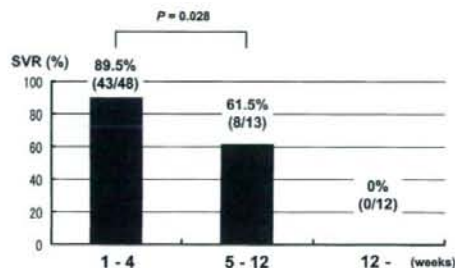


**Table 5** The rate of SVR according to HCV RNA levels more than 100 KIU/ml

Serotype 1	The rate of SVR
HCV RNA < 100 KIU/ml	85.7% (6/7)
HCV RNA 100 KIU/ml $\leq$	50.0% (6/12)
Serotype 2	The rate of SVR
HCV RNA < 100 KIU/ml	90.5% (19/21)
HCV RNA 100 KIU/ml $\leq$	60.0% (24/40)

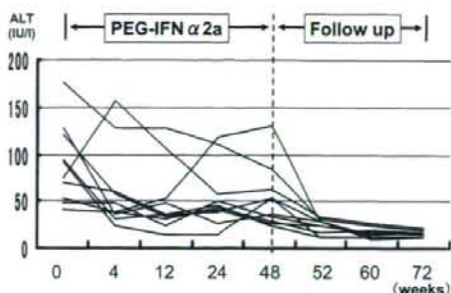


**Fig. 3** SVR was observed more frequently in cases with RVR\* than in cases with EVR\*\* ( $p = 0.028$ ).  
\*RVR: Rapid virological response. \*\*EVR: Early virological response

研究ではセロタイプ1型の症例数が少ないため、治療前の至適ウイルス量に関しては、今後も更なる検討が必要であると考えられた。

本研究ではBMIがSVR予測因子とならなかったのは、我々の対象症例では、欧米の報告に比べBMIが正常範囲内 (BMI: 18.5-25) の患者が多く、いわゆる肥満の症例が少なかったことなどより有意差を認めなかったものと考えられた。

治療前の肝線維化とSVRの検討では、線維化軽度例の方が、有意差をもってSVR率は高値であった。本邦における国内第II相臨床試験の結果では、肝線維化StagingとSVRの割合として、F1 38.9%, F2 39.4%, F3 20.0%であったが、統計学的検討は行われていない<sup>4)</sup>。以前我々は、インターフェロン単独療法において、高齢者や肝線維化進行例ではSVR率が低下することを報告したが<sup>12)</sup>、PEG-IFN単独療法においても線維化進行例ではSVR率が低いため、線維化軽度例ほど良い治療適応であると考えられた。また、F4ステージの肝硬変例 ( $n=3$ ) では、いずれもSVRは得られず、Zeuzem



**Fig. 4** Serum ALT levels were measured during PEG-IFN monotherapy and follow up period in cases that achieved SVR. In these 10 cases, serum ALT levels increased throughout the administration of PEG-IFN monotherapy, but steadily returned to normal levels during the follow up period. Whereas ALT levels decreased smoothly during both the treatment and follow up periods in 13 cases (Data not shown).

らの報告と一致した<sup>10)</sup>。このため、F4ステージであれば通常の48週投与ではウイルス陰性化の可能性が低いと考えられるため、48週以降の長期投与や治療方針の再考が検討されるべきと考えられた。

IFN治療開始後のSVR予測因子の検討では、早期のウイルス陰性化が重要であることが多数報告されている<sup>11)12)</sup>。本研究においても、治療開始後4週までにHCV RNA定性陰性化が得られたRVR群では、治療開始後5から12週までにHCV RNA定性陰性化が得られたEVR群よりも有意にSVR率が高く (Fig. 3)、また多変量解析でも有意差を認めた (Table 4)。以上より、PEG-IFN単独療法の治療効果予測因子として、治療開始4週以内のHCV RNA量定性陰性化 (RVR) が重要な因子であることが明らかとなった。また、治療開始5週から12週間にHCV RNA定性が陰性となったEVR症例では、SVRが得られる可能性がRVRに比べ低いため、投与期間を48週以後も延長することや、可能であれば、低用量のリバビリンを併用する、といった治療法の再考が必要になってくると考えられる。また、12週までにHCV RNA定性が陰性とならなかった症例では、SVRが得られる可能性が極めて低いと考えられるが、このような症例では発癌抑制のためにPEG-IFN治療を長期的に継続するといった選択肢も考えられる<sup>14)15)</sup>。

PEG-IFN単独治療に伴う血清ALT値上昇は少なからず報告されている<sup>6)16)</sup>。また国内第II相臨床試験によ

**Table 6** The rate of SVR according to HCV RNA levels in each serotypes

Serotype 1	The rate of SVR	
HCV RNA < 400 KIU/ml	78.5% (11/14)*	
HCV RNA 400 KIU/ml $\leq$	20.0% (1/5)*	* $p = 0.037$
Serotype 2	The rate of SVR	
HCV RNA < 500 KIU/ml	79.4% (31/39)**	
HCV RNA 500 KIU/ml $\leq$	54.5% (12/22)**	** $p = 0.047$

**Table 7** Multivariate logistic regression analysis of the factor associated with achievement of SVR

Factor	Odds ratio (95%CI)	$p$ value
RVR	17.2 (4.38-84.5)	0.0001

Only variables that achieved statistical significance ( $p < 0.05$ ) on multivariate logistic regression are shown.  
CI: Confidence Interval

Factor	Sensitivity	Specificity	PPV	NPV
RVR	82.3%	78.7%	89.6%	66.7%

PPV: Positive predictive value, NPV: Negative predictive value

ると、SVR、non-SVRにかかわらずPEG-IFN投与中に血清ALT値上昇を認めたものは180 $\mu$ g投与群で20.7%、90 $\mu$ g投与群で24.6%であった<sup>5)</sup>。原因として鉄過剰状態との関連も指摘されているが、はっきりとした機序は不明である<sup>17)</sup>。本研究ではSVRを来した症例のうち、経時的な血清ALT値が追跡可能であった23例について検討したが、そのうち10例(43.4%)でPEG-IFN投与中に血清ALT値の再上昇を来した。全例SVR例であるため、PEG-IFN投与後に血清ALT値は正常化している。従って、この10例においては、治療中の血清ALT値上昇はPEG-IFNによる副作用であったと考えられ、従来のインターフェロンより血清ALT値上昇率は高いことが明らかになった。PEG-IFN単独療法において治療中に血清ALT値上昇を来した場合、それが本剤の影響によるものか他の原因によるものかの見極めが重要であると考えられた。

## 結 語

1. C型慢性肝炎に対するペグインターフェロン $\alpha$ -

2a単独48週治療の適応は、若年で肝線維化が軽度の症例である。また、治療前HCV RNA量がセロタイプ1型では400 KIU/ml未満、セロタイプ2型では500 KIU/ml未満の症例が良い適応である。

2. 治療開始後のSVR予測因子として、治療開始4週以内のHCV RNA定性陰性が有用である。

3. ペグインターフェロン $\alpha$ -2a単独療法中の血清ALT値の上昇はSVR例の43.4%に認められ、ペグインターフェロン $\alpha$ -2aの副作用と考えられた。

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## The prognostic factors of sustained virologic response among patients of chronic hepatitis C treated with peg-interferon alpha 2a monotherapy

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A combination of peginterferon plus ribavirin is recommended therapy for patients with chronic hepatitis C. However, this treatment may influence the occurrence of adverse events induced by ribavirin including anemia or birth defects. This study aimed to reveal the outcome and prognostic factors of peginterferon alpha-2a (PEG-IFN) monotherapy in patients with chronic hepatitis C in Japan. The study included eighty-four patients who were treated with PEG-IFN monotherapy. Fifty-six patients (66.7%) achieved sustained virological response (SVR) in the ITT analysis. When comparing SVR and non-SVR groups, SVR predictable parameters included treatment at a younger age ( $p = 0.0464$ ) and early staging of fibrosis ( $p = 0.0002$ ). In addition, the most predictable parameter of SVR was serum HCV RNA levels undetectable within 4 weeks after the beginning of the treatment in the multivariate analysis (OR : 17.2, 95% CI : 4.38–84.5,  $p = 0.0001$ ). We suggested that PEG-IFN monotherapy is beneficial for patients who are younger, have mild fibrosis, and lower HCV RNA levels before treatment (<400 KIU/ml in serotype 1, <500 KIU/ml in serotype 2), and who achieve serum HCV RNA undetectable within 4 weeks from beginning the PEG-IFN monotherapy.

