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Figure legends

Figure 1. Amplification of HBV DNA and covalently closed circular HBV in resected liver. M, Marker (*pBR322/AluI*); cccHBV, covalently closed circular HBV.

Figure 2. Clinical courses of 7 patients with HCC who remained HCV free after interferon treatment. White bar indicates the period from the end of interferon treatment to the diagnosis of HCC. HCV RNA was not detected in any patient after treatment. Small dotted bar shows tumor-free period after liver resection. Meshed bar shows period with tumor recurrence. Two patients died of their tumors, and one died of a myocardial infarction at operation.

Table 1. Clinical characteristics of 7 patients

Case	Gender	Age at operation	Anti-HBs/ Anti-HBc	Anti-HCV/ HCV-RNA	HCV genotype before IFN	Alcohol intake	Body mass index (kg/m2)	Period from end of IFN to diagnosis of HCC*
1	M	65	-/-	+/-	2a	none	23.1	13 mon
2	M	59	+/+	+/-	1b	rare	23.7	45 mon
3	M	65	+/+	+/-	2a	rare	23.7	19 mon
4	M	61	+/+	+/-	2a	none	23.6	20 mon
5	M	59	-/-	+/-	2b	rare	23.4	41 mon
6	M	66	+/+	+/-	2a	none	18.1	80 mon
7	M	60	-/-	+/-	2a	none	21.5	103 mon

Table 3. Histological findings and the existence status of HBV DNA in patients with HCC

Case	Tumor size (mm)	Tumor Histology	Resected liver (Stage)	Resected liver (Grade)	HBV DNA in serum	HBV DNA in liver	ccc HBV	HBV DNA integration
1	19 x 18	Poorly	2	1	-	-	-	-
2	60 x 45	Poorly	2	1	-	+	+	chr. 11q22-23
3	50 x 45	Moderate	4	2	-	-	-	-
4	21x19	Poorly	4	2	-	-	-	-
5	30 x 22	Moderate	1	2	-	+	+	chr. 22q11
6	16 x 18	Moderate	2	2	-	+	-	chr. 11q12
7	40 x 30	Poorly	1	1	-	+	-	chr. 11q13 and 14q32

3

Table 4. Relations between clinical findings and HBV DNA integration in HCC

	HCC with HBV DNA integration (n = 4)		HCC without HBV DNA integration (n = 3)		*p*
Age	61 ± 3		64 ± 2		0.295
Interval (month)	67 ± 30		17 ± 4		0.0364*
Tumor size (mm)	32 ± 15		29 ± 16		0.7688
Tumor Histology	Moderately D. HCC	2	Moderately D. HCC	1	
	Poorly D. HCC	2	Poorly D. HCC	2	
Fibrosis of resected liver	Stage 1	2	Stage 1	0	
	Stage 2	2	Stage 2	1	
	Stage 3	0	Stage 3	0	
	Stage 4	0	Stage 4	2	

Results are shown as means ± SD. *statistically significant.

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Table 2. Oligonucleotides used as PCR primers

Sense primers				Antisense primers			
Name		Nucleotide sequence	Position	Name		Nucleotide sequence	Position
HBX							
HB-1	5´	CTCTCTCGGAAATACACCTC	220 - 1239	B-2	5´	GTAACTCCACAGAAGCTCCA	1818 - 1799
HB-3	5´	TGCCAACTGGATCCTGCGCGGGACGTCCTT	264 - 1293	B-4	5´	GGCTTGAACAGTAGGACATG	1742 - 1723
HBS							
HB-5	5´	AAGACCTGCACGATTCCT	391 - 408	B-6	5´	TAGAGGTAAAAAGGGACTC	672 - 654
HB-7	5´	TTCGCAAGATTCCTATGGG	99 - 517	B-8	5´	GCCCCCAATACCACATCA	634 - 617
HBC							
HB-9	5´	AACTTTTTCACCTCTGCCT	690 - 1708	B-10	5´	GCTTGCCTGAGTGCTGT	1945 - 1929
HB-11	5´	ACTGTTCAAGCCTCCAAGC	1731 - 1749	B-12	5´	AAGGAAAGAAGTCAGAAGGC	1848 - 1829
cccHBV							
P23	5´	CTGAATCCCGCGGACGACCC	1443-1462	P24	5´	ACCCAAGGCACAGCTTGGAGG	1891-1871
P25	5´	GTCTGTGCCTTCTCATCTGCC	1553-1573	P26	5´	AGATGATTAGGCAGAGGTGAAAAA	1846-1823
Cassette-ligation							
X-5	5´	ACTCTACCGTCCCCTTCTTCATCTGCCGTT	1351-1380	C-1	5´	GTACATATTGTCGTTAGAACGCGTAATACGACTCA	
X-6	5´	CTCTTTACGCGGTCTTTTTGTCTGTGCCTTC	1404-1434	C-2	5´	CGTTAGAACGCGTAATACGACTCACTATAGGGAGA	

