (72,314–88,765) | 1.43% |
| Tohoku | 12,047,975 | 104,736 (97,742–111,816) | 0.87% |
| Kanto | 41,247,892 | 231,799 (220,129–244,105) | 0.56% |
| Hokuriku/Tokai | 19,294,443 | 109,709 (101,722–117,581) | 0.56% |
| Kinki | 22,657,542 | 144,965 (134,387–155,464) | 0.64% |
| Chugoku | 7,650,977 | 59,948 (52,705–67,121) | 0.78% |
| Shikoku | 4,083,698 | 29,776 (26,080–33,437) | 0.73% |
| Kyushu | 14,682,313 | 141,639 (132,111–151,282) | 0.96% |
| Total | 127,285,653 | 903,145 (837,189–969,572) | 0.71% |

**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.


## Acknowledgements

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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 $7.0 \times 10^6$ 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 $8.4 \times 10^6$

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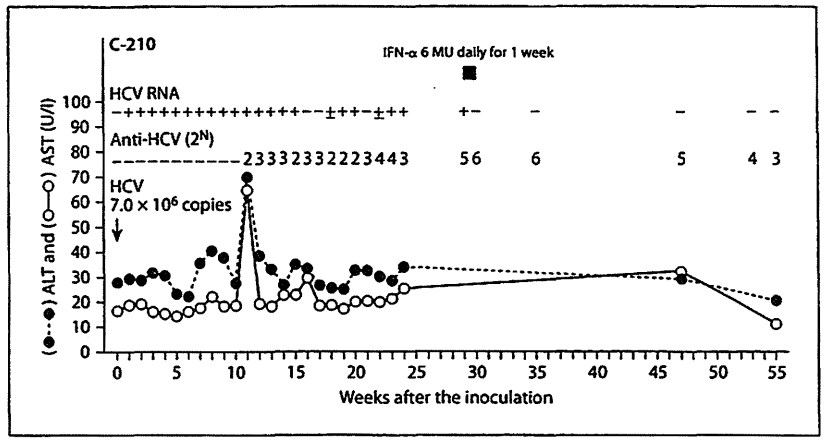


**Fig. 1.** Clinical course of chimpanzee No. 210 (C-210). The duration of IFN monotherapy is indicated at the top. HCV RNA was determined qualitatively by Amplinat MPX. Anti-HCV was determined by passive hemagglutination. Fluctuating levels of ALT and AST in the serum are shown below.

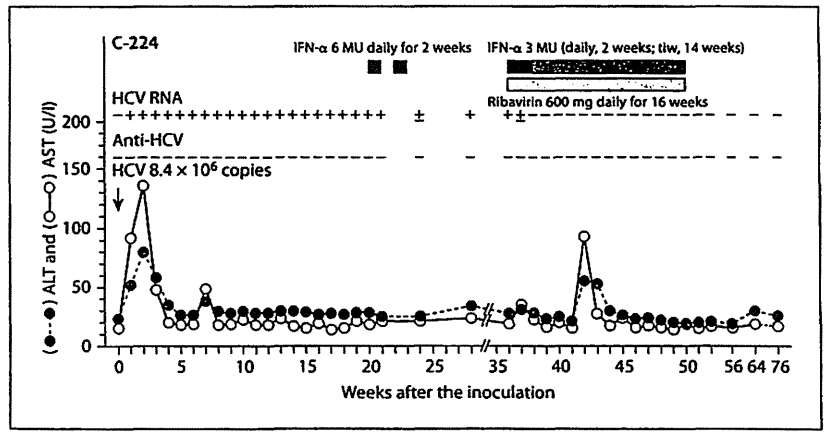


**Fig. 2.** Clinical course of chimpanzee No. 224 (C-224). The duration of 2 courses of IFN monotherapy (1 week each) as well as IFN (daily for 2 weeks and then 3 times a week for 14 weeks) combined with ribavirin (16 weeks) is indicated at the top.

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 $7.0 \times 10^6$ 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 $8.4 \times 10^6$ 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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