**Key words:** chronic hepatitis C PEG-IFN monotherapy SVR (Sustained virological response)  
RVR (Rapid virological response) liver fibrosis

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## Original Article

## Early decline of hemoglobin can predict progression of hemolytic anemia during pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C

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**Aim:** Ribavirin, used to treat chronic hepatitis C, can induce hemolytic anemia, forcing the discontinuance of treatment. To establish a predictive measure to help circumvent this, we evaluated the relationship of hemoglobin (Hb) decline with the discontinuance of treatment during the progression of ribavirin-induced anemia.

**Methods:** One hundred and sixteen patients (71% male) with genotype 1 chronic hepatitis C were treated with pegylated interferon (PegIFN)  $\alpha$ -2b and ribavirin. The mean age was 50.6 years and 55% were IFN naïve. A decline of Hb concentration by 2 g/dL at two weeks from the start of the treatment ("2 by 2" standard) was adopted as the predictive factor for the progression of anemia.

**Results:** By applying the "2 by 2" standard, with  $\Delta$ Hb  $\geq$  2 g/dL (34%,  $n = 39$ ), treatment was discontinued in 12 cases (31%), three of which (8%) because of severe anemia. For

$\Delta$ Hb < 2 g/dL (64%,  $n = 76$ ), treatment was discontinued in 11 (14%) cases; none due to severe anemia. Ten percent (4/39) of patients showed the minimum Hb  $\leq$  8.5 g/dL in the  $\Delta$ Hb  $\geq$  2 g/dL group, with none in the  $\Delta$ Hb < 2 g/dL group ( $P = 0.001$ ). Furthermore, the patients with minimum Hb  $\leq$  8.5 g/dL were found only in the "2 by 2" standard-positive and low CLF (<15) group (4/29, 14%).

**Conclusion:** Monitoring the Hb decline using the "2 by 2" standard can identify patients who are prone to developing severe anemia. Further prospective studies are needed using ribavirin reduction based on the "2 by 2" standard.

**Key words:** "2 by 2" standard, chronic hepatitis C, pegylated interferon and ribavirin combination therapy, progression of anemia

## INTRODUCTION

THE AIM OF antiviral therapy for hepatitis C virus (HCV) is to obtain a sustained viral response (SVR) and to reduce the occurrence rate of hepatocellular

carcinoma or hepatic disease-related mortality.<sup>1,2</sup> The current optimal therapy for patients with chronic hepatitis C is a combination of pegylated interferon (PegIFN) and ribavirin. This combination can significantly improve the SVR rate and is recommended as a standard regimen worldwide.<sup>3–8</sup> However, the SVR rates for the combination therapy of ribavirin with PegIFN for naïve patients with HCV genotype 1 has been reported to be 42–52%,<sup>6,9,10</sup> which means that eradication of HCV is not complete in approximately half of these patients. Recently, long-term treatment<sup>11</sup> and a higher dosage

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of drugs<sup>12,13</sup> have been used to try to raise the SVR rate for patients with HCV genotype 1. However, it remains to be established what constitutes satisfactory efficacy. In this study we focused on a treatment strategy to enable the prediction of severe side-effects in order to avoid the need to discontinue treatment and raise the SVR rate by PegIFN and ribavirin combination therapy. It is important that ribavirin, the key drug for eradicating HCV, is continued until the end of treatment in order to attain the maximum SVR rate. Hemolytic anemia induced by ribavirin is known as one of the most important adverse effects in the combination therapy of PegIFN and ribavirin.<sup>14–17</sup> To decrease the discontinuance rate of ribavirin due to severe anemia, epoetin alfa has been used for patients with progressing anemia, which can maintain the dose level of ribavirin as well as the quality of life of the patients.<sup>18–20</sup> However, from a cost-effectiveness standpoint, it would be difficult for this treatment strategy to become standard. Also, side-effects other than anemia arising from an overload of ribavirin mainly due to renal dysfunction cannot be avoided by the additional administration of epoetin alfa.

Hemolysis induced by ribavirin has been suggested to be related to a high plasma concentration of ribavirin.<sup>21</sup> The apparent clearance of ribavirin (CL/F), which reflects its plasma concentration at four weeks after the start of combination therapy, has been used as a predictive factor for ribavirin-induced hemolytic anemia before the start of treatment.<sup>22–24</sup> However, the progression of hemolytic anemia occurs due not only to hemolysis, but also impaired hematogenous function. On the other hand, hemoglobin (Hb) dynamics directly reflect the degree of progression of anemia. We have reported that the early decline of Hb correlates with the progression of anemia during IFN and ribavirin combination therapy.<sup>25</sup> It is necessary to verify that a similar early predictor for the progression of anemia can be adopted in PegIFN and ribavirin combination therapy, since PegIFN is known to induce less depression of bone marrow function than usual IFN.

In this study, we evaluated the utility of the early decline of Hb in comparison with the CL/F to predict the progression of anemia in the combination therapy of PegIFN and ribavirin.

## METHODS

### Patients

THIS STUDY WAS conducted at 12 institutions in Japan. A total of 116 patients with chronic hepatitis C were enrolled and treated with a combination of

**Table 1** Patient characteristics

Age (years)	50.6 ± 10.1 (24–70)
Gender (male/female)	82/34 (male 70.7%)
Body weight (kg)	64.5 ± 11.1
Previous IFN therapy (naïve/relapser/no responder)	64/38/14
HCV-RNA level (IU/L) (<500/500–850/850<)	18/27/71
ALT (IU/L)	110 ± 60 (33–76)
Crnn (mg/dL)	0.9 ± 0.2
Liver histology	
Fibrosis (F1/F2/F3/unknown)	35/49/31/1
Activity (A1/A2/A3/A4)	15/33/56/12
WBC (/mm <sup>3</sup> )	5317 ± 1207
Neutrocytes (/mm <sup>3</sup> )	2778 ± 902
Platelets (×10 <sup>3</sup> /mm <sup>3</sup> )	17.4 ± 4.0
RBC (×10 <sup>6</sup> /mm <sup>3</sup> )	459 ± 41
Hemoglobin (g/dL)	14.5 ± 1.2

Data are given as the mean ± SD.

ALT, alanine transaminase; RBC, red blood cells; WBC, white blood cells.

PegIFN and ribavirin. All patients were anti-hepatitis C virus antibody positive, had HCV-RNA detectable in their serum by the polymerase chain reaction (PCR) method, and showed elevated serum alanine transaminase (ALT) (above the upper limit of the normal), serum Hb concentration  $\geq 12$  g/dL, neutrocytes  $\geq 1500/\text{mm}^3$  and platelets  $\geq 10^5/\text{mm}^3$  within six months before the treatment. Exclusion criteria were the presence of hepatitis B surface antigen, antihuman immunodeficiency virus antibody and other forms of liver disease (alcoholic liver disease, hepatotoxic drugs, autoimmune hepatitis).

The baseline characteristics of the patients are shown in Table 1. The mean age was 50.6 ± 10.1 years, and 71% (82 patients) were male. All patients had HCV-RNA with genotype 1 and high viral loads (more than 10<sup>5</sup> copies/mL serum by Amplicor-HCV monitor assay). The mean ALT level was 110 ± 60 IU/L. Sixty-four patients (55%) were IFN naïve and the others were undergoing retreatment.