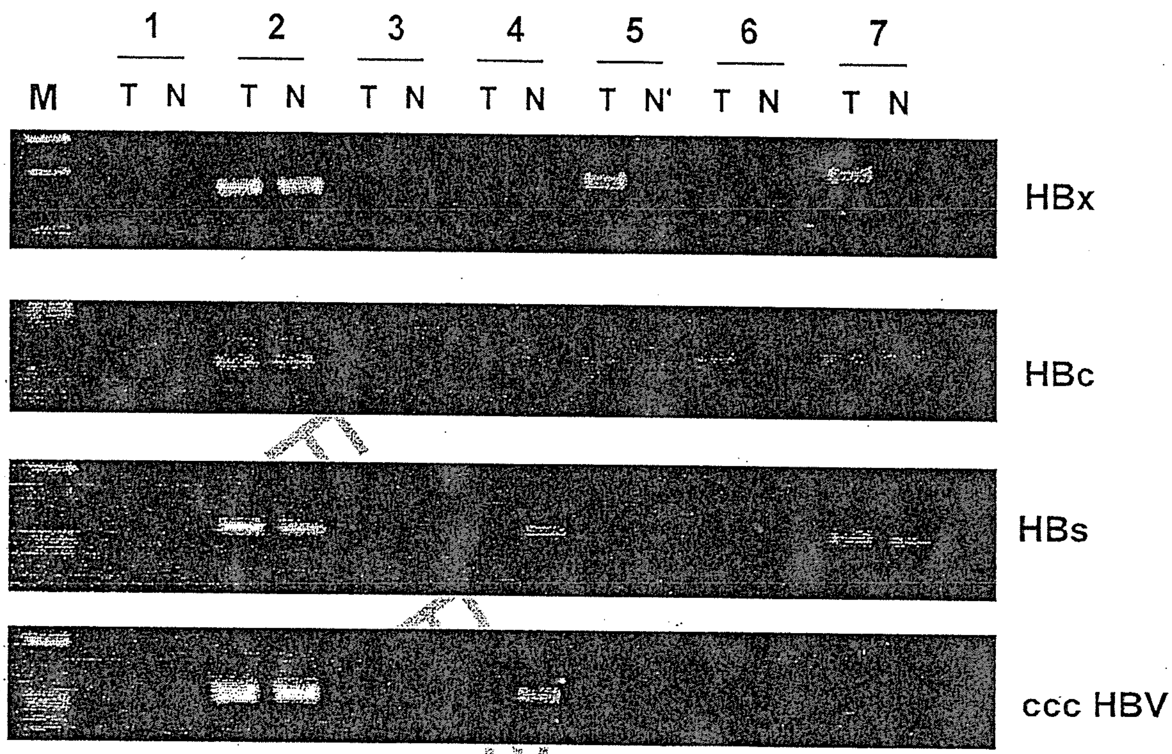

Figure 1

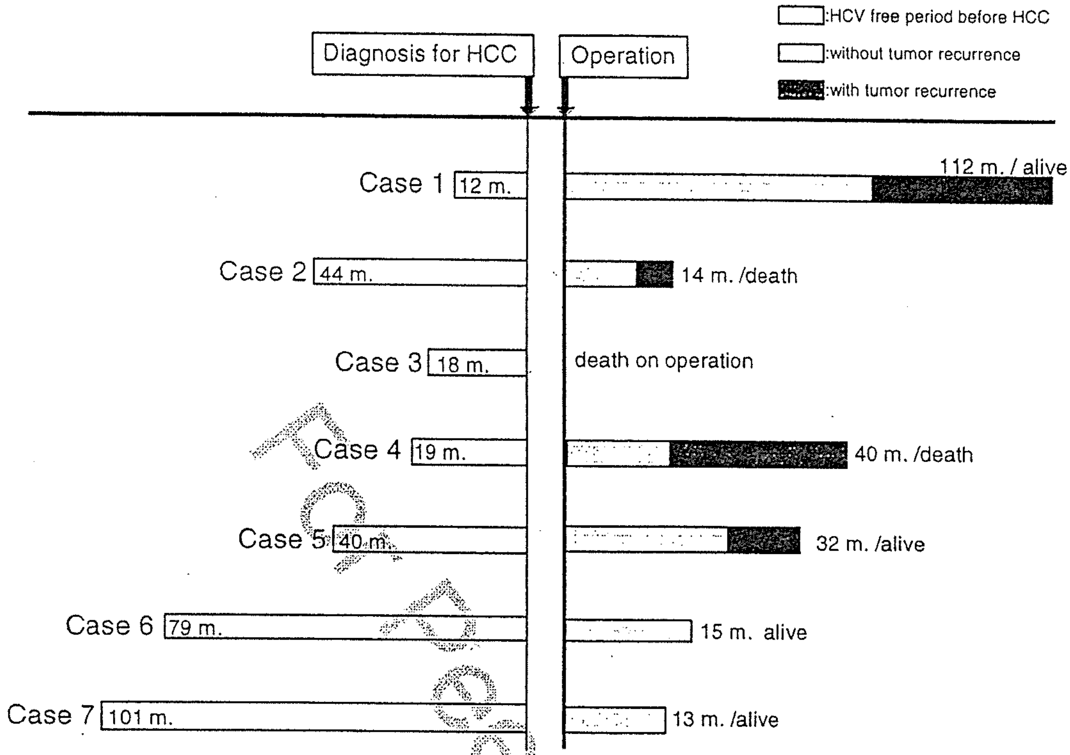
:HCV free period before HCC
:without tumor recurrence
:with tumor recurrence
Diagnosis for HCC
Operation
Case 1
12 m.
112 m. / alive
Case 2
44 m.
14 m. /death
Case 3
18 m.
death on operation
Case 4
19 m.
40 m. /death
Case 5
40 m.
32 m. /alive
Case 6
79 m.
15 m. alive
Case 7
101 m.
13 m. /alive

Figure 2

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LIVER

Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C

H Yoshida, R Tateishi, Y Arakawa, M Sata, S Fujiyama, S Nishiguchi, H Ishibashi, G Yamada, O Yokosuka, Y Shiratori, M Omata

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See end of article for authors' affiliations

Correspondence to: Dr H Yoshida, Department of Gastroenterology, University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113-8655, Japan; yoshida-2im@h.u-tokyo.ac.jp

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Background: An increase in the incidence of hepatocellular carcinoma (HCC) in Japan since the 1980s suggests an imminent outbreak in other countries where viral spread occurred more recently. Interferon therapy for chronic hepatitis C, in general, has been shown to prevent HCC.
Aims: To determine the scale of benefit in individual patients.
Subjects: Histologically proven chronic hepatitis C patients in the Inhibition of Hepatocarcinogenesis by Interferon Therapy (IHIT) cohort (*Ann Intern Med* 1999;**131**:174), as updated in March 2003.
Methods: The lifetime risk for HCC was calculated based on HCC incidence rates, stratified by sex, age, fibrosis stage, and outcome of interferon therapy. The gain in HCC free survival was defined as the difference between expected HCC free survival with sustained virological response and that without.
Results: The gain in HCC free survival was greater when a patient was younger and fibrosis was more advanced. For example, a 30 year old male with F3 fibrosis gained 12.4 years by attaining sustained response while a patient with F1 fibrosis older than 60 years gained less than one year. For a treatment protocol with a given sustained response rate, prior estimation of the gain can be obtained by multiplying the calculated HCC free survival for responders by the response rate.
Conclusions: The gain in HCC free survival may serve as an indicator of the benefit of interferon therapy in terms of HCC prevention and be useful in the consideration of indication and selection of treatment protocol for individual patients.

