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Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy?

Naoki Hiramatsu [a,*], Tsugiko Oze [a], Natsuko Tsuda [a], Nao Kurashige [a], Keisuke Koga [a], Takashi Toyama [a], Masakazu Yasumaru [a], Tatsuya Kanto [a], Tetsuo Takehara [a], Akinori Kasahara [a], Michio Kato [b], Harumasa Yoshihara [c], Kazuhiro Katayama [d], Taizo Hijioka [e], Hideki Hagiwara [f], Shinji Kubota [g], Masahide Oshita [h], Yoshimichi Haruna [i], Eiji Mita, Kunio Suzuki [j], Kazunobu Ishibashi [k], Norio Hayashi [a]

[a] *Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita City, Osaka 565-0871, Japan*
[b] *National Hospital Organization Osaka National Hospital, Osaka, Japan*
[c] *Osaka Rousai Hospital, Osaka, Japan*
[d] *Osaka Kouseinenkin Hospital, Osaka, Japan*
[e] *National Hospital Organization Osaka Minami Medical Center, Osaka, Japan*
[f] *Higashiosaka City Central Hospital, Osaka, Japan*
[g] *Kansai Rousai Hospital, Hyogo, Japan*
[h] *Osaka Police Hospital, Osaka, Japan*
[i] *Osaka General Medical Center, Osaka, Japan*
[j] *Saiseikai Senri Hospital, Osaka, Japan*
[k] *Kaizuka City Hospital, Kaizuka City, Osaka, Japan*

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Abstract

The aim of this study was to investigate the efficacy and safety of combination therapy of interferon and ribavirin for aged patients with chronic hepatitis C.

Methods: This study was conducted at Osaka University Hospital and institutions participating in the Osaka Liver Disease Study Group on 329 patients with chronic hepatitis C receiving interferon and ribavirin combination therapy (group A, under 60 year old, $n=199$; group B, 60–64 year old, $n=64$; group C, over 65 year old (mean age, 67.8 ± 2.2 year old, $n=66$)). Of the 293 patients who were tested for HCV serotype and HCV viral loads, 215 had HCV-RNA with serotype 1 and high viral loads (1H) and the other 78 had HCV-RNA with serotype 2 or low viral loads (non-1H).

Results: In per-protocol analysis, the overall SVR rate of 1H patients was 28% (51/184). Among the 1H patients, the SVR rate was significantly lower in group C (16%) and group B (17%) than in group A (34%) ($p<0.05$). The overall SVR rate of non-1H patients was 85% (57/67). No significant difference was found in the SVR rate among group C (79%), group B (100%), and group A (84%). On the other hand, the discontinuance of both drugs due to side effects was 29% (19/66) in group C, 20% (13/64) in group B, and 11% (21/199) in group A, with the discontinuance rates being higher in the older group ($p=0.002$).

Conclusions: In aged chronic hepatitis C patients, interferon and ribavirin combination therapy can be recommended for the non-1H patients who showed a high SVR rate of approximately 65%, but not for the 1H patients.

Keywords: Chronic hepatitis C; Aged patient; Interferon and ribavirin combination therapy

* Corresponding author. Tel.: +81 6 6879 3621; fax: +81 6 6879 3629.
E-mail address: hiramatsu@gh.med.osaka-u.ac.jp (N. Hiramatsu).

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1. Introduction

Hepatitis C virus (HCV) is estimated to infect up to 170 million people worldwide [1]. Long persistence of HCV infection can lead to progression of liver fibrosis causing liver cirrhosis and ultimately hepatocellular carcinoma (HCC) [2,3]. In Japan, it is estimated that two million people are infected with HCV, and more than 30,000 patients die of HCC every year, with approximately 80% being caused by HCV infection [4]. It has been reported that HCV carriers in Japan tend to be old [5], and liver fibrosis progresses in aged patients. Moreover, the risk of HCC increases with progression of liver fibrosis and older age, with the occurrence of HCV-related HCC reaching a peak at around the age of 65 years old [3]. Past studies have made clear that interferon (IFN) therapy is effective for eliminating HCV, and IFN therapy significantly reduces the progression of liver fibrosis [6,7] and the risk of HCC, especially among virologic or biochemical responders [8–10]. Furthermore, recently, several groups have reported that IFN therapy, specially the SVR group, improved the survival of patients with HCV [11,12], also in aged patients [13].

The combination therapy with IFN and ribavirin has been reported to be effective for eliminating HCV compared with IFN monotherapy [14–16], but additional side effects of ribavirin, such as hemolytic anemia, which is not found in IFN monotherapy have been reported, leading to discontinuance of the treatment [17]. For aged patients, sufficient informed consent should be obtained before the start of stronger antiviral therapy with possible severe side effects, because the function of the organs is generally poor, and the adverse effects of IFN therapy have been observed more frequently in older patients [18].

The question arises of whether aged patients with chronic hepatitis C should be treated with the combination therapy of IFN and ribavirin, while IFN monotherapy has been shown to be effective even in aged patients. In this study, we conducted a multi-center, retrospective study of patients with chronic hepatitis C treated by IFN and ribavirin combination therapy, and examined the efficacy and prevalence of side effects to clarify the adaptation of anti-viral treatment for aged patients.

2. Patients and methods

2.1. Patients

The current study was conducted at Osaka University Hospital and the institutions of the Osaka Liver Disease Study Group. The 329 patients with chronic hepatitis C included in this study were treated with combination IFN-α-2b and ribavirin between January 2001 and April 2004. All patients had HCV RNA detectable in serum by the polymerase chain reaction (PCR) method, had elevated ALT (above the upper limit of the normal) and had been histologically proven to have chronic hepatitis. None of the patients were positive for hepatitis B surface antigen and anti-human immunodeficiency virus antibody or had other forms of liver disease (alcoholic liver disease, hepatotoxic drugs, autoimmune hepatitis). This study protocol was carried out according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

2.2. Determination of HCV RNA levels

Serum HCV-RNA levels were quantified using branched DNA (bDNA) probe assay (version 2; Chiron, Dai-ichi Kagaku, Tokyo) [19,20] or combined PCR assay (Amplicor-HCV monitor assay) [21]. In this study, a high viral load was designated as the condition of a serum HCV-RNA level of more than 10^6 equivalents/ml by bDNA assay or more than 10^5 copies/ml serum by Amplicor-HCV monitor assay [22].

2.3. Treatment schedule

The 329 patients were treated with 10 MU ($n = 79$) or 6 MU ($n = 243$) or 3 MU ($n = 7$) IFN-α-2b intramuscularly every day for the first 2 weeks and the three times a week for the following 22 weeks in combination with ribavirin at a daily dose of 600 or 800 mg, depending on body weight (<60 or ≥60 kg, respectively). The starting doses of ribavirin were 800 mg per day for 178 patients, 600 mg per day for 148 patients, and 400 mg per day for three patients. The ribavirin dose was decreased or stopped in 91 patients (28%) due to side effects. The ribavirin dose of 200 mg was reduced if the hemoglobin value was below 10 g/dl. The ribavirin was stopped if Hb fell bellow 8.5 g/dl. One hundred and five patients continued only IFN therapy for 24 weeks after the combination therapy, because the combination therapy of IFN-α-2b and ribavirin for 48 weeks was not covered by medical insurance in Japan at that time. Patients with persistently undetectable HCV RNA 6 months after completion of treatment were considered to have achieved a sustained virological response.

2.4. Statistical analysis

Age, histological scores before IFN therapy, serum ALT levels, red blood cell (RBC) count, hemoglobin (Hb), white blood cell (WBC) count and platelet (Plt), and creatinine are expressed as mean ± S.D. Statistical analysis for group comparisons was performed by the χ^2-test. The SVR rate was evaluated using the probability proportional to size analysis (PPS analysis) and the intention-to-treat analysis (ITT analysis). A value of $p < 0.05$ (two-tailed) was considered to indicate significance.