### Treatment schedule

All patients were treated with a combination of PegIFN  $\alpha$ -2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA) and ribavirin (Rebetol; Schering-Plough) for 48 weeks. PegIFN was administered at a mean of 1.5  $\mu\text{g}/\text{kg}$  body weight subcutaneously once a week. Ribavirin was given orally twice a day for the total dose. Dosages of both medications were decided based on the

body weight of the patients: those with a body weight of 40–60 kilograms (kg) were given PegIFN 75 µg/body and ribavirin 600 mg/day, those with a body weight of 60–80 kg were given PegIFN 105 µg/body and ribavirin 800 mg/day, and those with a body weight of 80–100 kg were given PegIFN 135 µg/body and ribavirin 1000 mg/day. The PegIFN dose was reduced by 50% if the neutrocyte count was below 750/mm<sup>3</sup> or the platelet (Plt) count was below  $8 \times 10^3$ /mm<sup>3</sup>. The PegIFN was discontinued if the neutrocyte count was below 500/mm<sup>3</sup> or the Plt count was below  $5.0 \times 10^3$ /mm<sup>3</sup>. The ribavirin dose of 200 mg was reduced when the Hb concentration decreased to less than 10 g/dL and the ribavirin was discontinued when the Hb concentration decreased to less than 8.5 g/dL, in accordance with the drug information for ribavirin. No ferric medicine or erythropoietin to prevent anemia was administered.

Patients with persistently undetectable HCV-RNA six-months after the end of treatment were considered to have achieved SVR.

### Blood tests

All patients were examined for serum HCV-RNA level, hematological and biochemical tests just before therapy, at the end of week 2 and every four weeks during the treatment. When the treatment was completed, the patients were assessed every four weeks up to 24 weeks after the end of treatment.

### Total ribavirin clearance

Using the method of Kamar *et al.*, CL/F at the start of the treatment was calculated as follows:  $CL/F (L/h) = 32.3 \times BW \times (1 - 0.0094 \times age) \times (1 - 0.42 \times sex) / Scr$  (BW, body weight; sex = 0 for male and 1 for female; Scr = serum creatinine).<sup>17</sup>

### Definition of "severe anemia" leading to the discontinuance of ribavirin

In this study, the "discontinuance of ribavirin due to severe anemia" was defined as follows: discontinuance of ribavirin due to a decrease of Hb to less than 8.5 g/dL or clinical symptoms of anemia associated with a decrease of Hb of more than 3 g/dL from the start of the combination therapy.

### Statistical analysis

Age, body weight, ribavirin dosage/body weight, white blood cell count, red blood cell count, Hb concentration, Plt, serum ALT levels and serum creatinine are expressed as mean ± SD. The SVR rate was evaluated using the intention-to-treat analysis (ITT analysis). The

differences in proportions were tested by the  $\chi^2$ -test and Mantel-Haenszel  $\chi^2$ -test. A value of  $P < 0.05$  (two-tailed) was considered to indicate significance. All calculations were performed by SAS program 9.1 (SAS Institute, Cary, NC, USA).

## RESULTS

### Frequency and reasons for dose reduction or discontinuance of PegIFN and/or ribavirin

OF THE 116 patients, 92 completed 48 weeks of therapy, but 24 patients (21%) had to discontinue both PegIFN and ribavirin. Thirty-nine patients (34%) completed the entire treatment schedule without reduction or discontinuance of either drug. The ribavirin dose was decreased for 39 patients (34%) and the PegIFN dose was decreased for 33 patients (28%), including 19 patients for whom both drugs had to be reduced. The reasons for discontinuance of both drugs included anemia, thyroid dysfunction, skin eruption and neutropenia, with the major reasons being anemia (17%) and thyroid dysfunction (17%).

### Efficacy of the combination therapy with dose reduction or discontinuance of PegIFN and/or ribavirin

The SVR rate was 57% (66/116) for all according to ITT analysis. According to the category of response to previous IFN therapy, the SVR rates were 43% (6/14) in

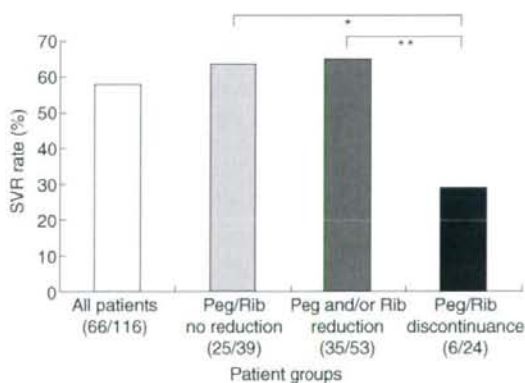


Figure 1 SVR rate due to PegIFN/ribavirin dose reduction or discontinuance. (□), All patients; (▨), patients without dose reduction; (▩), patients with dose reduction; (■), patients with drug discontinuance. Significant levels: \* $P = 0.003$ ; \*\* $P = 0.001$ .

**Table 2** Rate of the ribavirin reduction or discontinuance due to adverse effects according to CL/F level

	No reduction	Dose reduction	Discontinuance	
			All cases	Cases due to severe anemia
20 ≤ CL/F (n = 12)	67% (8/12)	25% (3/12)	8% (1/12)	0
15 ≤ CL/F < 20 (n = 23)	57% (13/23)	30% (7/23)	13% (3/23)	0
10 ≤ CL/F < 15 (n = 39)	46% (18/39)	31% (12/39)	23% (9/39)	5% (2/39)
CL/F < 10 (n = 42)	33% (14/42)	40% (17/42)	26% (11/42)	5% (2/42)

$P = 0.031$  (Mantel-Haenszel  $\chi^2$ -test).

**Table 3** Minimum hemoglobin levels during PegIFN/ribavirin combination therapy according to CL/F level

	10 g/dL < Hb	8.5 < Hb ≤ 10 g/dL	Hb ≤ 8.5 g/dL
20 ≤ CL/F (n = 12)	92% (11/12)	12% (1/12)	0
15 ≤ CL/F < 20 (n = 23)	83% (19/23)	17% (4/23)	0
10 ≤ CL/F < 15 (n = 39)	72% (28/39)	23% (9/39)	5% (2/39)
CL/F < 10 (n = 42)	50% (21/42)	43% (18/42)	7% (3/42)

$P = 0.009$  (Mantel-Haenszel  $\chi^2$ -test).

non-responders, 61% (23/38) in relapsers, and 58% (37/64) in naïve patients. The relationship between dose reduction or discontinuance of PegIFN and ribavirin and the SVR rate on IIT analysis is shown in Figure 1. Similar SVR rates were obtained in the groups without dose reduction of PegIFN and ribavirin (64%, 25/39) and with reduction of PegIFN and/or ribavirin (66%, 35/53); in detail, the SVR rate was 79% (11/14) in the group with reduction of only PegIFN, 55% (11/20) with reduction of only ribavirin, and 63% (12/19) with reduction of both PegIFN and ribavirin. In the group where both drugs were discontinued, the SVR rate was 25% (6/24), significantly lower than the group without reduction of both drugs ( $P = 0.003$ ), and the group with reduction of PegIFN and/or ribavirin ( $P = 0.001$ ).

#### CL/F and dose reduction or discontinuance of ribavirin

CL/F calculated for all patients showed a median of 12.6 L/h (range 4.5–27.9). At the start of the treatment, 36% (42/116) were under 10 L/h, 34% (39/116) were 10–15 L/h, 20% (23/116) were 15–20 L/h and 10% (12/116) were 20 L/h or more.

The rate of dose reduction or discontinuance of ribavirin is shown in Table 2 for different levels of CL/F. The rate of discontinuance of ribavirin in all cases was 8% (1/12) for the CL/F ≥ 20, 13% (3/23) for the 15 ≤ CL/F < 20, 23% (9/39) for the 10 ≤ CL/F < 15, and

26% (11/42) for the CL/F < 10 group. Ribavirin did not have to be discontinued due to severe anemia among patients with 15 ≤ CL/F, but did for the 18% (2/11) of those with CL/F < 10 and 22% (2/9) of those with 10 ≤ CL/F < 15. The rate of reduction and discontinuance of ribavirin correlated significantly with the CL/F level.

#### CL/F and minimum hemoglobin level during treatment

To examine the relationship between anemia and the cessation of ribavirin in further detail, we evaluated the minimum hemoglobin level during treatment. Table 3 presents the different levels in relation to CL/F. The patients with minimum Hb ≤ 8.5 g/dL, the criterion for discontinuance of ribavirin, accounted for 7% (3/42) of the group of CL/F < 10, and 5% (2/39) of the group of 10 ≤ CL/F < 15. No patients of the group of CL/F ≥ 15 showed minimum Hb ≤ 8.5 g/dL.