Various studies have indicated that a nationwide spread of hepatitis C virus (HCV) took place in Japan in the 1950s and 1960s.[1 2] As a result, there has been a rapid increase in hepatocellular carcinoma (HCC) incidence since 1980, now claiming more than 30 000 deaths each year. Simultaneously, there was a decline in the number of deaths assigned to cirrhosis. The decline may be partly due to advances in HCC diagnosis but the major cause seems to lie in the increasing risk of HCC as patients are getting older. The time lag of 30 years between the peaks of infection spread and HCC incidence corresponds to the observed interval between the time of blood transfusion and carcinogenesis in typical HCC patients.[3] These data strongly suggest an imminent outbreak of HCC incidence in other countries, including the USA, where HCV infection is thought to have been spread more recently.[4 5]

Shortly after the discovery of HCV in 1989,[6 7] interferon therapy was confirmed to be effective against HCV infection.[8–10] We and other groups have shown that interferon therapy significantly reduces the risk of HCC development among chronic hepatitis C patients.[11–15] In our previous study, 50% relative risk reduction, compared with untreated patients, was observed for conventional interferon monotherapy that showed an overall sustained virological response (SVR) rate of 33%, and a relative risk reduction to 20% was revealed among patients who achieved SVR.[15] As the antiviral efficacy of interferon therapy has been improved by recent advances such as combination with ribavirin[16–18] and introduction of pegylated interferons,[19–22] we can expect that the efficacy on HCC prevention has also been strengthened.

We have also shown that cirrhosis gradually resolves once SVR is achieved,[23] suggesting that interferon therapy will also prevent death due to liver failure or variceal rupture. However, HCC is clearly the dominant cause of death in patients with chronic hepatitis C, at least among our cohort in Japan where the average age is over 50 years and most patients abstain from heavy alcohol consumption.[24] Thus we have focused on HCC prevention as the primary object of interferon therapy.

Considering the current status of therapeutics, interferon therapy is clearly recommended only in a selected group of patients.[25] Since the benefit of antiviral therapy differs among individual patients, the indication as well as the choice of regimen should be decided based on the expected benefit for each patient. Quantification of benefit requires reasonable assessment of the lifetime risk of HCC and the expected reduction in it with treatment. In this study, we propose an indicator, the gain in HCC free survival, to quantify the benefit specific to individual patients. The value is calculated based on both life expectancy and individualised risk of HCC, and applicable to distinct protocols with varying efficacy. It may serve as the gold standard for the benefit of antiviral therapy in terms of HCC prevention.

MATERIALS AND METHODS

Incidence rates of HCC

Crude data were obtained from the IHIT (Inhibition of Hepatocarcinogenesis by Interferon Therapy) database,[15 23 24] as updated on 31 March 2003. Every patient underwent liver biopsy in 1990 or later, and liver fibrosis was staged according to the classification system of Desmet and colleagues.[26] Patients had no history of HCC, and were positive for HCV antibody and negative for hepatitis B surface antigen. We excluded those who developed HCC or dropped out of surveillance within one year after liver biopsy, and the start

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; SVR, sustained virological response; NNT, number needed to treat of observation was set at exactly one year after liver biopsy. Entry into the cohort was closed in 1999. The cohort population analysed in this study consisted of 2392 patients who received interferon monotherapy within one year of liver biopsy, and 395 patients who did not. Among 2392 interferon treated patients, 836 (34.9%) showed SVR, as determined six months after cessation of interferon administration. After undergoing liver biopsy, 90% of patients abstained from alcohol except for infrequent social occasions, and only 2% continued drinking alcohol (>80 g daily).

Patients underwent abdominal ultrasonography every 3–6 months, and contrast enhanced computed tomography was also performed every 6–12 months in patients with advanced fibrosis. A final diagnosis was made based on haemodynamic patterns on contrast enhanced computed tomography, abdominal angiography, or computed tomography during angiography. Ultrasound guided tumour biopsy was performed in ambiguous cases. The SVR group showed 27 events of HCC development during an observation period of 4767 person years; in the non-SVR group, 214 events in 9922 person years; and in the untreated group, 67 events in 2168 person years.

HCC incidence rates, stratified by age, sex, and fibrosis stage at entry, were calculated in each group by the person year method. Risk ratios were analysed using Cox proportional hazard regression. Age, as ranked by 10 years, and fibrosis stage were represented by dummy variables in the analysis. Adjusted HCC incidence rates were calculated so that the sum of squares of differences between the adjusted and observed values, weighted by the number of patients in each category, was minimised while conserving the risk ratios obtained by proportional hazard regression.

HCC free survival

The probability that a patient remains free of HCC at nth year of observation was calculated as:

$$(1-Q1)(1-P1)\times(1-Q2)(1-P2)\times(1-Q3)\times(1-P3)\times\ \ldots\ \times(1-Qn)(1-Pn)$$

where Qi is the age and sex specific death rate in the general population and Pi is the annual incidence of HCC specific to the patient in the ith year. Age and sex specific death rates were those published by the Ministry of Health, Welfare, and Labour of Japan for the vital statistics surveyed in 2000.[27] The gain in HCC free survival by interferon therapy was defined as the area between the cumulative HCC free survival curves. This model is based on an assumption that fibrosis stage remains constant with time (see model limitations in the results section).

Statistics

Values are expressed as mean (SD) unless otherwise specified. All statistical analyses were performed with SAS Software version 6.12. We used an original program coded in Object Pascal to calculate cumulative HCC free survival.

RESULTS

Incidence rates of HCC

Demographic data of the patients analysed in the current study are summarised in table 1, and observed HCC development and deaths are shown in table 2, illustrating that HCC was the major sequela among the cohort. Crude incidences of HCC did not differ between the untreated group and the non-SVR group in the corresponding category (data not shown). Patients with advanced fibrosis (stages F3 or F4) in the non-SVR group or in the untreated group showed a very high incidence rate. In fact, values obtained were greater than those found in 1999,[15] suggesting that the risk of HCC has increased with time. Fibrosis stage was determined at the time of liver biopsy and had possibly progressed during the observation period. As previously described, HCC incidence rates were substantially lower in the SVR group.

Cox proportional hazard regression analysis revealed that male sex, older age, and advanced fibrosis were associated with a higher risk of HCC, both in the non-SVR groups (table 3) and in the untreated group (data not shown). Multivariate analysis showed that the risk ratio of non-SVR to no treatment was 0.835 (95% confidence interval (CI) 0.625–1.1125; p = 0.2214). We previously showed that the risk of HCC was decreased in patients who showed normalised serum alanine aminotransferase levels in spite of continued viraemia after interferon therapy.[24] However, active hepatitis recrudesces not infrequently in a short period[28] and the effect on HCC prevention in those patients appears to decline in the long run. Thus we assumed that interferon therapy without attaining SVR had no effect on HCC prevention. Table 4 shows the incidence rates of HCC, as fitted to the crude data by the least squares method. These values were used in modelling of the estimated HCC free survival of individual patients.