3. Results

3.1. Clinical characteristics before combination therapy

The baseline clinical features of the 329 patients are shown in Table 1. At the start of the treatment, 130 patients were 60

Table 1
Baseline characteristics of patients according to age

	Group A ($n=199$)	Group B ($n=64$)	Group C ($n=66$)	p-value
Age (years old)	49.0 ± 8.7	62.0 ± 1.4	67.8 ± 2.2	
Sex (M/F)	142/54[a]	36/28	43/23	[a]$p<0.05$
HCV serotype (1/2/unknown)	142/51/6	53/10/1	54/12/0	N.S.
HCV-RNA (H/L/unknown)	173/12/14	58/2/4	60/5/1	N.S.
1H/non 1H/unknown	125/53/21	45/8/11	45/17/4	
Fibrosis (F 1/F2/F3/F4/unknown)	75/46/33/6/39	26/15/10/2/11	19/15/17/4/11	N.S.
ALT (IU/L)	112 ± 85[b]	91 ± 49	90 ± 57	$p<0.05$[b]
WBC	5330 ± 1570[b]	4970 ± 1390	4760 ± 1120	$p<0.05$[b]
RBC ($\times 10^4$ μl)	458 ± 47[b]	433 ± 45	431 ± 47	$p<0.01$[b]
Hb (g/dl)	14.6 ± 1.5[b]	14.0 ± 1.2	13.7 ± 1.4	$p<0.01$[b]
Plt ($\times 10^4$ μl)	16.0 ± 7.0[b]	14.9 ± 5.3	14.2 ± 4.9	$p<0.05$[b]

Note: Data are given as the mean ± S.D. N.S., not significant. Group A, patients under 60 years of age (gender of three patients were unknown); group B, patients older than 60 years but under 65 years of age; group C, patients older than 65 years of age; 1H group, patients with genotype 1 and high viral load; non-1H group, patients other than 1H group.

[a] Significant level was compared with group B.

[b] Significant levels were compared with group B and group C.

years old or older. One hundred ninety-nine patients were under 60 years old (group A), sixty-four patients were 60–64 years old (group B) and sixty-six patients were 65 years old or older (group C). No significant difference was found in serotype, viral load and histological stage among the three groups. In aged patients, ALT, RBC, Hb, WBC, and Plt were less than in young patients (ALT, $p<0.05$; RBC and Hb, $p<0.01$; WBC and Plt, $p<0.05$). Among the patients, 215 had HCV-RNA with genotype 1 and high viral loads (1H group) and 114 had HCV-RNA with genotype 2 or low viral loads (non-1H group).

3.2. Initial dosage and treatment duration of interferon

Three kinds of IFN dosage were used in this study. Among group A, 10MU, 6MU, and 3MU were administered for 60 patients, 134 patients, and 5 patients; 12, 52, and none among group B, and 8, 56, and 2 among group C. No significant difference was found in the distribution of IFN dosage among each group. The 24 and 48-week treatments (IFN and ribavirin treatment for 24 weeks followed by IFN monotherapy for 24 weeks) were carried out for 102 patients and 75 patients among group A; 37 and 14 among group B; 32 and 16 among group C. The rates of patients receiving the 48-week treatment were similar for the three groups.

3.3. PPS analysis

On PPS analysis, the overall SVR rate of 1H patients was 28% (51/184). The SVR rates were 34% (40/117) for group A, 17% (6/36) for group B, and 16% (5/31) for group C. Among the 1H patients, the SVR rates of group B and C were significantly lower than that for group A ($p<0.05$). The overall SVR rate of non-1H patients was 85% (57/67). No significant difference was found in the SVR rates among group A (84%; 36/43), group B (100%; 5/5), and group C (79%; 11/14) (Fig. 1).

3.4. ITT analysis

On ITT analysis, the SVR rate was 24% (51/215) in 1H patients, being 32% (40/125) for group A, 13% (6/45) for group B, and 11% (5/45) for group C. Among the 1H patients, the SVR rates of group B and C were significantly lower than that for group A (A versus B; $p<0.05$, A versus C; $p<0.01$).

On the other hand, in the non-1H group, the SVR rate was 73% (57/78), being 77% (41/53) for group A, 63% (5/8) for group B, and 65% (11/17) for group C. No significant difference was found among the groups (Fig. 2).

3.5. Adverse effects

The entire treatment schedule without reduction and discontinuance of both drugs was completed by 174 patients (53%). Sixty-two percent (123/199) of the patients in group A, 42% (27/64) in group B, and 36% (24/66) in group C com-

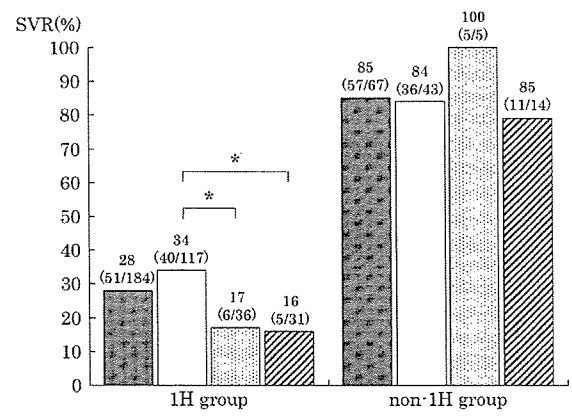

Fig. 1. Efficacy of the combination therapy according to age (PPS analysis). 1H group, patients with genotype 1 and high viral load. Non-1H group, patients not in the 1H group. (▩) all patients; (□) group A, patients under 60 years of age; (▨) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: *$p<0.05$.

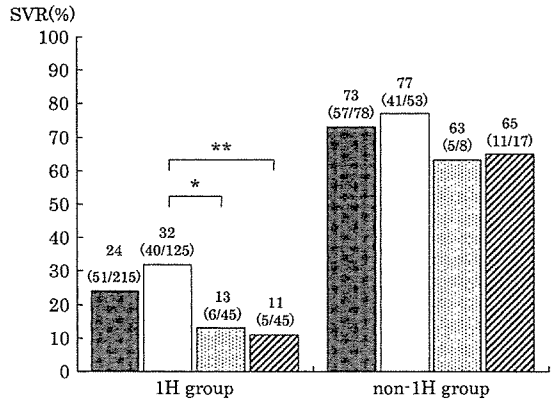

Fig. 2. Efficacy of the combination therapy according to distinction of age (ITT analysis). 1H group, patients with genotype 1 and high viral load. Non-1H group, patients not in the 1H group. (■) all patients; (□) group A, patients under 60 years of age; (▩) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: $^{*}p<0.01$; $^{**}p<0.05$.

pleted all treatment schedules (A versus B; $p<0.0001$, A versus C; $p<0.001$). IFN treatment was stopped along with ribavirin in 52 patients (16%), and the IFN dose was decreased in 20 patients (6%). The ribavirin dose was decreased in 72 patients (22%), and stopped without discontinuance of IFN in 20 patients (6%). The discontinuance rate of both drugs was significantly higher in group C (29%, 21/199) and B (20%, 13/64) than group A (11%, 19/66) (Fig. 3).

The reasons for dose reduction and discontinuance of the treatment were anemia, general fatigue, digestive disorder, eczema, neutropenia, and psychological disorder. Among the patients discontinuing both drugs, for those under 60 years old, the major reasons were anemia (32%), general fatigue

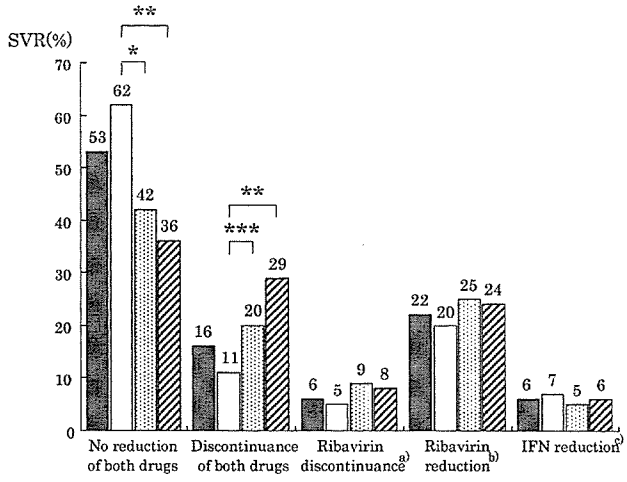

Fig. 3. Dose reduction or discontinuance of IFN and ribavirin. (a) Ribavirin discontinuance without discontinuance of IFN, (b) ribavirin reduction without discontinuance of IFN, and (c) IFN reduction regardless of discontinuance or reduction of ribavirin. (■) all patients; (□) group A, patients under 60 years of age; (▩) group B, patients from 60 years and older but under 65 years of age; (▨) group C, patients older than 65 years. Significant levels: $^{*}p<0.0001$; $^{**}p<0.001$; $^{***}p<0.005$.

(18%), digestive disorder (14%), and psychological disorder (14%). On the other hand, among the patients aged 60 years and older, the discontinuance of therapy due to anemia accounted for approximately 60% (17/28), which was twice as much as those of younger patients, with the difference being significant ($p<0.05$). Other reasons of the discontinuance of therapy among the patients aged 60 years and older were following; digestive disorder (14%), general fatigue (7%), eruption, granulocytopenia, thrombocytopenia, and psychological disorder (4%, respectively). Vascular diseases, such as cerebral bleeding did not appear in this study.