#### Early decline of Hb and progression of anemia during combination therapy

Following the initiation of combination therapy, the Hb concentration decreased rapidly until the end of four-weeks. At the end of two weeks, Hb had decreased by  $1.1 \pm 1.0$  g/dL among the patients without dose reduction of ribavirin ( $n = 53$ ),  $1.6 \pm 1.2$  g/dL among those with dose reduction ( $n = 39$ ), and  $1.8 \pm 1.0$  g/dL among



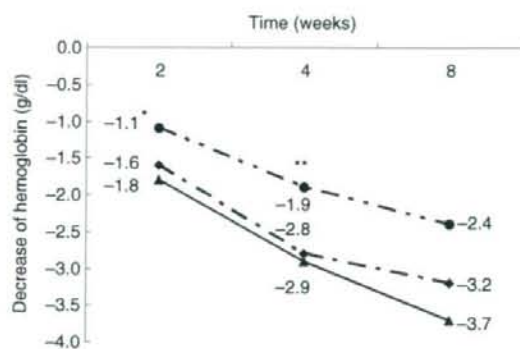


Figure 2 Course of  $\Delta$ Hb in the initial phase. (---), No reduction; (-·-·-), reduction; (—), discontinuance. \*Significantly different between patients with discontinuance and patients with no reduction ( $P=0.04$ ). \*\*Significantly different between patients with discontinuance and patients with no reduction ( $P=0.008$ ), and between patients with discontinuance and patients with reduction ( $P=0.003$ ).

those who had discontinued ribavirin ( $n=24$ ). It was significantly different between the patients with no reduction and those with discontinuance of therapy ( $P=0.04$ ). At the end of four weeks, Hb had decreased by  $1.9 \pm 1.2$  g/dL among the patients without dose reduction of ribavirin,  $2.8 \pm 1.2$  g/dL among those with dose reduction, and  $2.9 \pm 1.2$  g/dL among those who had discontinued ribavirin. Hb decline at the end of four weeks was significantly greater in the patients who had discontinued treatment and those who had reduced it, than in those with no reduction ( $P=0.008$ ,  $P=0.003$ , respectively) (Fig. 2).

In this study, we selected the Hb decrease at the end of two weeks as the predictive factor for anemia progression. This is because the judgment of Hb decrease at the end of four weeks is too late to prevent progression of anemia or to perform appropriate counter-measures, such as the administration of epoetin or reduction of ribavirin. Next, we tried to use two borderlines of  $\Delta$ Hb:

$\Delta$ Hb 2.0 indicates a 2 g/dL Hb decrease at the end of two weeks and  $\Delta$ Hb 1.5 indicates a 1.5 g/dL Hb decrease. When  $\Delta$ Hb 2.0 was adopted, the rate of discontinuance of drugs was 31% (12/39) in the  $\Delta$ Hb  $\geq 2.0$  and 14% (11/76) in the  $\Delta$ Hb  $< 2.0$ . When  $\Delta$ Hb 1.5 was adopted, it was 23% (14/60) in the  $\Delta$ Hb  $\geq 1.5$  and 16% (9/55) in the  $\Delta$ Hb  $< 1.5$ . Comparison of the  $\Delta$ Hb 2.0 and  $\Delta$ Hb 1.5 standards showed the sensitivity to be 52% (12/23) and 61% (14/23), and the specificity to be 71% (65/92) and 50% (46/92), respectively. With respect to discontinuance due to anemia, both  $\Delta$ Hb 2.0 and  $\Delta$ Hb 1.5 gave 100% sensitivity (3/3), and the specificities were 68% (76/112) using  $\Delta$ Hb 2.0 and 49% (55/112) using  $\Delta$ Hb 1.5. We decided to adopt the standard of  $\Delta$ Hb 2 g/dL at the end of two weeks from the start of the pegylated IFN and ribavirin combination therapy as the predictive factor for anemia progression ("2 by 2" standard), which has been taken as a predictive factor for anemia in the IFN and ribavirin combination therapy.<sup>25</sup>

Applying the "2 by 2" standard to PegIFN plus ribavirin combination therapy, the rate of reduction or discontinuance of the ribavirin dose was examined with respect to the Hb decrease level (Table 4). Only one patient was excluded from this study, because the treatment was discontinued on the 11th day. In the group of  $\Delta$ Hb (the decrease in Hb concentration at two weeks from the baseline)  $\geq 2$  g/dL ( $n=39$ ), the doses were reduced for 18 patients (46%) and discontinued for 12 (31%), three of whom (8%) had severe anemia. For the group of  $\Delta$ Hb  $< 2$  g/dL (76 patients), the doses were reduced for 21 patients (28%) and discontinued for 11 (14%); none due to severe anemia.

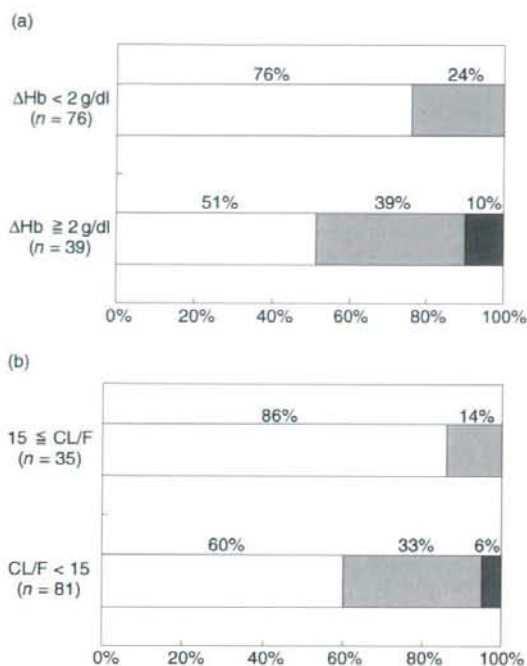
### Early decline of Hb and minimum hemoglobin level during treatment

As in the case of  $\Delta$ Hb, we evaluated the minimum hemoglobin level during treatment, as shown in Figure 3. The patients with minimum Hb  $\leq 8.5$  g/dL accounted for 10% (4/39) of the group of  $\Delta$ Hb  $\geq 2$  g/dL, and there was no patient with minimum Hb  $\leq 8.5$  g/dL.

Table 4 Rate of the ribavirin reduction or discontinuance due to adverse effects according to Hb decrease levels

	No reduction	Dose reduction	Discontinuance	
			All cases	Cases due to severe anemia
$\Delta$ Hb $< 2$ g/dL ( $n=76$ )	58% (44/76)	28% (21/76)	14% (11/76)	0
$\Delta$ Hb $\geq 2$ g/dL ( $n=39$ )	23% (9/39)	46% (18/39)	31% (12/39)	8% (3/39)

$P=0.004$  (Mantel-Haenszel  $\chi^2$ -test).



**Figure 3** Minimum hemoglobin levels during PegIFN/ribavirin combination therapy. (□), 10 g/dL < minimum Hb; (▒), 8.5 < minimum Hb ≤ 10 g/dL; (■), minimum Hb ≤ 8.5 g/dL. (a) According to the "2 by 2" standard (Hb 2 g/dL decrease at two weeks from the baseline).  $P = 0.009$  (Mantel-Haenszel  $\chi^2$ -test). (b) according to CL/F levels.  $P = 0.001$  (Mantel-Haenszel  $\chi^2$ -test).

in the  $\Delta\text{Hb} < 2 \text{ g/dL}$  group (Fig. 3a). The patients with minimum Hb ≤ 8.5 g/dL accounted for 6% (5/81) of the group of  $\text{CL/F} < 15$ , and there was no patient with minimum Hb ≤ 8.5 g/dL in the  $15 \leq \text{CL/F}$  group (Fig. 3b). The number of patients with minimum Hb ≤ 8.5 g/dL during PegIFN and ribavirin combination therapy according to "2 by 2" standard and CL/F levels is shown in Table 5. The patients with minimum Hb ≤ 8.5 g/dL were found only in the "2 by 2" standard-positive and low CL/F (<15) group (4/29, 14%).