HCC free survival

Using the fitted HCC incidence rates and the age and sex specific death rates, we estimated the lifetime cumulative HCC free survival with or without SVR. Figure 1 shows an example of a 30 year old male patient with chronic hepatitis C with stage F3 fibrosis. The area under the curve indicates the expected HCC free survival and the area between the two curves is the gain in HCC free survival when the patient achieves SVR. The gain, or the area, was calculated to be 12.4 years in this case.

We similarly calculated the gain in HCC free survival under various conditions (see fig 2, table 5). By definition, these values are applicable only after SVR has been achieved. The gain in HCC free survival that can be expected before the virological outcome is known is the product of the value in table 5 and the prior probability of achieving SVR.

The gain in HCC free survival was greater when a patient was younger or fibrosis was more advanced. Judging by the expected gain, indications for treatment are questionable in patients with fibrosis stage F0 or F1 and older than 60 years because they would gain less than one year even if they

Table 1 Demographic data for the patients analysed in the current study

		Interferon treated	
	Untreated	SVR	Non-SVR
No of patients	395	836	1556
Age (y)	55.0 (10.7)	47.7 (11.9)	50.5 (6.4)
Sex (M/F)	204/191	583/253	942/614
Fibrosis stage			
(F0-1/F2/F3/F4) (n)	128/141/42/84	278/331/173/54	440/568/381/167
(F0-1/F2/F3/F4) (%)	32/36/11/21	33/40/21/6	28/37/24/11

SVR, sustained virological response.

Table 2 Incidence of hepatocellular carcinoma (HCC) and death in the study cohort

		Interferon treated	
	Untreated	SVR	Non-SVR
No of patients	395	836	1556
Follow up (y)	6.5 (2.8)	6.7 (3.0)	7.4 (2.9)
HCC development (n)	67	27	214
Death (n)	33	11	89
With HCC (n)*	22	6	59
Hepatic deaths			
Without HCC (n)	4	1	8
Non-hepatic deaths (n)	7	4	22

*Includes deaths not directly related to HCC in patients who had developed the cancer.
SVR, sustained virological response.

attained SVR. On the other hand, patients with fibrosis stage F3 or F4 and younger than 50 years will gain more than 10 years with SVR, and more than five years even if the probability of attaining SVR is 50%.

Recently, the efficacy of interferon therapy has been improved by the introduction of peginterferon and ribavirin. However, more effective protocols will be accompanied by an increase in cost and incidence of untoward effects. These must be counterbalanced by an increase in expected benefit. While the increase in cost is the same, that in benefit is directly proportional to the values shown in table 5 and differs in each patient. The SVR rate for type 1b genotype high viral load infection was approximately 7% among the current cohort where six months of interferon monotherapy was the main protocol. The combination of peginterferon and ribavirin for 48 weeks, which is still under clinical trials in Japan, is expected to show a response rate of 40% or better for those patients. This difference (33%) corresponds to five years of the gain in HCC free survival in 40 year old patients with fibrosis stage F4 and to approximately one year in 60 year olds with fibrosis stage F2 (one third of the values given in table 5). Although these values are a hypothetical extrapolation, they may be clinically useful in choosing treatment protocols.

Model limitations

The model described in this article is based on several assumptions. Firstly, we assumed that interferon therapy that failed to achieve SVR had no beneficial effect, as described above, and this may result in underestimation of the benefit. However, the difference cannot be large: a 30 year old male with fibrosis stage F4 has a gain of 16.59 years instead of 15.98 years, and an 80 year old male with fibrosis stage F0/1 has a gain of 0.18 years instead of 0.15 years if we based the calculations on the incidence observed in the untreated group.

Secondly, we assumed that the benefit of interferon therapy was limited to HCC prevention. This is certainly an underestimation: successful interferon therapy improves liver function and may prevent death from liver failure. Several studies, failing to find an effect on HCC incidence, still indicated improvement in liver function with interferon therapy.[29 30] However, hepatic death without developing HCC was rare in the current cohort; one patient in the SVR group (variceal rupture (n = 1)), eight in the non-SVR group (liver failure (n = 4), variceal rupture (n = 3), not specified (n = 1)), and four in the untreated group (liver failure (n = 2), variceal rupture (n = 2)) died of a liver related cause without developing HCC, indicating annual mortality rates of 0.02%, 0.08%, and 0.18%, respectively (table 2). These values were small relative to the observed incidence of HCC.

Thirdly, we assumed that fibrosis stage remained constant, with the risk of HCC unchanged except for the increment due to aging. This may not be true: in fact, we previously indicated fibrosis progression in untreated patients and amelioration in interferon responders.[23] However, we did

Table 3 Annual hepatocellular carcinoma (HCC) incidence rates according to age and sex

Age (y)	F0/1	F2	F3	F4
SVR, male				
≤39	0.05% (0/65)	0.09% (0/59)	0.16% (0/14)	0.24% (0/4)
40–49	0.05% (0/57)	0.09% (0/66)	0.16% (1/29)	0.24% (0/9)
50–59	0.39% (0/38)	0.69% (3/62)	1.21% (5/51)	1.86% (1/18)
60+	0.70% (3/29)	1.18% (3/38)	2.01% (4/35)	3.20% (1/9)
SVR, female				
≤39	0.02% (0/32)	0.05% (0/38)	0.10% (0/7)	0.15% (0/1)
40–49	0.03% (0/25)	0.05% (0/23)	0.10% (0/3)	0.15% (1/1)
50–59	0.23% (0/20)	0.41% (1/33)	0.73% (1/20)	1.12% (1/6)
60+	0.40% (0/6)	0.71% (1/18)	1.25% (0/14)	1.93% (1/6)
Non-SVR, male				
≤39	0.05% (0/83)	0.13% (0/72)	0.28% (2/29)	0.56% (0/6)
40–49	0.35% (2/85)	1.00% (4/101)	2.16% (7/46)	4.26% (10/32)
50–59	0.82% (6/82)	2.33% (19/111)	5.06% (26/74)	10.0% (17/33)
60+	1.03% (4/36)	2.93% (13/59)	6.35% (17/64)	12.5% (15/29)
Non-SVR, female				
≤39	0.02% (0/37)	0.07% (0/21)	0.14% (0/10)	0.29% (0/2)
40–49	0.18% (0/41)	0.51% (2/44)	1.10% (3/18)	2.17% (0/6)
50–59	0.42% (1/53)	1.19% (8/96)	2.57% (19/80)	5.08% (5/32)
60+	0.52% (1/23)	1.49% (11/64)	3.23% (10/60)	6.37% (12/27)

The percentages indicate the annual incidence rates fitted by the least squares method using the risk ratios shown in table 4. Numbers in parentheses are the observed events/number at risk in each category.
SVR, sustained virological response; F0–F4, fibrosis stage.