4. Discussion

In Japan, randomized control studies have been performed on the combination therapy of IFN and ribavirin for 24 weeks in patients with chronic hepatitis C, and the combination therapy was approved in 2001. However, the patients in these studies were under 60 years of age. Accordingly, the efficacy and adverse effects of combination therapy for aged patients has been still unclear. Since HCV carriers in Japan are older by 10–20 years than those in the United States and the European countries, it is very important to clarify the actual state of affairs for aged patients with chronic hepatitis C receiving the combination therapy, especially in Japan. These findings should be applicable for patients with chronic hepatitis C in other countries in a few decades, because almost the same efficacy and adverse effects are expected in patients treated by pegylated interferon (peg-IFN) and ribavirin combination therapy. In this study, we examined the efficacy and prevalence of the side effects with the focus on patient age.

The aged patients showed higher rates of discontinuance of IFN and ribavirin and lower rates for no reduction of both drugs than younger patients. The most frequent reason for the discontinuance of both drugs was hemolytic anemia which accounted for 60% of the cases in patients 60 years or older. The progress of anemia was frequently noted in aged patients and resulted in the discontinuation of ribavirin. Hemolytic anemia induced by ribavirin administration has been reported to depend on the plasma ribavirin concentration [23], with a high ribavirin concentration leading to it, and the plasma clearance of ribavirin depending on renal function [24]. A major cause for the advance of anemia in aged patients is due to the fact that renal function is poorer than in younger patients, leading to lower ribavirin clearance. As a result, severe hemolytic anemia can be induced by higher ribavirin concentrations. Therefore, the dosage of ribavirin should be reduced at the beginning of treatment in the aged patients with chronic hepatitis C in order to avoid the discontinuance of ribavirin, because the reduction of ribavirin does not decrease the SVR rate of this therapy.

The SVR difference according to age was observed for 1H patients, but not non-1H patients, when only the patients who completed the treatment were examined (PPS analysis).

That is, the SVR rates were still high for the aged patients of the non-1H group, but lower for the aged patients than the young patients in the 1H group. There are two possible reasons for this. First, the number of patients with no reduction of both drugs was significantly fewer for the patients aged 60–64 years and <60 years than for the patients aged ≥65 years, and the older patients tended to require ribavirin reduction or discontinuance (Fig. 3). Second, the liver fibrosis score tended to be higher in aged patients than in young patients, although the significant difference was not seen in this study (Table 1). These factors can decrease the SVR rates in aged patients in the 1H group, from which it is difficult to eliminate the virus, although the aged patients in the non-1H group whose viruses are easily eliminated were not affected. The results on ITT analysis account for the conclusion of the indication for IFN and ribavirin combination therapy of 24 weeks for aged patients; the patients of the 1H group do not have good application whose SVR is approximately 10%. On the other hand, patients of the non-1H group should be given the combination therapy because of the higher SVR rates of about 65%.

Better efficacy of treatments using new drugs, such as peg-IFN and ribavirin combination therapy or NS3/4 protease inhibitor, is greatly anticipated.

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Early decline of hemoglobin correlates with progression of ribavirin-induced hemolytic anemia during interferon plus ribavirin combination therapy in patients with chronic hepatitis C

Tsugiko Oze[1], Naoki Hiramatsu[1], Nao Kurashige[1], Natsuko Tsuda[1], Takayuki Yakushijin[1], Tatsuya Kanto[1], Tetsuo Takehara[1], Akinori Kasahara[1], Michio Kato[2], Harumasa Yoshihara[3], Kazuhiro Katayama[4], Shinji Kubota[5], Taizo Hijioka[6], Kazunobu Ishibashi[7], Masahide Oshita[8], Hideki Hagiwara[9], Yoshimichi Haruna[10], Eiji Mita[11], Shinji Tamura[1], and Norio Hayashi[1]

[1] Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita 565-0871, Japan
[2] National Hospital Organization Osaka National Hospital, Osaka, Japan
[3] Osaka Rousai Hospital, Sakai, Japan
[4] Osaka Kouseinenkin Hospital, Osaka, Japan
[5] Kansai Rousai Hospital, Amagasaki, Japan
[6] National Hospital Organization Osaka Minami Medical Center, Kawachinagano, Japan
[7] Kaizuka City Hospital, Kaizuka, Japan
[8] Osaka Police Hospital, Osaka, Japan
[9] Higashiosaka City Central Hospital, Higashiosaka, Japan
[10] Osaka General Medical Center, Osaka, Japan
[11] Saiseikai Senri Hospital, Suita, Japan

Background. The aim of this study was to examine the factors correlated with the progression of ribavirin-induced hemolytic anemia in patients with chronic hepatitis C treated by interferon and ribavirin combination therapy. ***Methods.*** This study was conducted on 505 patients by the Osaka Liver Disease Study Group. A decline of hemoglobin (Hb) concentration by 2 g/dl at the end of 2 weeks from the start of the treatment ("2 by 2" standard) was adopted as a predictive factor for progression to severe anemia. The ribavirin apparent clearance (CL/F) was also examined. ***Results.*** Of 482 patients whose Hb value was more than 12 g/dl before the treatment, 68 patients (14%) had to discontinue ribavirin owing to severe anemia. Patients in the "2 by 2"-positive group (Hb decline over 2 g/dl) and the group with lower CL/F were significantly more likely to discontinue ribavirin owing to severe anemia. Discontinuation was more common among patients aged 60 years or older than for those under 60 years old (21% vs. 9%, $P < 0.001$). Among patients aged 60 years or older, only the "2 by 2" standard was significantly associated with the discontinuance of ribavirin owing to severe anemia in a multivariate analysis (odds ratio, 4.18; $P < 0.001$). ***Conclusions.*** The "2 by 2" standard of Hb decline can be used to identify patients likely to develop severe anemia. The early reduction of ribavirin can help prevent progression to severe anemia, thus allowing ribavirin therapy to be completed even in older patients.

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Reprint requests to: N. Hiramatsu

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

Introduction

Hepatitis C virus (HCV) is estimated to infect up to 170 million people worldwide,[1] and two million people in Japan. Long persistence of HCV infection can lead to progression of liver fibrosis, causing liver cirrhosis and ultimately hepatocellular carcinoma.[2,3] Past studies have made clear that interferon (IFN) therapy is effective for eliminating HCV,[4,5] but the sustained viral response (SVR) rate of IFN monotherapy is not sufficient. The addition of the nucleoside analog ribavirin to IFN in the treatment of patients with chronic hepatitis C can significantly improve the SVR rate, and combination therapy with IFN or pegylated-IFN (Peg-IFN) has been recommended as a standard regimen worldwide.[6–10] However, additional side effects of ribavirin have been reported, such as hemolytic anemia, which have not been found with IFN monotherapy, leading to discontinuance of the treatment.[11–14]

In previous studies, the discontinuance rate of IFN and ribavirin combination treatment due to severe side effects has been reported to be 6%–13%.[6,7] Ribavirin-induced hemolytic anemia has been suggested to depend on a high plasma concentration of ribavirin.[15] The ribavirin apparent clearance (CL/F), which reflects the plasma concentration of ribavirin at 4 weeks after the start of combination therapy, has been used as a

predictive factor for ribavirin-induced hemolytic anemia before the start of treatment.[16-18] Furthermore, in the manufacturer's drug information for ribavirin,[19] a dose reduction is recommended when hemoglobin (Hb) levels decrease to less than 10 g/dl, and discontinuance of ribavirin is recommended when Hb levels fall to less than 8.5 g/dl during combination therapy with IFN and ribavirin. However, according to this guideline, not a few patients are forced to discontinue ribavirin because the dose reduction to avoid severe anemia does not occur in time.

What is needed is a convenient guideline for avoiding ribavirin discontinuance due to severe anemia. In this study, we evaluated the correlation of Hb decline at 2 weeks after the start of combination therapy with the discontinuance of treatment due to progression of ribavirin-induced hemolytic anemia. We also assessed the utility of an early decline of Hb in comparison with the CL/F standard for predicting the progression to severe anemia.

Patients and methods

Patients

The current study was conducted at Osaka University Hospital and other institutions participating in the Osaka Liver Disease Study Group. The 505 patients with chronic hepatitis C included in this study were treated with a combination of interferon-α-2b and ribavirin between January 2001 and December 2005. All patients were anti-hepatitis C virus antibody positive, had HCV RNA detectable in their serum by the polymerase chain reaction method, and had elevated serum alanine transaminase (ALT) (above the upper limit of normal) within the 6 months prior to treatment. Excluded from this study were patients who were positive for hepatitis B surface antigen or anti-human immunodeficiency virus antibody or those with other forms of liver disease (alcoholic liver disease, hepatotoxic drugs, autoimmune hepatitis). Twenty-three patients whose Hb was under 12 g/dl before the treatment were also excluded because the aim of this study was to analyze the progression of anemia; patients with a low Hb level before treatment are known to have a tendency toward progression of anemia. The remaining 482 patients were followed in this study.