## DISCUSSION

**P**REDICTION OF THE progression of anemia is necessary to decide whether drugs can be continued, with minimization of the disadvantages induced by anemia. Recently, CL/F has been used as a marker of

**Table 5** The number of patients with minimum hemoglobin ≤ 8.5 g/dL during PegIFN/ribavirin combination therapy according to "2 by 2" standard and CL/F levels

	$\Delta\text{Hb} < 2 \text{ g/dL}$ (n = 76)	$\Delta\text{Hb} \geq 2 \text{ g/dL}$ (n = 39)
$\text{CL/F} \geq 15$ (n = 35)	0/25	0/10
$\text{CL/F} < 15$ (n = 80)	0/51	4/29 (14%)

progressing anemia that necessitates discontinuance of treatment. For example, if the patients have a low CL/F level, they should start treatment with a low ribavirin dose. In this study, we attempted to use the CL/F level measurement for our patients. To predict which patients might have to discontinue the treatment, the target range had to be  $\text{CL/F} < 15$  because 6% of patients (n = 5) in this range showed minimum Hb ≤ 8.5 g/dL, which is the level at which ribavirin should be discontinued. No patients of the  $\text{CL/F} \geq 15$  group showed minimum Hb ≤ 8.5 g/dL. Our findings showed that 70% of the patients (81/116) with  $\text{CL/F} < 15$  should be discriminated from the others (Table 3). In the same manner, using  $\Delta\text{Hb}$  as the marker, 34% of the target patients in the  $\Delta\text{Hb} \geq 2 \text{ g/dL}$  group were identified because 10% in this range showed minimum Hb ≤ 8.5 g/dL. Compared to CL/F,  $\Delta\text{Hb}$  is considered to be more sensitive and convenient for identifying the high risk patients for whom treatment would need to be discontinued. Furthermore, the application of "2 by 2" standard in the group with low level of CL/F < 15 can be the most sensitive method for this (Table 5), since no patients with progression of anemia were found in the "2 by 2" standard-negative group with  $\text{CL/F} < 15$ .

In Japan, ribavirin doses are set at 600 mg for <60 kg, 800 mg for 60-80 kg, and 1000 mg for ≥80 kg, which are lower doses than those used in Europe and the USA. In this study, the mean ribavirin level at the start of treatment was 743 mg per day, while the AASLD practice guideline for genotype 1 hepatitis C is a daily dose of 1000 mg for body weight ≤ 75 kg and 1200 mg if >75 kg<sup>26</sup>. In Japan, the use of lower doses is why fewer patients treated with PegIFN and ribavirin combination therapy are forced to discontinue the treatment due to severe anemia. Since the "2 by 2" model and/or CL/F can identify the patients who are prone to develop severe anemia, the other patients could be candidates for ribavirin dose-up strategies to raise SVR rates.

A considerable number of patients with chronic hepatitis C are over 60 years old in Japan (mean age is

around 55 years old),<sup>27</sup> although the mean age of this study was 50.6 years old. The number of aged patients with chronic hepatitis C is expected to increase in Europe and the USA, as well as in Japan. In IFN and ribavirin combination therapy, the discontinuance rate due to anemia was significantly higher in aged patients ( $\geq 60$  years old, 21%) than in younger patients ( $< 60$  years old, 9%) ( $P < 0.001$ ).<sup>25</sup> Earlier prediction of anemia is necessary to reduce the ribavirin dose in order to prevent the progression of severe anemia or to start epoetin alfa administration as needed, especially with aged patients. The "2 by 2" standard in PegIFN and ribavirin combination therapy should be a useful and convenient device for predicting the progress of anemia and treatment discontinuance in Europe and the USA, as well as in Japan.

## CONCLUSION

**I**N CONCLUSION, THIS paper has shown that the SVR rate can be raised by preventing the discontinuance of ribavirin in PegIFN and ribavirin combination therapy. What is now needed is a prospective study of whether the early reduction of ribavirin in "2 by 2" standard-positive patients can improve the SVR rates, to ascertain the utility of the "2 by 2" standard in PegIFN and ribavirin combination therapy.

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## Declining Incidence of Hepatocellular Carcinoma in Osaka, Japan, from 1990 to 2003

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**Background:** Japan has the highest incidence rate of primary liver cancer attributed to chronic hepatitis C virus (HCV) infection among developed countries. Molecular clock analysis of HCV sequences revealed that the spread of HCV took place earlier in Japan than in other countries. This might influence recent temporal trends in hepatocellular carcinoma (HCC) incidence.

**Objective:** To characterize the contribution of HCV-related hepatocellular carcinoma (HCC) to recent changes in HCC incidence in Osaka, Japan.

**Design:** Population-based survey.

**Setting:** Osaka Cancer Registry and 10 hospitals in Osaka.

**Participants:** 63 862 patients with HCC that was diagnosed between 1981 and 2003 in Osaka Prefecture, including 5253 HCV-seropositive patients with HCC that was diagnosed between 1990 and 2003 at 10 hospitals.

**Measurements:** Incidence of HCC and estimated incidence rate of HCV-related HCC, measured by multiplying the prevalence of anti-HCV by the corresponding HCC incidence rate.

**Results:** Between 1981 and 2003, peak incidence of HCC among men age 50 to 59 years, 60 to 69 years, and 70 to 79 years occurred in 1986, 1995, and 2000, respectively, with marked downward trends thereafter (average annual change,  $-7.9$ ,  $-22.3$ , and  $-12.4$  per 100 000 persons, respectively). Similar trends were observed in women. Estimated sex- and age-specific incidence of HCV-related HCC (per 100 000 persons) decreased from 255 to 92 cases at the maximum in men age 60 to 69 years and from 61 to 34 cases in women age 60 to 69 years, whereas estimated incidence of non-HCV-related HCC did not change between 1990 and 2003.

**Limitation:** Infection was determined only by HCV seropositivity.

**Conclusion:** The incidence of HCC in Osaka started to decrease by 2000, mainly because of decreased HCV-related HCC.

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Primary liver cancer was the fifth most common cancer worldwide by 2000, with approximately 551 000 new cases recorded (1). In most countries, hepatocellular carcinoma (HCC) comprises 85% to 90% of primary liver cancer cases. With some exceptions, developed countries, including the United States, have been experiencing an increase in the incidence of primary liver cancer, considered to be due at least in part to increased prevalence of chronic hepatitis C virus (HCV) infection (2).

Japan has had one of the highest incidence rates of primary liver cancer among developed countries (age-standardized incidence rate in 1995, 25.5 per 100 000 men and 7.7 per 100 000 women) (3). Approximately 90% of liver cancer cases are HCC, which, in Japan, is mainly caused by chronic HCV infection rather than chronic hepatitis B virus infection (4). A recent report on the age-standardized incidence of primary liver cancer among Japanese men, which was calculated from 6 population-based

cancer registries, showed a sharp increase that started in the mid-1970s but leveled off in the mid-1990s (5). These distinctive trends were thought to be due to the spread of HCV infection, which began in the 1920s and increased after World War II (6–8). Thus, HCV penetrated Japan earlier than Spain, Egypt, the United States, the former Soviet Union, South Africa, and Hong Kong, as evidenced by molecular clock analysis of the sequences of HCV isolates (8). However, recent temporal trends regarding incidence rates of HCC and the contribution of HCV infection have not been clearly documented in the Japanese population.

We analyzed temporal trends for HCC incidence rates between 1981 and 2003 in Osaka Prefecture (population in 2005, 8.8 million) and interpreted these in the context of HCV infection rates.

### METHODS

#### Data Collection on Incident HCC Cases

We obtained data on incident HCC cases from the Osaka Cancer Registry, which was established by the Osaka Prefectural Government in 1962. The registry collects reports on patients with newly diagnosed cancer, including demographic and cancer-related information, from all medical institutions in Osaka Prefecture (9). These have been routinely supplemented by death certificates gathered

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by the Osaka Prefectural Government (9). For patients with cancer who were enrolled in the registry on the basis of their death certificate, we contacted the issuing hospital to obtain information on diagnosis and treatment and to establish the date of HCC incidence, which we determined to be the time of diagnosis at that hospital. We site-coded the data according to the International Classification of Diseases for Oncology, Third Edition (10). We included patients with HCC (codes 8170 through 8180). The protocol was approved by the ethics committee of the Osaka Medical Center for Cancer and Cardiovascular Diseases.