Table 4 Risk of hepatocellular carcinoma (HCC) development

	Relative risk (95% CI)	
Factor	SVR	Non-SVR
Male v female	1.66 (0.67–4.13)	1.97 (1.48–2.62)
Age (y)		
≤39	1	1
40–49	1	7.61 (1.81–31.93)
50–59	7.67 (1.69–34.72)	17.84 (4.39–72.49)
60+	13.20 (2.93–59.53)	22.36 (5.48–91.26)
Fibrosis stage		
F0/1	1	1
F2	1.76 (0.47–6.67)	2.86 (1.59–5.13)
F3	3.10 (0.86–11.26)	6.19 (3.50–10.95)
F4	4.78 (1.13–20.18)	12.23 (6.81–21.95)

Relative risks were calculated by Cox proportional hazard regression separately in each group.

not have enough samples to calculate age stratified rates of fibrosis progression. The long term changes in HCC risk in interferon responders have not yet been clearly elucidated. Thus using the incidence rates observed in the seven year observation period was a compromise. All of the assumptions listed here may have underestimated, but not overestimated, the benefit of interferon therapy.

Lastly, we did not analyse the effect of alcohol consumption as there were very few heavy drinkers among the cohort. Alcohol is one of the major risk factors for HCC development and liver failure. The merit of successful interferon therapy may be greater in drinkers if they wish to continue alcohol. However, we recommend abstention to chronic hepatitis C patients whether or not they receive antiviral therapy.

DISCUSSION

To date, large scale cohort studies conducted in Japan, including ours, have unanimously indicated that by far the most important sequela of chronic hepatitis C is HCC development, and that interferon therapy significantly reduces its incidence. In contrast, several studies performed in Western countries found that HCC was less common, and interferon therapy did not have significant effects. The reason for this discrepancy has not been clarified. In this study, we showed that the risk of HCC substantially increased with age when patients of the same sex and fibrosis stage were compared (table 2). The prevalence of HCV infection in Japan is highly skewed to the older generations, and this may partly explain the high incidence of HCC in Japan. If this is the case, HCC incidence will increase substantially in Western countries in the near future, as it did in Japan in the 1980s.

The clinical importance of interferon therapy should be measured in terms of its efficacy in HCC prevention, at least in countries where HCC is the predominant complication of HCV infection. A popular indicator of efficacy of therapy in preventing a disease is the number (of patients) needed to treat (NNT), which is identical to the inverse of absolute risk reduction. Mathematically, NNT for one decrement in HCC development during a lifetime is equivalent to the life expectancy divided by the gain in HCC free survival. Supposing that the SVR rate is 100%, NNT is 3.92 (48.7/12.4; table 5) for a Japanese 30 year old male patient with fibrosis stage F3. This value should be divided by the expected SVR rate if the outcome is not known. As NNT is directly proportional to life expectancy, older patients have smaller values for NNT, indicating "higher efficacy", if the gain in HCC free survival is the same. This may not be intuitive for individualised consideration of indications for treatment.

Several authors have performed cost effective analyses of interferon therapy for chronic hepatitis C based on the Markov model.[31–35] In fact, our current model can be considered as a simplified Markov model where a chronic hepatitis C patient either achieves or does not achieve SVR with interferon therapy, and has the corresponding risk of HCC thereafter. Also, the HCC free survival is equivalent to the gain in quality adjusted life year, where a year before HCC development scores 1 and one after it scores 0. Owing to those simplifications, the model is not dependent on assumptive parameters but on observed data.

In conclusion, by using data obtained in a real cohort, we established an indicator of the benefit of interferon therapy—the gain in HCC free survival. This indicator may be useful in assessing the indications for interferon therapy and in selecting the best treatment protocol for individual patients.

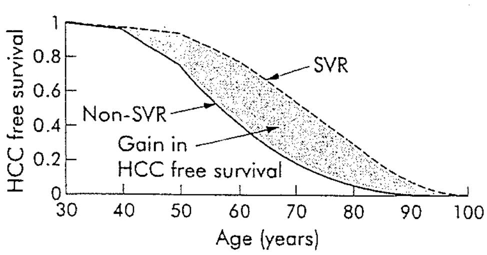

Figure 1 Gain in hepatocellular carcinoma (HCC) free survival by interferon therapy. The case of a 30 year old male patient with fibrosis stage F3. Cumulative HCC free survival curves were determined based on the patient specific HCC incidence rates and age and sex specific death rates in case of sustained virological response (SVR) and non-SVR. The area surrounded by the two curves indicates the gain in HCC free survival obtained by achieving SVR.

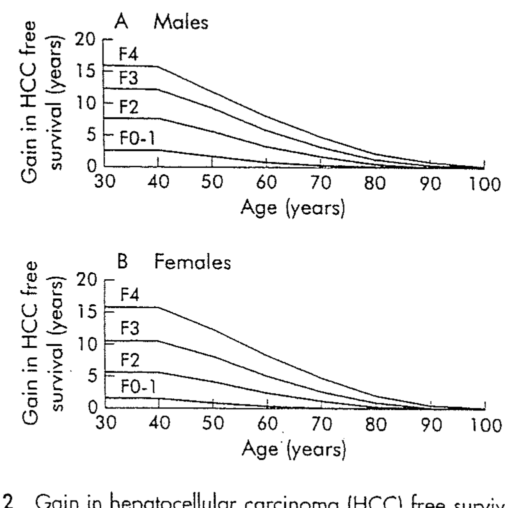

Figure 2 Gain in hepatocellular carcinoma (HCC) free survival by sustained virological response as a function of age and fibrosis stage.

Table 5 Gain in hepatocellular carcinoma (HCC) free survival by sustained virological response as a function of age and fibrosis stage

Age (y)	Life expectancy	F0/1	F2	F3	F4
Males					
30	48.7	2.48	7.66	12.40	15.98
40	39.1	2.52	7.68	12.41	15.96
50	29.9	1.68	5.75	9.45	12.14
60	21.4	0.84	3.38	5.95	8.14
70	14.0	0.40	1.70	3.26	4.98
80	8.0	0.15	0.67	1.40	2.38
Females					
30	55.3	1.45	5.60	10.52	15.73
40	45.5	1.46	5.61	10.51	15.69
50	36.0	0.93	4.24	8.17	12.44
60	26.9	0.44	2.52	5.17	8.39
70	18.2	0.22	1.30	2.81	4.95
80	10.6	0.08	0.52	1.18	2.24

Expressed in years, life expectancy was that in the Japanese general population in 2000. The gain in HCC free survival was the difference in expected cumulative HCC free survival with and without attaining a sustained virological response.