The baseline clinical features of the 482 patients are shown in Table 1. Their mean age was 55.2 ± 10.9 years, and 66% were men. Among the patients, 347 had HCV RNA with genotype 1 and high viral loads (1H group) and 130 had HCV RNA with genotype 2 or low viral loads (non-1H group). The mean ALT level was 100 ± 74 IU/l. In this study, a high viral load was defined as a serum HCV-RNA level of more than 10^6 equivalents/ml by branched DNA assay or more than 10^5 copies/ml serum by Amplicor-HCV monitor assay.

Treatment schedule

Of the 482 patients treated with a combination of interferon-α-2b and ribavirin, 273 were IFN naïve and 209 were undergoing retreatment. All patients were scheduled to receive interferon-α-2b (Intron-A, Schering-Plough, Kenilworth, NJ, USA) at a dose of 6 (n = 371) or 10 (n = 111) MU intramuscularly every day for the first 2 weeks and three times a week thereafter. Ribavirin (Rebetol; Schering-Plough) was given orally twice a day for a total dose of 800 mg (n = 261), 600 mg (n = 215), or 400 mg (n = 6) per day. The IFN dose was decreased from 10 to 6 MU or from 6 to 3 MU when the

Table 1. Baseline characteristics of patients

Number	482	
Age (y.o)	55.2 ± 10.9	(21–75)
Sex (male/female)	320/162	
Body weight (kg)	62.3 ± 9.9	(35–94)
HCV serotype (1/2/unknown)	364/111/7	
(1H/non-1H/unknown)	347/130/5	
Fibrosis (0/1/3/4/unknown)	19/192/202/13/56	
WBC (/mm^3)	5184 ± 1531	(2100–13200)
RBC (×10^4/mm^3)	449 ± 42	(329–617)
Hb (g/dl)	14.4 ± 1.2	(12.0–19.2)
Plt (×10^4/mm^3)	15.4 ± 5.4	(4.4–36.1)
ALT (IU/l)	100 ± 74	(17–736)
Serum creatinine (mg/dl)	0.8 ± 0.2	(0.3–1.7)
Ribavirin dosage/body weight (mg/kg)	11.4 ± 1.5	(4.6–17.8)

Data are shown as means ± SD
HCV, hepatitis C virus; 1H group, patients with genotype 1 and high viral load; non-1H group, patients not in the 1H group; Fibrosis, Knodell's histological score (category4); WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; Plt, platelets; ALT, alanine aminotransferase

white blood cell (WBC) count was below 1500/mm^3, the neutrocyte count below 750/mm^3, or the platelet (Plt) count below 5×10^4/mm^3. IFN was discontinued when the WBC count was below 1000/mm^3, the neutrocyte count below 500/mm^3, or the Plt count below 2.5×10^4/mm^3. 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 manufacturer's drug information for ribavirin.[19] Ferric medicine or erythropoietin to prevent anemia was not administered. Ribavirin was scheduled to be administered for 24 weeks for all patients, and IFN for 24 weeks for 307 patients and for 48 weeks for 175 patients.

Patients with persistently undetectable HCV RNA 6 months after completion of treatment were considered to have achieved SVR.

Blood tests

All patients were examined for serum HCV-RNA level and underwent hematological and biochemical tests just before therapy, at the end of week 2, and every 4 weeks thereafter during treatment. When treatment was completed, the patients were assessed every 4 weeks until 24 weeks after the end of treatment.

Total ribavirin clearance

Using the method of Kamar et al.,[17] CL/F at the start of the treatment was calculated as follows:

$$\text{CL/F (l/h)} = 32.3 \times \text{BW} \times (1 - 0.0094 \times \text{Age}) \times (1 - 0.42 \times \text{Sex})/\text{Scr},$$

where BW = body weight; sex = 0 for male and 1 for female; and Scr = serum creatinine.

Definition of "severe anemia" leading to discontinuance of ribavirin

In this study, "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 combination therapy.

Liver histology

Hepatic fibrosis was assessed by Knodell's histological score (category 4).[20] Fibrosis stage was evaluated on a scale from 0 to 4: 0 = no fibrosis; 1 = fibrosis portal expansion; 3 = bridging fibrosis (portal–portal or portal–central linkage); 4 = cirrhosis.

Statistical analysis

Age, body weight, ribavirin dosage/body weight, WBC count, red blood cell (RBC) count, Hb concentration, Plt, serum ALT levels, and Scr are expressed as means ± SD. The SVR rate was evaluated using an intention-to-treat (ITT) analysis. The differences in proportions were tested by the χ-squared test. For univariate and multivariate analyses, a logistic regression analysis was used to predict ribavirin-induced severe anemia. A value of $P < 0.05$ (two-tailed) was considered to indicate significance.

Results

Efficacy of the combination therapy with dose reduction or discontinuance of ribavirin

The relationship between dose reduction or discontinuance of ribavirin and the SVR rate on ITT analysis is shown in Fig. 1. The SVR rate was 20% (71/347) for all 1H patients and 72% (93/130) for all non-1H patients. Among the 1H patients, SVR was achieved for 24% (45/189) without dose reduction of ribavirin and for 26% (20/76) with dose reduction. Significantly lower SVR rates were observed for patients who had to discontinue ribavirin treatment owing to adverse effects (7%, 6/82) in comparison with those with ($P < 0.01$) or without ($P < 0.01$) dose reduction. In the non-1H group, similar SVR rates were found with dose reduction of ribavirin [SVR rate without dose reduction, 83% (58/70), vs. SVR rate with dose reduction, 82% (23/28)], and the SVR rate of patients who had to discontinue ribavirin owing to adverse effects was significantly lower (38%, 12/32) than that for those with ($P < 0.001$) or without ($P < 0.0001$) dose reduction.

The same tendency was observed even in the 307 patients treated with IFN for 24 weeks. Among the 1H patients treated for 24 weeks, SVR was achieved for 19% (17/91) without dose reduction of ribavirin, 15% (6/41) with dose reduction, and 3% (2/75) with discontinuance. There were significant differences between the patients with discontinuance and those without ($P < 0.01$) or with ($P < 0.05$) dose reduction. Among the non-1H patients treated for 24 weeks, SVR rates were 85% (39/46) for the patients without dose reduction of ribavirin, 85% (17/20) for those with dose reduction, and 33% (10/30) for those with discontinuance. Significantly lower SVR rates were observed for patients who had to discontinue ribavirin than for those with ($P = 0.05$) or without ($P < 0.05$) dose reduction.

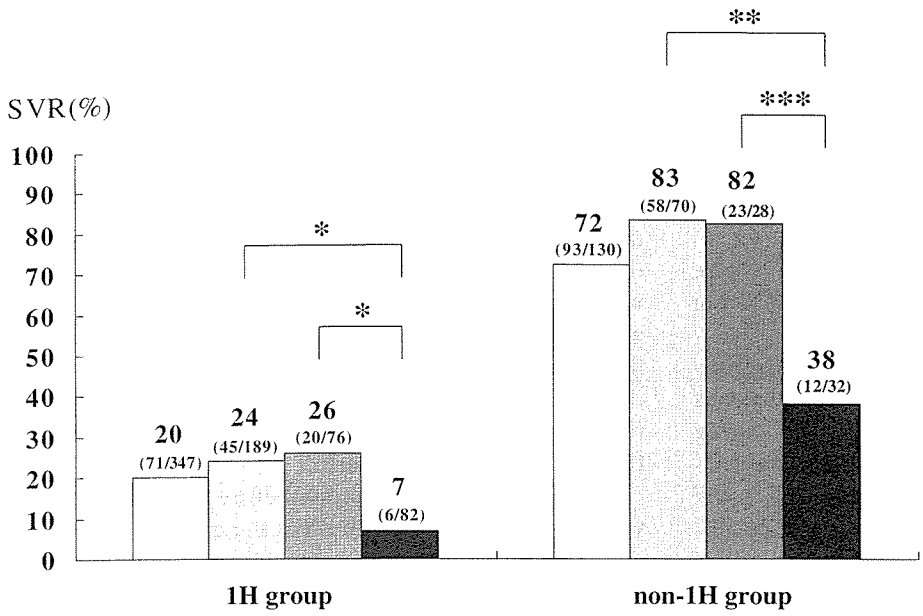

Fig. 1. Efficacy of combination therapy with dose reduction or discontinuance of ribavirin (intention-to-treat analysis). *1H group*, patients with genotype 1 and high viral load; *non-1H group*, patients not in the 1H group; *SVR*, sustained viral response. □ all patients; ▒ patients without dose reduction of ribavirin; ▒ patients with dose reduction of ribavirin; ■ patients with discontinuance of ribavirin. *, $P < 0.01$; **, $P < 0.0001$; ***, $P < 0.001$