From 1981 to 2003, 48 166 men and 15 696 women with HCC were documented in the Osaka Cancer Registry. We calculated the annual age-standardized incidence rates of HCC (world population as a standard population) by sex between 1981 and 2003. To characterize temporal trends for HCC, we assessed 10-year, age-specific incidence rates of HCC between 1981 and 2003 in individuals age 50 to 79 years. We studied these particular age-specific rates because most HCV-related HCC cases in the Japanese population occur between the ages of 50 and 79 years (4). We used the annual population estimates from 1981 to 2003, which were based on the average population in each sex and age category for the Osaka Prefecture during the particular period, as denominators for calculating incidence rates. The annual population estimates were based on data from the 1980, 1985, 1990, 1995, 2000, and 2005 Japanese population censuses, with linear interpolation for the years in between.

#### Statistical Analysis

To identify years when a statistically significant change in the slope of the temporal trend in the incidence occurred, we applied the joinpoint regression model by using the Joinpoint Regression Program, version 3.0 (U.S. National Cancer Institute, Bethesda, Maryland). We assumed constant variance and uncorrelated errors (11) because we could not detect heteroskedasticity by the White test or autocorrelation by the Durbin-Watson test in men or women in any age group.

We computed the estimated slopes describing the average annual change of incidence rate per 100 000 persons and the corresponding 95% CIs for each trend by fitting a piecewise regression line to the rates, using calendar year as a regression variable. We used the permutation test method to identify years when a statistically significant change had occurred ( $P < 0.05$ ) and set the number of randomly permuted data sets at 4499. We set the number of joinpoints to a minimum of 0 and a maximum of 3 in the Joinpoint Regression Program.

#### Data Collection on Prevalence of HCV Infection among Patients with HCC

The Osaka Cancer Registry does not collect serologic data on HCV infection in the registered patients. Therefore, we used data on HCV seropositivity from patients with HCC that was diagnosed at 10 hospitals in Osaka

#### Context

Hepatitis C virus (HCV) infection in Japan began to spread during the 1920s, increased after World War II with an explosion in parenteral amphetamine use and paid blood donation, and decreased in the 1950s to 1960s with voluntary blood donation and penalties against amphetamine use. Evidence linking the trends in HCV infection to hepatocellular carcinoma rates in Japan is limited.

#### Contribution

Data from the Osaka Cancer Registry and 10 Osaka hospitals suggest that hepatocellular carcinoma rates began to decrease in 2000, mainly because of a decrease in HCV-associated cancer.

#### Implication

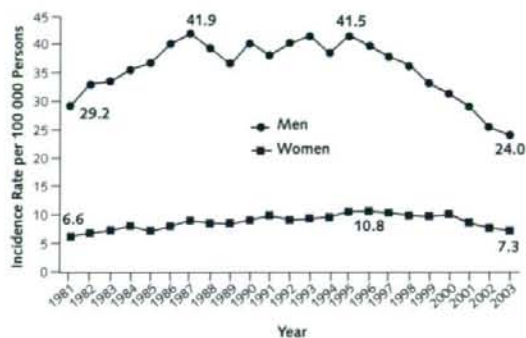
Control of HCV transmission within a population seems to be followed by a decrease in hepatocellular carcinoma.

—The Editors

Prefecture (1 university hospital, 2 cancer centers, and 7 general hospitals) to estimate the prevalence of HCV infection in patients with HCC. We considered the HCC diagnosis confirmed when the patient had positive histologic or positive radiologic results by enhanced computed tomography or hepatic angiography. We collected data on the patient's sex, date of birth, date of diagnosis between 1990 and 2003, first Chinese letter of the family name, and presence of hepatitis B surface antigen and antibody to hepatitis C (anti-HCV) as assessed by any commercially available kit. We did not collect the full first and family name for reasons of confidentiality. Because anti-HCV testing first became available in Japan in 1990, we collected data on patients whose HCC diagnosis was between 1990 and 2003. One investigator checked for duplication of the data set, because some patients might have been registered multiple times among the participating hospitals as a result of referrals and recurrence of HCC. We defined HCV-related HCC as occurring in patients who were HCV-seropositive at the time of diagnosis.

We calculated the sex-specific, age-specific (50 to 59, 60 to 69, or 70 to 79 years), and period-specific (1990 to 1992, 1993 to 1995, 1996 to 1998, 1999 to 2001, or 2002 to 2003) prevalences of HCV seropositivity for patients with HCC. We then multiplied prevalence rates by the corresponding strata of the HCC incidence rate obtained from the Osaka Cancer Registry data. Thus, we derived the denominators from the general population in Osaka through the denominators of the HCC incidence rate and obtained the numerators by multiplying the prevalence rates by the HCC incidence rate. We calculated the incidence rate of non-HCV-related HCC by subtracting HCV-related HCC from total HCC. Thus, we describe trends for the estimated incidence rates of HCV-related

**Figure 1.** Trends in age-standardized (world population) incidence of hepatocellular carcinoma in Osaka, Japan, 1981–2003.



and non-HCV-related HCC between 1990 and 2003 in Osaka Prefecture. We calculated the CI of the estimated rates by multiplying the lower and upper limits of the CI of the prevalence based on SE by the corresponding HCC incidence rate.

#### Role of the Funding Source

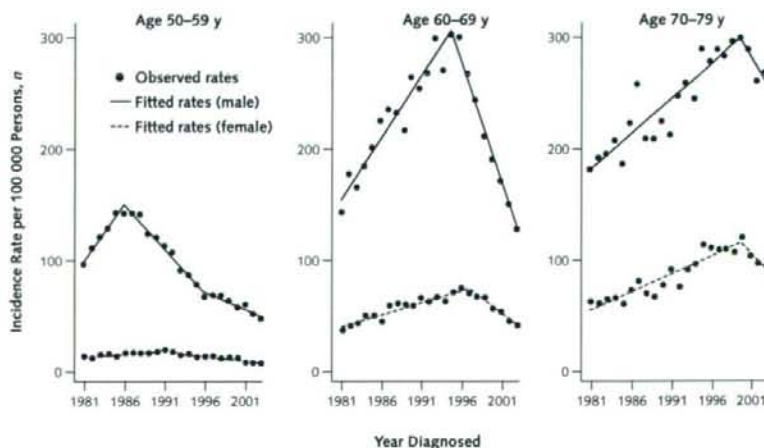
This study was supported by the Osaka Prefectural Government between 1990 and 2000 and Grants-in-Aid for Hepatitis Research of the Japanese Ministry of Health, Labor, and Welfare. There is no conflict of interest in the study. The funding sources had no role in the collection, management, or analysis of data.

#### RESULTS

The age-standardized incidence rate of HCC in men increased between 1981 and 1987 from 29.2 to 41.9 cases per 100 000 persons, then fluctuated until 1995. After that, it steadily decreased to 24.0 cases per 100 000 persons in 2003 (Figure 1). Among women, the age-standardized incidence rate of HCC increased between 1981 and 1996 from 6.6 to 10.8 cases per 100 000 persons, then gradually decreased to 7.3 cases per 100 000 persons in 2003 (Figure 1).

Figure 2 shows the trends in the incidence of HCC among men and women age 50 to 59 years, 60 to 69 years, and 70 to 79 years in Osaka between 1981 and 2003. The HCC incidence rate increased from 1981 to 1986 among men age 50 to 59 years, from 1981 to 1995 among men age 60 to 69 years, and from 1981 to 2000 among men age 70 to 79 years (average annual change of the incidence rate [per 100 000 persons], 10.0, 10.7, and 6.2, respectively) (Table 1). A striking downward trend occurred after the year of peak incidence in the 3 age groups (−7.9 until 1996, −22.3 until 2003, and −12.4 until 2003, respectively). Among men age 50 to 59 years, there was a second joinpoint (a change from rapid to moderate decrease) in 1996, resulting in a slope of −3.1 until 2003. Among women age 50 to 59 years, 60 to 69 years, and 70 to 79 years, the incidence rates of HCC peaked in 1991, 1997, and 2000, respectively (Table 1). The rates in women seemed to increase slightly from 1981 until the year of the joinpoint, with slopes of 0.43, 2.07, and 3.10, respectively. Thereafter, HCC incidence rates in women decreased through 2003 at a statistically significant average annual rate of −0.9, −5.7, and −7.9, respectively (Table 1).