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Authors' affiliations

H Yoshida, R Tateishi, Y Shiratori*, M Omata, Department of Gastroenterology, University of Tokyo, Tokyo, Japan
Y Arakawa, Third Department of Internal Medicine, Nihon University, Tokyo, Japan
M Sata, Second Department of Internal Medicine, Kurume University, Fukuoka, Japan
S Fujiyama, Third Department of Internal Medicine, Kumamoto University, Kumamoto, Japan
S Nishiguchi, Third Department of Internal Medicine, Osaka City University, Osaka, Japan
H Ishibashi, Department of Clinical Research, National Nagasaki Medical Centre, Nagasaki, Japan
G Yamada, Centre of Liver Disease, Kawasaki Hospital Kawasaki Medical School, Okayama, Japan
O Yokosuka, First Department of Internal Medicine, Chiba University, Chiba, Japan

*Present address: Department of Gastroenterology, Hepatology, and Infectious Diseases, Okayama University, Okayama

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Effects of ribavirin combined with interferon-α2b on viral kinetics during first 12 weeks of treatment in patients with hepatitis C virus genotype 1 and high baseline viral loads

M. Enomoto,[1] S. Nishiguchi,[1] M. Kohmoto,[1] A. Tamori,[1] D. Habu,[1] T. Takeda,[1] S. Seki[1] and S. Shiomi[2] [1]*Department of Hepatology, Graduate School of Medicine, Osaka City University Medical School, Osaka; and* [2]*Department of Nuclear Medicine, Graduate School of Medicine, Osaka City University Medical School, Osaka, Japan*

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SUMMARY. This study aimed to find how ribavirin increases viral disappearance in patients with hepatitis C virus (HCV) of genotype 1 and high baseline viral loads (>5.0 × 10^5 copies/mL) when given with interferon (IFN). Using the real-time quantitative polymerase chain reaction, we measured serum HCV in 20 patients during the first 12 weeks of therapy with IFN-α2b and ribavirin. Controls were 10 similar patients given IFN-α2b alone. IFN-α2b was given at 6 MU daily for 2 weeks, and then three times weekly. Ribavirin was given at 600 or 800 mg daily. Serum HCV RNA decreased rapidly in the first phase, during the first 24 h of therapy (day 0), and more slowly in the early second phase (days 1–14). The median decrease was by 1.41 and 0.078 log 10/day in these two phases in the combination therapy group, and 0.90 and 0.081 log 10/day in the monotherapy group. The difference between groups in the first phase was not significant ($P = 0.24$), nor was that in the next phase ($P = 0.68$). Later in the second phase, between days 14 and 84, the median decrease was larger in the combination therapy group (0.030 log 10/day) than in the monotherapy group (0.015 log 10/day, $P = 0.035$). In patients with HCV genotype 1 and high viral loads, the effects of ribavirin with IFN-α appeared slowly, after the earliest days of treatment. A long-term favourable outcome of combination therapy may be associated with a rapid viral decline in this later phase of therapy.

Keywords: hepatitis C, interferon, polymerase chain reaction, ribavirin, viral kinetics.

INTRODUCTION

Hepatitis C virus (HCV) infects some 170 million people worldwide and is an important health care problem [1]. Persistent infection with HCV often progresses to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma over the course of several decades. As the report by Hoofnagle *et al.* [2] in 1986 on the effects of interferon (IFN) therapy on chronic hepatitis C, this drug has been the only approved agent for eradication of HCV and perhaps reduction of the incidence of hepatocellular carcinoma [3–5]. Several factors identifiable before therapy are independent predictors of the response to IFN, including the baseline level of serum HCV [6,7] and the HCV genotype [8]. In addition, analysis of the changes in HCV titres in the early part of IFN treatment is useful for prediction of the therapeutic response. After a delay of 7–10 h after IFN administration starts, the amount of viral RNA declines rapidly, with an estimated half-life of 5.0–7.2 h, during the first 1 or 2 days of therapy, and then declines more slowly [9,10]. In patients with HCV genotype 1, viral decline in the early phase of IFN treatment is slower than that in patients with genotype 2 [11], which may explain in part their lower rate of sustained virological response. We reported earlier [12] that the changes in serum levels of HCV genotype 1 during the first 2 weeks of IFN-α treatment can be used for prediction of the long-term outcome of therapy.

Abbreviations: HCV, hepatitis C virus; IFN, interferon; TaqMan PCR, real-time quantitative polymerase chain reaction; PEG, polyethyleneglycol.

Correspondence: Dr Shuhei Nishiguchi, Department of Hepatology, Graduate School of Medicine, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan.
E-mail: snishiguch@med.osaka-cu.ac.jp

Ribavirin is a synthetic guanosine nucleoside analogue that inhibits the replication of various RNA and DNA viruses. In patients with chronic hepatitis C, the combination of IFN-α and ribavirin gives a higher rate of sustained virological response than IFN-α alone [13–17]. The combination has become the standard therapy, especially for patients with HCV genotype 1 and high baseline viral loads. However, the

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synergistic effects of ribavirin when combined with IFN on the changes in HCV levels have not been fully identified. Zeuzem *et al.* reported that viral kinetics do not differ between patients treated with IFN-α alone and those given ribavirin also [18]. One possible reason for this unexpected finding may be that viral kinetics could be analysed in that study only in the first few weeks of therapy. That is, patients with genotype 2 or with low viral loads were included, and the analytical sensitivity of the assay used to measure HCV RNA was limited, so viral RNA became undetectable in most subjects within the first 4 weeks of therapy. Pharmacokinetic studies showed that serum ribavirin concentrations take 4–8 weeks to reach a plateau [19,20]; the synergistic effects of this drug with IFN might become evident slowly, after the earliest phase of treatment. To test this hypothesis, a study of the kinetics of HCV in patients with genotype 1 and high viral loads monitored by a more sensitive quantitative method during a longer term of therapy with IFN alone and in combination with ribavirin is needed.

The aim of this study was to find how ribavirin increases viral decline in patients with chronic HCV infection when given with IFN-α. Using a sensitive real-time quantitative polymerase chain reaction (TaqMan PCR), we monitored serum HCV levels in patients with genotype 1 and high viral loads during the first 12 weeks of combination therapy with IFN-α2b and ribavirin. The results were compared with those in patients with similar baseline characteristics treated with IFN-α2b alone.