Table 2. Rate of the ribavirin reduction or discontinuance due to adverse effects with different levels of CL/F

			Discontinuance	
	No reduction	Dose reduction	All cases	Cases due to severe anemia
20 ≤ CL/F	94%	2%	4%	0%
(n = 45)	(42/45)	(1/45)	(2/45)	(0/45)
15 ≤ CL/F < 20	66%	19%	15%	6%
(n = 100)	(66/100)	(19/100)	(15/100)	(6/100)
10 ≤ CL/F < 15	54%	24%	23%	14%
(n = 179)	(96/179)	(42/179)	(41/179)	(25/179)
CL/F < 10	37%	28%	35%	23%
(n = 158)	(58/158)	(44/158)	(56/158)	(37/158)

Frequency of and reasons for dose reduction or discontinuance of ribavirin during combination therapy

We examined the rate of discontinuance of therapy due to adverse effects up to the end of 24 weeks, because all cases of discontinuation occurred before the end of 24 weeks. Of the 482 patients, 401 patients completed 24 weeks of therapy, and 81 patients (17%) had to discontinue both IFN and ribavirin before the end of the 24 weeks. Of the 401 patients undergoing 24 weeks of therapy, the entire treatment schedule without reduction or discontinuance of either drug was completed by 262 patients (54%). The ribavirin dose was decreased for 106 patients (22%) and was stopped without discontinuance of IFN for 33 patients (7%). Overall, 114 patients (24%) discontinued ribavirin treatment. The reasons for dose reduction or discontinuance of ribavirin were anemia, general fatigue, digestive disorder, eczema, neutropenia, thrombocytopenia, or psychological disorder. Among the patients discontinuing ribavirin, the major reasons were anemia (14%), general fatigue (2%), or digestive disorder (2%).

CL/F and dose reduction or discontinuance of ribavirin

CL/F calculated for all patients was 4.6–32.5 l/h. The mean CL/F was 13.0 l/h, and the median was 11.9 l/h. At the start of treatment, CL/F was less than 10 l/h for 33% (158/482) of patients, 10–15 l/h for 37% (179/482), 15–20 l/h for 21% (100/482), and more 20 l/h for 9% (45/486).

Table 2 shows the rates of dose reduction or discontinuance of ribavirin in relation to different levels of CL/F. The rate of discontinuance of ribavirin among all patients was 4% (2/45) for patients with CL/F ≥ 20, 15% (15/100) for those with 15 ≤ CL/F < 20, 23% (41/179) for those with 10 ≤ CL/F < 15, and 35% (56/158) for those with CL/F < 10. The rate of discontinuance of ribavirin due to severe anemia was 14% (68/482) among all pa-

tients. There was no discontinuance of ribavirin due to severe anemia among patients with CL/F ≥ 20, but the rate of discontinuance was 6% (6/100) among those with 15 ≤ CL/F < 20, 14% (25/179) among those with 10 ≤ CL/F < 15, and 23% (37/158) among those with CL/F < 10. The rate of continuance of ribavirin without dose reduction decreased in proportion to the decline of CL/F. In this study, we adopted two categories of CL/F, below 15 l/h (CL/F < 15) and below 10 l/h (CL/F < 10), to assess CL/F as a factor for predicting anemia progression.

We also analyzed the predictive factor of anemia progression according to patient age, because CL/F varies widely with patient age and tends to be lower among older patients. Among patients under 60 years old (n = 288), 17% (48/288) had CL/F under 10 l/h, 38% (109/288) had CL/F 10–15 l/h, 30% (86/288) had CL/F 15–20 l/h, and 16% (45/288) had CL/F over 20 l/h. On the other hand, among those 60 years old or older (n = 194), 57% (110/194) had CL/F under 10 l/h, 36% (70/194) had CL/F 10–15 l/h, 7% (14/194) had CL/F 15–20 l/h, and none had CL/F over 20 l/h. Thus, the majority (93%) of the patients 60 years old or older had a low CL/F (<15), whereas only 55% of those under 60 years old had CL/F < 15.

Early decline of Hb and progression of anemia during combination therapy

Figure 2 shows the decline of Hb from the start of combination therapy. We conducted this analysis for the 433 patients: those who did not need a dose reduction of ribavirin (n = 262), those who needed a dose reduction owing to a decrease of Hb to less than 10 g/dl (n = 103), and those who discontinued ribavirin due to "severe anemia" (n = 68). We excluded 49 patients from this analysis: 46 patients stopped combination therapy for reasons other than anemia, such as general fatigue or digestive disorder, and the other three patients were not responding to antiviral treatment and stopped therapy before 24 weeks without a dose reduction of ribavirin. Following the initiation of combination therapy, Hb concentration decreased rapidly until the end of the 4th week. At the end of 2 weeks, Hb had decreased by 0.9 ± 1.2 g/dl among the patients without dose reduction of ribavirin, by 1.8 ± 1.3 g/dl among those with dose reduction, and by 2.3 ± 1.4 g/dl among those who discontinued ribavirin. At the end of 4 weeks, Hb had decreased by 2.1 ± 1.5 g/dl among the patients without dose reduction of ribavirin, by 3.2 ± 1.5 g/dl among those with dose reduction, and by 3.9 ± 1.5 g/dl among those discontinuing ribavirin.

ΔHb [ΔHb = (Hb value just before treatment) − (Hb value during treatment)] both at the end of 2 weeks and at the end of 4 weeks were significantly larger among the patients discontinuing ribavirin than among those without dose reduction of ribavirin (P < 0.0001, P < 0.0001, respectively). In this study, we adopted the category of ΔHb at the end of 2 weeks because it allowed the progression of anemia to be estimated at an earlier phase of treatment than did ΔHb at the end of 4 weeks.

To establish the cutoff value of ΔHb at the end of 2 weeks, we used two categories of ΔHb: a decrease in Hb concentration at 2 weeks to 2 g/dl below the baseline (ΔHb2.0) or to 1.5 g/dl below the baseline (ΔHb1.5). We conducted this analysis for 480 patients, because two patients stopped combination therapy before 2 weeks for reasons other than anemia. With the ΔHb2.0 standard, the rate of discontinuance of ribavirin due to severe anemia was 10% (32/338) in the ΔHb < 2.0 group and 25% (36/142) in the ΔHb ≥ 2.0 group, with the difference being significant (P < 0.0001) (Table 3). With the ΔHb1.5 standard, the rate of discontinuance of ribavirin due to severe anemia was significantly higher

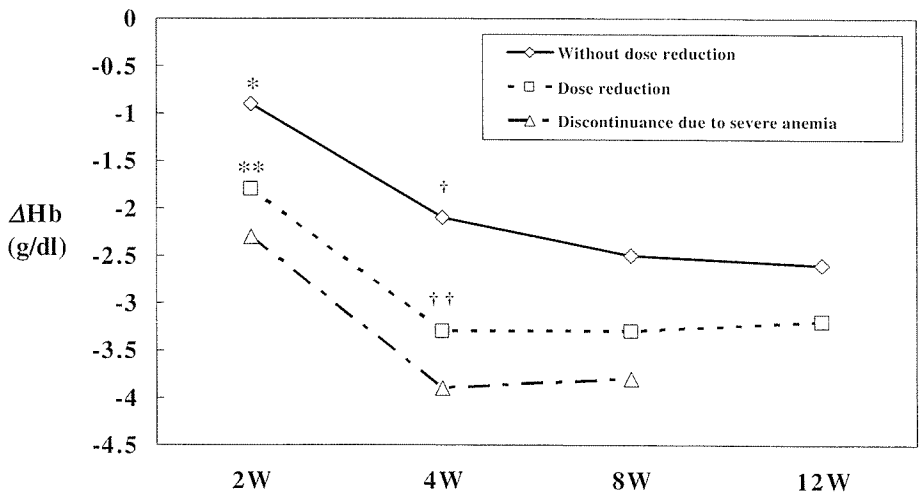

Fig. 2. Decline of hemoglobin according to dose reduction or discontinuance of ribavirin. *Significantly different from patients with dose reduction (P < 0.0001) and patients with discontinuance (P < 0.0001); **significantly different from patients with discontinuance (P < 0.02); †significantly different from patients with dose reduction (P < 0.0001) and patients with discontinuance (P < 0.0001); ††significantly different from patients with discontinuance (P < 0.01)