**Figure 2.** Joinpoint analysis of the incidence rate of hepatocellular carcinoma among individuals age 50 to 79 years in Osaka, Japan, 1981–2003.



**Table 1. Joinpoint Analysis of the Hepatocellular Carcinoma Incidence Rate per 100 000 Persons in Osaka, Japan, 1981–2003**

Age Range	Peak Year	Incidence Rate per 100 000 Persons	Trend 1		Trend 2		Trend 3	
			Years	Slope (95% CI)	Years	Slope (95% CI)	Years	Slope (95% CI)
<b>Men</b>								
50–59 y	1986	142.0	1981–1986	10.0 (8.2 to 11.8)*	1986–1996	-7.9 (-8.6 to -7.1)*	1996–2003	-3.1 (-4.2 to -2.1)*
60–69 y	1995	299.6	1981–1995	10.7 (9.1 to 12.3)*	1995–2003	-22.3 (-26.0 to -18.6)*	-	-
70–79 y	2000	296.4	1981–2000	6.2 (4.8 to 7.5)*	2000–2003	-12.4 (-35.7 to 10.9)	-	-
<b>Women</b>								
50–59 y	1991	19.7	1981–1991	0.4 (0.2 to 0.7)*	1991–2003	-0.9 (-1.1 to -0.7)*	-	-
60–69 y	1997	68.5	1981–1997	2.1 (1.7 to 2.4)*	1997–2003	-5.7 (-7.3 to -4.1)*	-	-
70–79 y	2000	118.1	1981–2000	3.1 (2.5 to 3.7)*	2000–2003	-7.9 (-18.1 to 2.4)	-	-

\*  $P < 0.001$ .

Table 2 shows the prevalence of anti-HCV antibodies among 5253 patients age 50 to 79 years with HCC that was diagnosed at 10 hospitals in Osaka between 1990 and 2003. The prevalence was highest in men with HCC that was diagnosed in 1993 to 1995 (82.4%). The proportion of HCV-seronegative patients ranged from 18% to 29% through the observation period. The prevalence of anti-HCV was almost constant (81% to 83%) among women with HCC that was diagnosed between 1993 and 2003 (Table 2).

Figure 3 shows changes in the estimated incidence rate of HCV-related and non-HCV-related HCC from 1990 to 2003. Among men, the estimated incidence rate of HCV-related HCC steadily decreased among Osaka residents age 50 to 59 years from 83 (95% CI, 77 to 89) cases per 100 000 persons in 1990 to 1992 to 26 (CI, 21 to 30) cases per 100 000 persons in 2002 to 2003. Among men

age 60 to 69 years, incidence seemed to peak (255 [CI, 247 to 264] cases per 100 000 persons) from 1993 to 1995. Among men age 70 to 79 years, the incidence rate increased from 1990 to 1992 (169 [CI, 153 to 186] cases per 100 000 persons) to 1999 to 2001 (246 [CI, 234 to 258] cases per 100 000 persons) and leveled off afterward. The estimated incidence rate of HCV-related HCC among women age 50 to 59 years decreased from 12.4 (CI, 10.1 to 14.7) cases per 100 000 persons during 1990 to 1992 to 4.2 (CI, 2.5 to 5.8) cases per 100 000 persons during 2002 to 2003, whereas among women age 60 to 69 years, the incidence peaked (61 [CI, 57 to 64] cases per 100 000 persons) during 1996 to 1998. The trend in women age 70 to 79 years seemed to be similar to that in men of the same age: increasing during the 1990s and leveling off in the early 2000s (Figure 3). The estimated incidence rate of non-HCV-related HCC was lower than that of HCV-

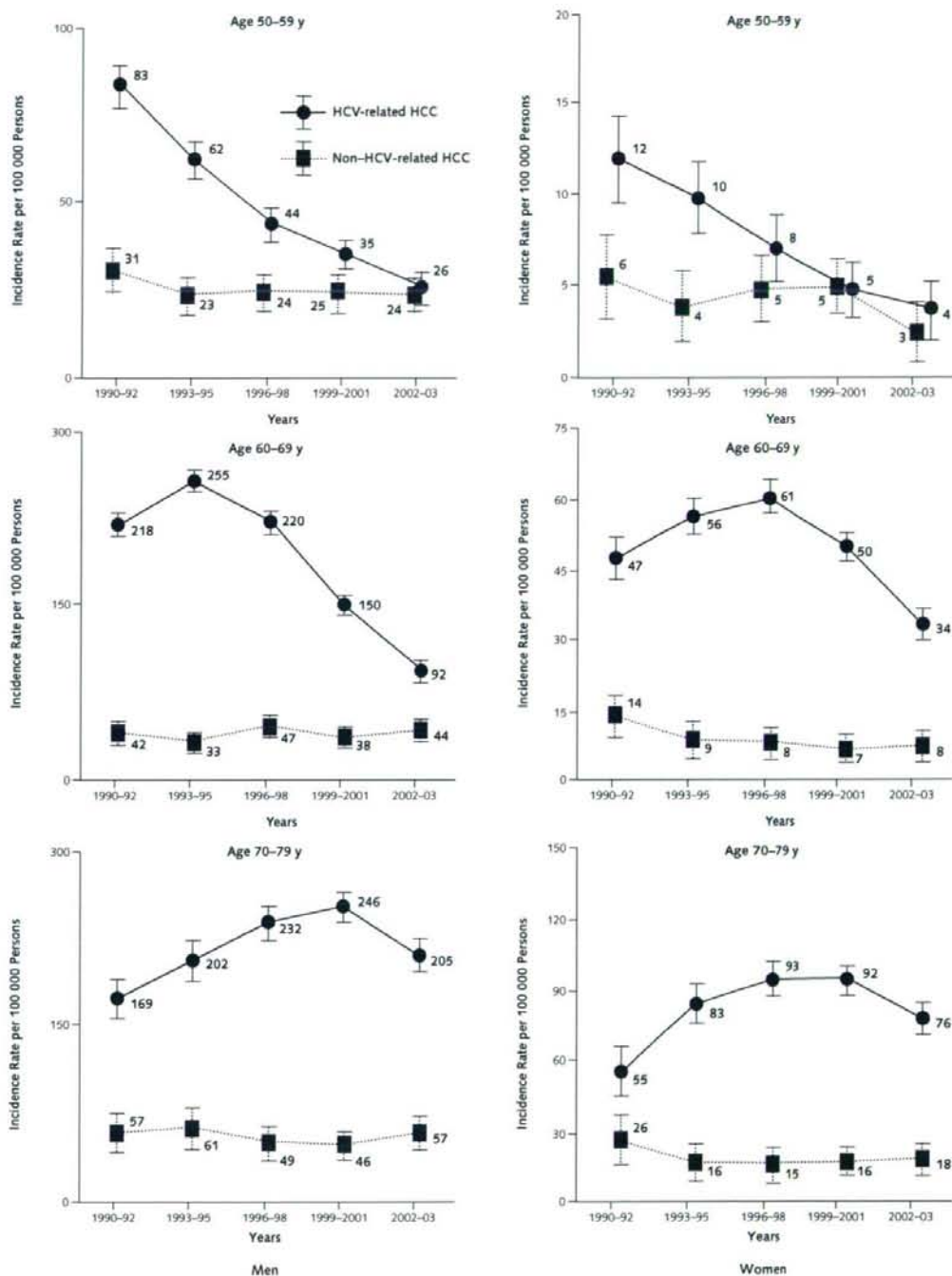
**Table 2. Prevalence of Anti-HCV among 5253 Patients Age 50 to 79 Years with Hepatocellular Carcinoma at 10 Hospitals in Osaka, Japan, 1990–2003\***