MATERIALS AND METHODS

Patients

The subjects were 20 patients with chronic hepatitis C (13 men and seven women; mean age, 55 ± 11 years) who started combination therapy with IFN-α2b and ribavirin at our hospital between March 1999 and December 2002. Ten patients with chronic hepatitis C (six men and four women; mean age, 56 ± 9 years) who were treated with IFN-α2b alone between October 1998 and July 2001 were used as historic controls. The inclusion criteria were as follows: persistent elevation of serum alanine aminotransferase activity for at least 6 months before therapy, presence of genotype 1 of HCV in serum, presence of serum HCV RNA of more than 5.0×10^5 copies/mL by TaqMan PCR, absence of serum hepatitis B surface antigen and signs of other likely causes of chronic liver disease, histological features of chronic hepatitis found in liver biopsy specimens taken within 6 months before the start of therapy, and no evidence of hepatocellular carcinoma on ultrasonography or computed tomography. Serum samples were obtained from the patients before the administration of the drug(s) on the first day of therapy (day 0) and on days 1, 7, 14, 28, and 84, and were stored at −80 °C before being tested. Procedures of the study were in accord with the Helsinki Declaration of 1975 (1983 revision) and were approved by our hospital ethics committee.

Treatment

Patients were treated with recombinant IFN-α2b (Intron A, Schering-Plough, Kenilworth, NJ, USA) by intramuscular injection at the dosage of 6 MU every day for 2 weeks, followed by 6 MU three times a week for 22–46 weeks. Ribavirin (Rebetol; Schering-Plough) was given orally twice a day at a total daily dose of 600 mg for the 10 patients who weighed 60 kg or less and 800 mg for the remaining 10 patients who weighed more than 60 kg for 24 weeks. The most common adverse effect of ribavirin is haemolysis. The dose of ribavirin was reduced by 200 mg per day in patients whose haemoglobin concentrations fell below 10 g/dL.

Assays

Routine haematological and biochemical tests were performed by the standard procedures. Genotypes of HCV were identified by direct sequencing of the amplification products generated during the Amplicor Monitor test (Roche Diagnostics, Branchburg, NJ, USA) [21] with an ABI 3700 DNA sequencer (Perkin Elmer Corp./Applied Biosystems, Foster City, CA, USA) [22]. Serum HCV RNA was measured by TaqMan PCR as described by Takeuchi *et al.* [23]. In brief, HCV RNA was extracted from 250 mL of a serum sample, converted to complementary DNA with reverse transcriptase, and amplified by PCR with a TaqMan EZ RT-PCR kit and the ABI Prism 7700 sequence detection system (Perkin Elmer). A TaqMan probe, labelled with fluorescent reporter and quencher dyes, annealed specifically to the template between the primers and then was digested during the PCR, resulting in the emission of fluorescence. Successive PCR cycles exponentially amplified the PCR product and increased the fluorescence intensity. The detection range of the assay was between 2.0×10^2 and 1.0×10^9 copies/mL of HCV RNA. For comparison, a second generation version of the Amplicor Monitor test [21] was used also to measure HCV RNA in serum. The detection range of the assay was between 0.5 and 500 kIU/mL (a standard sample containing 10^5 copies/mL of HCV was assigned a titre of 10^5 IU/mL).

Histology

Liver biopsy was performed for each patient within 6 months before the start of therapy. The histopathological findings were assessed by grading of inflammatory activity and staging of fibrosis by the classification of Desmet *et al.* [24] by an experienced pathologist blinded to the clinical data.

Statistical analysis

Statistical analysis was performed with the Statview SE + Graphics program, version 5.0 (SAS Institute, Cary, NC, USA). Distributions of continuous variables were ana-

Table 1 Baseline characteristics of patients with chronic hepatitis C treated with IFN-α2b with or without ribavirin

Characteristics	Combination therapy group (*n*= 20)	Monotherapy group (*n*= 10)	*P*-value
Age (years)	55 ± 11	56 ± 9	0.96
Sex (M/F)	13/7	6/4	0.93
Previous IFN treatment (+/–)	13/7	6/4	0.93
Haemoglobin (g/dL)	14.3 ± 1.2	14.2 ± 1.5	0.86
ALT (IU/L)	102 (74–135)	94 (73–109)	0.64
HCV RNA (log 10 copies/mL)	7.07 ± 0.35	6.78 ± 0.51	0.11
Grade of inflammation			
Mild	11	7	0.52
Moderate	8	3	
Severe	0	0	
Stage of fibrosis			
Mild	8	6	0.42
Moderate	5	3	
Severe	6	1	

Values represented as mean ± SD for normally distributed variables, and medians (with the interquartile range) for non-normally distributed variables.
Serum HCV RNA was measured by TaqMan PCR.
IFN, interferon; ALT, alanine amino transferase; HCV, hepatitis C virus.

lysed by the Mann–Whitney *U*-test. Differences in proportions were tested by Fisher's exact test. The significance of correlation was evaluated by Spearman's rank analysis. A two-tailed *P*-value of <0.05 was taken to indicate statistical significance.

RESULTS

Baseline characteristics of patients

The baseline characteristics of patients in the two groups were similar (Table 1). All patients were infected with genotype 1b of HCV, which is the most common kind in this country. In one patient in the combination therapy group, the biopsy sample was too small for evaluation, except for the finding of chronic hepatitis.

Changes in HCV RNA in first 12 weeks of treatment

In the first 12 weeks of treatment, no patient needed reduction in the dose of IFN-α2b. The dose of ribavirin was reduced in one patient at week 10, because the haemoglobin concentration was <10 g/dL. The proportions of patients without HCV RNA detectable by the Amplicor Monitor test and by TaqMan PCR at different times during therapy are shown in Tables 2 and 3, respectively. Of the patients in whom serum HCV RNA decreased to under the detection limit, none had relapse of viraemia during the 12 weeks. Changes in serum HCV RNA as monitored by TaqMan PCR during the first 12 weeks of therapy are shown in Fig. 1. As previously reported, serum HCV levels decreased rapidly during the first 24 h of therapy and more slowly thereafter. For the analysis here, we tentatively defined the period between 0 and 24 h of therapy (day 0) as the first phase, the period between days 1 and 14 as the early second phase, and the period between days 14 and 84 as the late second phase (see Discussion).