Table 3. Rate of the ribavirin reduction or discontinuance due to adverse effects with rate of anemia progression

			Discontinuance	
	No reduction	Dose reduction	All cases	Cases due to severe anemia
ΔHb ≥ 2.0 (n = 142)	37% (53/142)	29% (41/142)	34% (48/142)	25%* (36/142)
ΔHb < 2.0 (n = 338)	61% (209/338)	19% (65/338)	20% (64/338)	10% (32/338)

*$P < 0.0001$

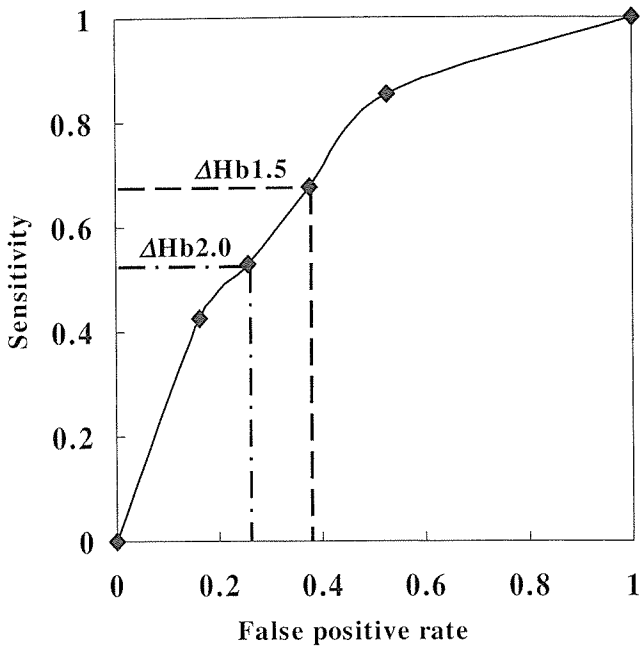

Fig. 3. Receiver-operating characteristic curve for ΔHb at the end of 2 weeks for discontinuance of ribavirin due to severe anemia

in the ΔHb ≥ 1.5 group than in the ΔHb < 1.5 group (8%, 22/279 vs. 23%, 46/201; $P < 0.0001$). Figure 3 shows the receiver-operating characteristic curve using ΔHb at the end of 2 weeks for the discontinuance of ribavirin due to severe anemia. Between the ΔHb2.0 and ΔHb1.5 standards, no significant difference was found in sensitivity (53%, 36/68, vs. 68%, 46/68; NS). On the other hand, the false positive rate was significantly lower with the ΔHb2.0 standard than with the ΔHb1.5 standard (26%, 93/360, vs. 38%, 136/360; $P < 0.001$), and accuracy was significantly higher with the ΔHb2.0 standard than with the ΔHb1.5 standard (71%, 303/428, vs. 63%, 270/428; $P = 0.02$). Therefore, we adopted ΔHb2.0 at the end of 2 weeks (the "2 by 2" standard) as a predictive factor for discontinuance of ribavirin due to severe anemia because of the higher specificity rate of ΔHb2.0 (lower false positive rate).

Logistic regression analysis for discontinuance of ribavirin in combination therapy

We assessed the factors correlated with the discontinuance of ribavirin due to severe anemia by logistic regression analysis. The following factors were evaluated: age, sex, body weight, ribavirin dosage/body weight, IFN dosage, Scr, Hb value at the start of the therapy, CL/F category, and early decline of Hb ("2 by 2" standard). Older age, lower body weight, lower Hb at the start of the therapy, lower CL/F (CL/F < 10 or CL/F < 15), and "2 by 2"-positive (the patients whose Hb had decreased by more than 2 g/dl at 2 weeks from the start of the treatment) were factors significantly associated with discontinuance of ribavirin due to severe anemia by univariate logistic regression analysis (Table 4). Next, we assessed the factors correlated with the discontinuance of ribavirin due to severe anemia by multivariate logistic regression analysis. Among the factors selected as significant by the univariate analysis, we omitted age and body weight from the multivariate analysis because they were included as parameters in the numerical formula for CL/F. Therefore, we evaluated the Hb value at the start of therapy, the CL/F category, and the "2 by 2" category by multivariate analysis. The CL/F borderline values of 10 l/h and 15 l/h were evaluated separately. In the multivariate logistic regression analysis, lower Hb at the start of therapy, lower CL/F (CL/F < 10 or CL/F < 15), and "2 by 2"-positive were significantly associated with discontinuance of ribavirin due to severe anemia (Table 5).

Useful predictive factors for discontinuance of ribavirin among older patients

Among the 288 patients under 60 years old, 50 (17%) had discontinued ribavirin by the end of 24 weeks for various reasons, including anemia, general fatigue, digestive disorder, and psychological disorders. Among the 194 patients aged 60 years and older, 64 (33%) had discontinued ribavirin, with severe anemia accounting for approximately 65% (41/64). More than twice as many patients aged 60 years and older discontinued ribavirin treatment compared with younger patients;

Table 4. Univariate analysis for the discontinuance of ribavirin due to severe anemia

Factor	Category	Odds ratio	95% CI	*P* value
Age			1.045–1.117	<0.0001
Sex	Male/Female	1/1.18	0.663–2.029	0.56
Body weight			0.928–0.981	<0.001
Serum creatinine			0.551–9.492	0.25
Ribavirin/Body weight			0.945–1.357	0.18
IFN dosage	6MU/10MU	1/1.03	0.557–1.893	0.93
Hb			0.480–0.780	<0.0001
CL/F	≥15/<15	1/5.56	0.076–0.427	0.0001
	≥10/<10	1/3.14	0.187–0.540	<0.0001
"2 by 2"	Negative/Positive	1/3.23	0.182–0.527	<0.0001

CI, confidence interval; IFN, interferon; CL/F, apparent clearance; "2 by 2", ΔHb2.0 at the end of 2 weeks; "2 by 2"-positive means ΔHb ≥ 2.0; "2 by 2"-negative means ΔHb < 2.0

Table 5. Multivariate analysis for the discontinuance of ribavirin due to severe anemia

Factor	Category	Odds ratio	95% CI	*P* value
Hb			0.446–0.785	0.0003
CL/F	≥15/<15	1/3.18	0.126–0.786	0.01
"2 by 2"	Negative/Positive	1/4.35	0.127–0.419	<0.0001
Hb			0.440–0.784	0.0003
CL/F	≥10/<10	1/1.98	0.278–0.923	0.03
"2 by 2"	Negative/Positive	1/4.63	0.119–0.393	<0.0001

this difference was significant (21%, 41/194, vs. 9%, 27/288; $P = 0.0003$) (Table 6).

We assessed the analysis for discontinuance of ribavirin due to severe anemia among the patients aged 60 years or older. Older age, lower CL/F (CL/F < 10), and "2 by 2"-positive were factors significantly associated with discontinuance of ribavirin due to severe anemia by univariate logistic regression analysis (Table 7A). Next, we assessed the factors correlated with the discontinuance of ribavirin due to severe anemia by multivariate logistic regression analysis. Among the three factors selected as significant by univariate analysis, we omitted the factor of age from the multivariate analysis as it was included as a parameter in the numerical formula for CL/F. In the multivariate logistic regression analysis of the CL/F category (CL/F < 10) and the "2 by 2" category, the latter was the only significant factor associated with the discontinuance of ribavirin due to severe anemia (Table 7B). Using the "2 by 2" standard, the rate of discontinuance of ribavirin due to severe anemia was 14% (18/133) in the "2 by 2"-negative (the patients whose Hb decreased by less than 2 g/dl from the start of treatment) group and 38% (23/60) in the "2 by 2"-positive group, with the difference being significant ($P < 0.0001$) (Table 8).

We next compared the sensitivity, specificity, and accuracy of the CL/F category with those of the "2 by 2" category as predictive factors for discontinuance of ribavirin due to severe anemia among patients aged 60 years or older. Table 9 shows the comparison between the CL/F < 15 category and the "2 by 2" category (Table 9A) and that between the CL/F < 10 category and the "2 by 2" category (Table 9B). Although sensitivity was higher for the lower CL/F category [CL/F < 15, 100% (41/41); CL/F < 10, 71% (29/41)] than for the "2 by 2" category (56%, 23/41), specificity and accuracy were significantly higher for the "2 by 2" category than for the CL/F category [specificity: "2 by 2," 77% (96/125) vs. CL/F < 15, 7% (9/125), $P < 0.0001$; "2 by 2" vs. CL/F < 10, 47% (59/125), $P < 0.0001$; accuracy: "2 by 2," 72% (119/166) vs. CL/F < 15, 30% (50/166), $P < 0.0001$; "2 by 2" vs. CL/F < 10, 53% (88/166), $P < 0.001$].