Variable	1990–1992		1993–1995		1996–1998		1999–2001		2002–2003	
	Patients, n	Prevalence (±SE), %	Patients, n	Prevalence (±SE), %	Patients, n	Prevalence (±SE), %	Patients, n	Prevalence (±SE), %	Patients, n	Prevalence (±SE), %
<b>Men</b>										
Anti-HCV(+)	602	78.3 ± 1.5	677	82.4 ± 1.3	651	78.7 ± 1.4	709	76.6 ± 1.4	385	70.9 ± 1.9
Anti-HCV(+) and HBsAg(+)	18	2.3 ± 0.5	17	2.1 ± 0.5	11	1.3 ± 0.4	16	1.7 ± 0.4	8	1.5 ± 0.5
Anti-HCV(+) and HBsAg(-)	584	75.9 ± 1.5	660	80.3 ± 1.4	640	77.4 ± 1.5	693	74.8 ± 1.4	377	69.4 ± 2.0
Anti-HCV(-)	167	21.7 ± 1.5	145	17.6 ± 1.3	176	21.3 ± 1.4	217	23.4 ± 1.4	158	29.1 ± 1.9
Anti-HCV(-) and HBsAg(+)	60	7.8 ± 1.0	57	6.9 ± 0.9	71	8.6 ± 1.0	106	11.4 ± 1.0	68	12.5 ± 1.4
Anti-HCV(-) and HBsAg(-)	107	13.9 ± 1.2	88	10.7 ± 1.1	105	12.7 ± 1.2	111	12.0 ± 1.1	90	16.6 ± 1.6
Total	769	100.0	822	100.0	827	100.0	926	100.0	543	100.0
<b>Women</b>										
Anti-HCV(+)	165	73.0 ± 3.0	211	82.7 ± 2.4	248	82.9 ± 2.2	274	80.8 ± 2.1	200	81.0 ± 2.5
Anti-HCV(+) and HBsAg(+)	8	3.5 ± 1.2	2	0.8 ± 0.6	5	1.7 ± 0.7	2	0.6 ± 0.4	2	0.8 ± 0.6
Anti-HCV(+) and HBsAg(-)	157	69.5 ± 3.1	209	82.0 ± 2.4	243	81.3 ± 2.3	272	80.2 ± 2.2	198	80.2 ± 2.5
Anti-HCV(-)	61	27.0 ± 3.0	44	17.3 ± 2.4	51	17.1 ± 2.2	65	19.2 ± 2.1	47	19.0 ± 2.5
Anti-HCV(-) and HBsAg(+)	21	9.3 ± 1.9	17	6.7 ± 1.6	29	9.7 ± 1.7	29	8.6 ± 1.5	18	7.3 ± 1.7
Anti-HCV(-) and HBsAg(-)	40	17.7 ± 2.5	27	10.6 ± 1.9	22	7.4 ± 1.5	36	10.6 ± 1.7	29	11.7 ± 2.0
Total	226	100.0	255	100.0	299	100.0	339	100.0	247	100.0

\* HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus.



**Figure 3.** Trends in estimated incidence rates of hepatitis C virus (HCV)-related and non-HCV-related hepatocellular carcinoma (HCC) in Osaka, Japan, 1990–2003.



Information on anti-HCV status only became available after 1989. Error bars indicate 95% CIs.

related HCC in most strata. We observed no distinctive changes in the temporal trends for non-HCV-related HCC during the study period.

## DISCUSSION

Our analysis of HCC incidence in the Japanese population between 1981 and 2003 identified calendar years in which significant changes in temporal trends occurred. The HCC incidence rates in men and women age 50 to 59 years peaked during 1986 and 1991, respectively; in men and women age 60 to 69 years during 1995 and 1997, respectively; and in men and women age 70 to 79 years in 2000. We also found that temporal trends for HCC incidence between 1990 and 2003 by age group were mainly determined by trends in the incidence rates of HCV-related HCC.

The most likely explanation for these observations is the particular mode of HCV transmission in Japanese society. According to a study on molecular tracing of endemic HCV (8), the exponential spread of HCV-1b infection, a dominant genotype of HCV in Japan, started in the 1920s. This was associated with treatment of *Schistosoma japonicum* beginning in 1921 (12). Later, HCV infection coincided with an increase in parenteral amphetamine use in the devastated country during and after World War II (6, 7). Subsequently, viral spread was considered to be amplified through blood transfusions and parenteral medical procedures in the 1950s and 1960s (6, 7). Data on first-time blood donor candidates in Osaka indicate that the prevalence of anti-HCV antibodies among those born in 1925 to 1935 was much higher (7% to 10%) than that in the younger generation born in 1936 to 1955 (13). It is plausible that Japanese people born between 1925 and 1935, who were adolescents in the early 1950s, were most susceptible to HCV transmission under these circumstances. Age groups with peak incidence of HCC in men and women in the current study (1986 and 1991, respectively, for 50 to 59 years; 1995 and 1997, respectively, for 60 to 69 years; and 2000 for 70 to 79 years) included the generation for which prevalence of anti-HCV was high in Osaka (born in 1925 and 1935) (13). Stiffening of legal penalties against amphetamine use starting in 1954 and conversion from paid to voluntary blood donation in the late 1960s may have reduced HCV transmission, thereby resulting in the lower prevalence of HCV infection in generations born after 1935. Indeed, the spread of HCV in Japan essentially ended by the early 1990s at the latest, as evidenced by the current very low incidence of HCV infection among repeat blood donors (14, 15). Better detection methods introduced in the early 1980s for HCC in patients with cirrhosis through ultrasonography and measurement of  $\alpha$ -fetoprotein may have contributed to the apparent increase in the incidence of HCC found in this study. However, the distinctive changes we observed in the age-specific incidence of HCC during the 1990s through

the early 2000s cannot be explained by the increased ability to detect HCC, because the different joinpoints in age-specific incidence rates would not be derived from a single period effect of detection of HCC.

Increases in the incidence of and deaths from liver cancer in the 1970s to 1990s have been reported in Japan (5, 16), Australia (2), the United Kingdom (17), France (2, 18), Italy (2, 18), and the United States (2, 19). The increases in Japan and the United States are attributable to increased seroprevalence of HCV (6, 13, 20, 21), whereas this relationship has not been clearly established in the other countries.

Certain limitations of this study should be considered. First, because cancer reporting in Osaka is not mandated by law, HCC could have been underreported. However, because it is fatal, most of the unreported cases should have been detected by examination of the death certificate. In addition, because the proportion of persons with HCC included only on the basis of their death certificate was almost constant (22% to 25%) during the observation period (22–24), such underreporting would not be expected to affect the temporal trends for HCC incidence rates shown in our study. Second, the proportion of HCV-seropositive patients among the 5253 cases diagnosed at 10 hospitals might differ somewhat from the entire cohort of patients with HCC in Osaka. However, all Japanese patients, including those with HCC, have easy access to hospitals because of the national medical insurance system, and the 10 participating hospitals did not select patients with HCC on the basis of their etiologic background. Therefore, it is realistic to suppose that selection bias on prevalence of anti-HCV among these 5253 patients would have been limited. Finally, the temporal trends seen in the present study might differ from those among the entire Japanese population. We previously reported age-specific incidence rates of liver cancer by birth year in Japanese men between 1962 and 1997 (5) by using 6 population-based cancer registries from Cancer Incidence in Five Continents (9) (registries for Miyagi, Yamagata, Osaka, Hiroshima, Saga, and Nagasaki). Our previous study found the peak incidence of HCC among those born between 1931 and 1935 (5). In addition, the age-dependent prevalence of anti-HCV among first-time blood donors in Osaka (13) was similar to those in other areas of Japan (25). These findings may indicate that the timing of the outbreak of HCV infection and its reduction were similar in the different geographic areas of the country.

In conclusion, our calculation of HCC incidence rates demonstrated that they are already decreasing in both sexes in Osaka, Japan. That the outbreak of HCV infection in Japan after World War II and its termination occurred earlier in Japan than in the rest of the world is the most likely explanation for these observations. These findings confirm that HCV-related HCC is a preventable disease that can be decreased by controlling parenteral HCV transmission. In the early 1990s, interferon therapy for patients

with chronic HCV infection was started in Japan to reduce the risk for HCC (26, 27). A nationwide, community-based anti-HCV screening system targeting individuals age 40 to 70 years was introduced by municipal governments in Japan in 2002. Further observation of the temporal trends of HCC incidence is needed to assess the efficacy of these interventions in Japan.

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