Decline of HCV RNA in different phases of treatment

The rate of decrease in serum HCV RNA as monitored by TaqMan PCR in each phase of treatment with IFN-α2b alone

Table 2 Patients without detectable serum HCV RNA by Amplicor Monitor test four times during treatment

Group	*n*	Numbers (%) on Day 7	Day 14	Day 28	Day 84
Combination therapy	20	0 (0)	3 (15)	8 (40)	13 (65)
Monotherapy	10	0 (0)	0 (0)	0 (0)	6 (60)

Table 3 Patients without detectable serum HCV RNA by TaqMan PCR four times during treatment

Group	*n*	Numbers (%) on Day 7	Day 14	Day 28	Day 84
Combination therapy	20	0 (0)	1 (5)	3 (15)	12 (60)
Monotherapy	10	0 (0)	0 (0)	0 (0)	4 (40)

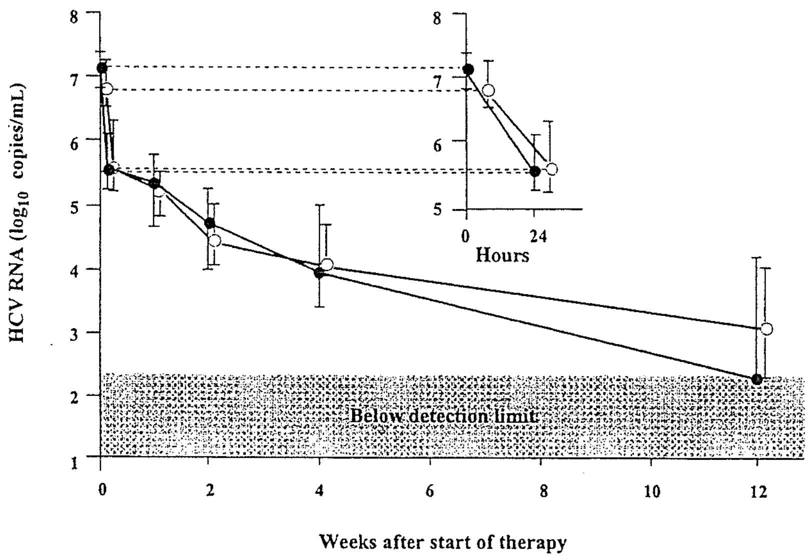

Fig. 1 Changes in serum hepatitis C virus (HCV) RNA during the first 12 weeks of therapy: (●) in 20 patients treated with interferon (IFN)-α2b in combination with 600 or 800 mg of ribavirin daily depending on body weight, and (○) in 10 patients treated with IFN-α2b alone. IFN-α2b was given at the dosage of 6 MU every day for 2 weeks, followed by 6 MU three times a week. Serum HCV RNA was measured by TaqMan PCR. Values are medians, with bars showing the interquartile range. The lower detection limit of TaqMan PCR was 2.0×10^2 copies/mL.

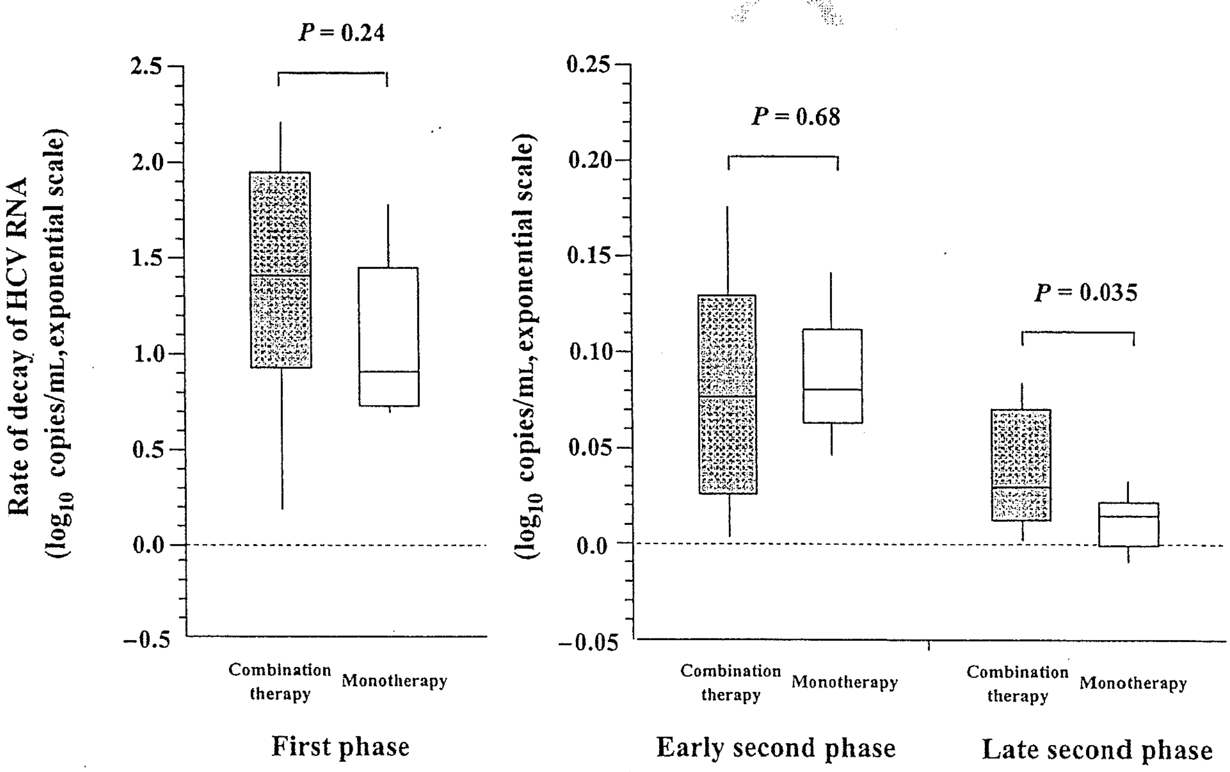

Fig. 2 Rate of decrease in serum hepatitis C virus (HCV) RNA per day in patients treated with interferon (IFN)-α2b alone or in combination with ribavirin: in the first phase between 0 and 24 h of therapy (day 0), in the early second phase between days 1 and 14, and in the late second phase between days 14 and 84. Serum HCV RNA was measured by TaqMan PCR. The boxes show medians and interquartile ranges; the vertical bars show the ranges. In the late second phase between days 14 and 84, the rate of decrease in the combination therapy group was significantly higher than that in the monotherapy group (P = 0.035).

or in combination with ribavirin is shown in Fig. 2. The differences in the first 14 days between the two groups were not significant (first phase P = 0.24, and second phase 0.68). In the late second phase (between days 14 and 84, or between days 14 and 28, if HCV RNA was not detected on day 84), the decrease in the combination therapy group was