Table 6. Major causes of discontinuance of ribavirin

	Age < 60	Age ≥ 60
Severe anemia	27 (9%)	41 (21%)*
General fatigue	7	3
Digestive disorders	5	3
Neutropenia	1	1
Thrombocytopenia	2	4
Eruption with itching	2	4
Psychological disorders	3	3
Others	3	5
Total	50/288 (17%)	64/194 (33%)

*$P < 0.001$

Table 7. Univariate and multivariate analysis for the discontinuance of ribavirin due to severe anemia among the patients aged 60 years and older

A. Univariate analysis

Factor	Category	Odds ratio	95% CI	*P* value
Age			1.007–1.250	0.04
Sex	Male/Female	1/1.67	0.280–1.286	0.19
Body weight			0.947–1.021	0.37
Serum creatinine			0.865–33.586	0.07
Ribavirin/Body weight			0.775–1.205	0.76
IFN dosage	6 MU/10 MU	1/1.92	0.803–4.579	0.14
Hb			0.537–1.106	0.16
CL/F	≥15/<15	—	—	0.97
	≥10/<10	1/2.16	0.217–0.989	0.047
"2 by 2"	Negative/Positive	1/4.24	0.112–0.497	0.0001

B. Multivariate analysis

Factor	Category	Odds ratio	95% CI	*P* value
CL/F	≥10/<10	1/2.12	0.213–1.042	0.063
"2 by 2"	Negative/Positive	1/4.18	0.112–0.507	0.0002

Table 8. Rate of the ribavirin reduction or discontinuance due to adverse effects with the rate of anemia progression among the patients aged 60 years and older

			Discontinuance	
	No reduction	Dose reduction	All cases	Cases due to severe anemia
ΔHb ≥ 2.0 ("2 by 2"-positive) (n = 60)	27% (16/60)	23% (14/60)	50% (30/60)	38%* (23/60)
ΔHb < 2.0 ("2 by 2"-negative) (n = 133)	46% (61/133)	29% (39/133)	25% (33/133)	14% (18/133)

*P < 0.0001

Table 9. Comparison of "2 by 2" standard and CL/F standard for the discontinuance of ribavirin due to severe anemia among the patients aged 60 years and older

A.

	"2 by 2"-positive	CL/F < 15	*P* value
Sensitivity	56% (23/41)	100% (41/41)	<0.0001
Specificity	77% (96/125)	7% (9/125)	<0.0001
Accuracy	72% (119/166)	30% (50/166)	<0.0001

B.

	"2 by 2"-positive	CL/F < 10	*P* value
Sensitivity	56% (23/41)	71% (29/41)	0.17
Specificity	77% (96/125)	47% (59/125)	<0.0001
Accuracy	72% (119/166)	53% (88/166)	<0.001

Discussion

Ribavirin, developed in 1972, is a synthetic nucleic acid analog, which has antiviral activity in vitro against a wide variety of RNA and DNA viruses. Combination therapy of ribavirin with IFN or Peg-IFN led to remarkable progress in antiviral therapy for chronic hepatitis C. To raise the SVR rate for such combination therapy, it is very important to predict the discontinuance of the therapy due to an adverse effect and prevent it. In this study, we observed the incidence of hemolytic anemia, the major side effect of ribavirin. The factors correlated with the progression of anemia were analyzed to avert the need to discontinue ribavirin treatment of patients with chronic hepatitis C receiving combination therapy.

Several studies in the United States and European countries have reported that higher ribavirin dosage or a higher plasma concentration of ribavirin increases the SVR rate.[21,22] However, a higher ribavirin dose or higher plasma concentration of ribavirin entails the risk of having to discontinue ribavirin treatment. In Japan, analysis of the relationship between the SVR rate and a dose reduction or discontinuance of ribavirin, has shown that reducing the dose of ribavirin does not affect the SVR rate. In the present study, the SVR rate of the patients discontinuing ribavirin was also shown to be significantly lower than the patients who did not discontinue it

in both the 1H group and the non-1H group ($P < 0.01$ and $P < 0.01$, respectively). The SVR rate was almost the same between patients without a dose reduction of ribavirin and those with a dose reduction in both groups (1H, 24% vs. 26%; non-1H, 83% vs. 83%). Therefore, averting ribavirin discontinuance, even if its dose must be reduced, can lead to improvement of the SVR rate. This means that it is important to identify patients prone to develop severe anemia leading to ribavirin discontinuance while they are still in the early phase of treatment, and to consider ribavirin dose reduction before anemia progression.

CL/F relating to the plasma concentration of ribavirin at the end of 4 weeks after initiation of the combination therapy has been used as a predictive factor for the progression of anemia.[16-18] In this study, the patients with a lower CL/F value, which is thought to be correlated with a high plasma concentration of ribavirin, showed a higher rate of discontinuance of ribavirin due to severe anemia than those with a higher CL/F value. This indicates that prediction of anemia progression using the CL/F is useful before the initiation of combination therapy. We analyzed predictive factors for discontinuance of ribavirin due to severe anemia using two CL/F categories, CL/F < 10 and CL/F < 15, taking into account that the mean CL/F was 13.0 l/h and the median was 11.9 l/h, and compared the usefulness of those categories with that of the "2 by 2" standard.

We focused on the early decline of the Hb concentration after the initiation of combination therapy. Monitoring of the Hb decline allowed clear assignment of the patients into three groups: patients without dose reduction of ribavirin, those with dose reduction, and those who discontinued ribavirin. At the end of 2 weeks, a significant relationship was already observed among the three groups. Therefore, we examined the relationship between the beginning of a progression to severe anemia and the decrease in the Hb concentration at the end of 2 weeks (ΔHb). Since a standard value of ΔHb for dose reduction of ribavirin must be established, we compared ΔHb2.0 with ΔHb1.5, and found that the specificity and accuracy of ΔHb2.0 as a predictive factor for the discontinuance of ribavirin due to severe anemia was higher than those of ΔHb1.5. We therefore adopted ΔHb2.0 at the end of 2 weeks from the start of treatment (the "2 by 2" standard) as the predictive factor for discontinuance of ribavirin due to severe anemia, because an early reduction of ribavirin should be limited to those patients with a higher specificity rate for the progression of anemia. Furthermore, ΔHb2.0 is easier to calculate.

In the multivariate logistic regression analysis, both the CL/F category and the "2 by 2" category were useful for all patients as independent predictive factors for discontinuing ribavirin due to severe anemia (Table 5). Patients with lower CL/F (CL/F < 10 or CL/F < 15) and those who were "2 by 2" positive were significantly associated with the discontinuance of ribavirin due to severe anemia. Thus, the CL/F standard should be used as a predictive factor before combination therapy is begun, and the "2 by 2" standard should be used during the combination therapy. We also assessed which would be the more useful predictive factor for discontinuance of ribavirin due to severe anemia among older patients. Multivariate analysis showed that only the "2 by 2" standard was significantly related to the discontinuance of ribavirin due to severe anemia among older patients (Table 7B). Moreover, the "2 by 2" standard showed higher specificity (77%) and accuracy (72%) for the discontinuance of ribavirin due to severe anemia among older patients than either CL/F value (Table 9). The ribavirin dose of 200 mg should be reduced for aged patients whose Hb decreases over 2 g/dl from the start of combination therapy in order to avoid having to discontinue ribavirin administration altogether.

Hemolytic anemia has been reported to be induced by ribavirin administration, depending on the plasma ribavirin concentration[15] and the fragile membrane of RBC in which ribavirin accumulates.[23] Furthermore, the plasma clearance of ribavirin has been reported to depend on renal function.[24,25] The anemia associated with IFN and ribavirin therapy is a "mixed anemia," in which both hemolysis and bone marrow suppression occur simultaneously. In this study, many patients, especially older ones, had to discontinue ribavirin due to severe anemia, as previously reported.[26] A major reason for this was thought to be the tendency of the plasma concentration of ribavirin to rise due to lower renal function and impaired hematogenous function as the anemia progressed. In predicting the discontinuance of ribavirin due to severe anemia using the CL/F category, the lower CL/F implies that older patients and patients with low renal function are high-risk groups. However, CL/F does not account for the fragile membrane of RBC or the hematogenous function. Therefore, the CL/F standard cannot be a good marker for individual patients, because CL/F does not reflect in vivo phenomena triggered by ribavirin. CL/F is related simply to the plasma concentration of ribavirin at the end of 4 weeks after the initiation of combination therapy. On the other hand, the "2 by 2" standard can be useful as a predictive factor of ribavirin discontinuance forces by severe anemia for all patients, including older patients. It indicates that the "2 by 2" standard reflects plural factors, such as the occurrence of hemolysis and hematogenous functions. We suggest that the "2 by 2" standard is more useful than the CL/F category as a predictive factor for discontinuance of ribavirin due to severe anemia, especially among older patients.

In conclusion, it is important to monitor the early decline of the Hb concentration after initiation of combination therapy and to reduce the dose of ribavirin at the end of 2 weeks based on the magnitude of the Hb decline. An early reduction of ribavirin before progression to severe anemia can reduce the number of patients who are destined to discontinue ribavirin therapy. This should help improve the patients' quality of life by preventing the progression to severe anemia. Further prospective study is necessary to evaluate the antiviral outcome by ITT analysis using early reduction of ribavirin based on the "2 by 2" standard.

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Case report

Squamous cell carcinoma as a rare entity of primary liver tumor with grave prognosis

Nobukazu Yuki [a,*], Yasuki Hijikata [a], Michio Kato [a], Kunimitsu Kawahara [b], Kenichi Wakasa [c]

[a] *Department of Gastroenterology, Osaka National Hospital, Hoenzaka 2-1-14, Chuo-ku, Osaka 540-0006, Japan*
[b] *Department of Pathology, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino 583-8588, Japan*
[c] *Department of Pathology, Osaka City University Hospital, Osaka 545-8586, Japan*

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Abstract

The development of primary squamous cell carcinoma (SCC) of the liver has only rarely been reported in association with pre-existing hepatic cysts and biliary tract diseases. This report describes an unusual case of SCC originating in a cirrhotic liver. A 63-year-old male alcoholic was incidentally found to have a 6-cm liver tumor which showed mixed echogenic by sonography and a low-density area with rim enhancement by computed tomography. Tumor biopsy led to a diagnosis of SCC with a sarcomatoid change. The tumor showed fatal rapid growth accompanied by abdominal pain. Transcatheter arterial embolization and chemotherapy were not effective, and the tumor increased to 13 cm in diameter over an 8-month period. A post-mortem search revealed no alternative primary tumor site other than liver. Review of the literature shows that abdominal pain is a chief symptom of primary liver SCC. Only three of the nine patients treated with hepatic resection survived without recurrence during 8 months to 4 years of follow-up. We propose that SCC with a grave prognosis should be considered as a rare entity of primary liver tumor even in cirrhotic patients.

Keywords: Liver cirrhosis; Liver tumor; Squamous cell carcinoma

1. Introduction

Squamous cell carcinoma (SCC) rarely arises from the liver. However, when it does occur, the prognosis can be worse than that of hepatocellular carcinoma, the most common primary liver tumor. Here, we describe an unusual case of SCC primarily arising from a cirrhotic liver. In this paper, we combine our experience with data obtained from the literature, and try to summarize this rare entity of primary liver tumor based on its clinical presentation, diagnostic findings and results of subsequent therapy.

2. Case report

2.1. Clinical features

The entire 8-month clinical course is shown in Fig. 1. A 63-year-old Japanese male with alcohol abuse (>80 g/day over 40 years) underwent a health checkup at a local hospital. He was aymptomatic, but abdominal sonography disclosed a cirrhotic liver with a 6-cm mixed echogenic tumor in segment 6. He had no confounding etiology of liver disease other than alcohol. He was negative for hepatitis B surface antigen and hepatitis C virus antibody, had no history of administration of hepatotoxic drugs and showed no evidence of autoimmune liver disease. Computed tomography confirmed the tumor as a low-density area showing rim enhancement after contrast injection. A needle biopsy demonstrated the possibility

* Corresponding author. Tel.: +81 6 6942 1331; fax: +81 6 6943 6467.
E-mail address: yuki@onh.go.jp (N. Yuki).

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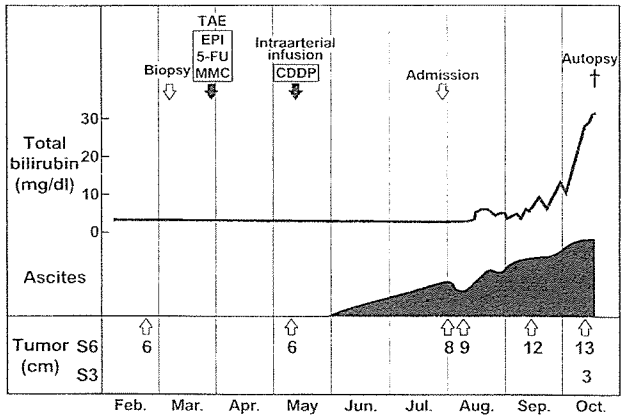

Fig. 1. Clinical course of a case with primary squamous cell carcinoma of the liver. The tumor showed no response to transcatheter arterial embolization (TAE) and chemotherapy resulting in fatal rapid growth.

of the tumor being SCC. A thorough search for a primary tumor was undertaken including chest radiography, computed tomography of the lungs, upper gastrointestinal endoscopy, colonoscopy, urological examination, magnetic resonance imaging of the brain and ear, nose and throat screening. No other tumor source was found. Hepatic angiography showed a 6-cm hypovascular mass in the lower part of the right liver lobe (Fig. 2). Epirubicin hydrochloride (EPI) 50 mg, fluorouracil (5-FU) 250 mg and mitomycin C (MMC) 4 mg were infused followed by transcatheter arterial embolization (TAE). Thereafter, the tumor was further treated by continuous transarterial infusion of cicplatin (CDDP) 100 mg over 90 min.

Five months after the initial diagnosis, the patient was referred to Osaka National Hospital, complaining of abdominal distension and vague right upper quadrant pain. He had an intermittent fever of up to 38.4 °C. Physical examination was significant for marked ascites and right upper quadrant tenderness. Abdominal sonography showed an 8-cm mixed echogenic liver tumor in segment 6 (Fig. 3). By computed tomography, the tumor appeared again as a low-density area showing rim enhancement (Fig. 4). Aspi-

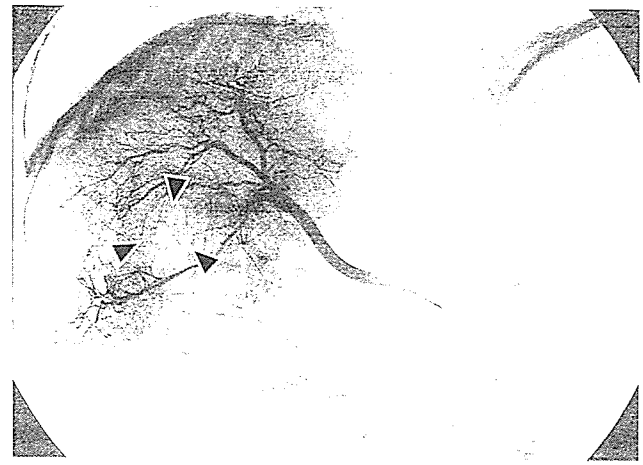

Fig. 2. Hepatic angiography disclosed a 6-cm hypovascular mass in the lower part of the right liver lobe (arrows).

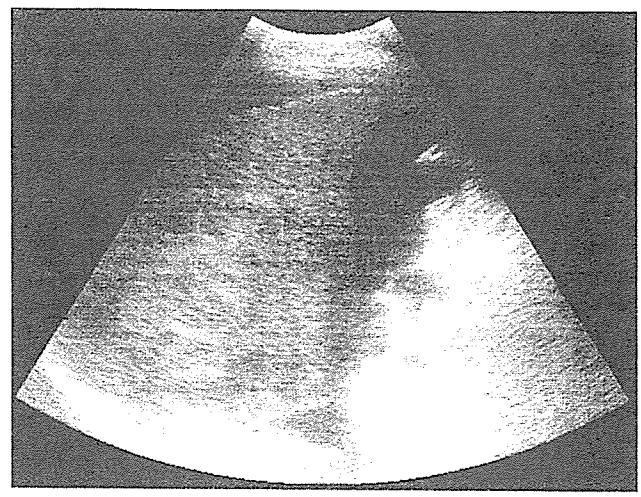

Fig. 3. On admission to our institution, abdominal sonography showed marked ascites and a cirrhotic liver with an 8-cm mixed echogenic tumor in segment 6.

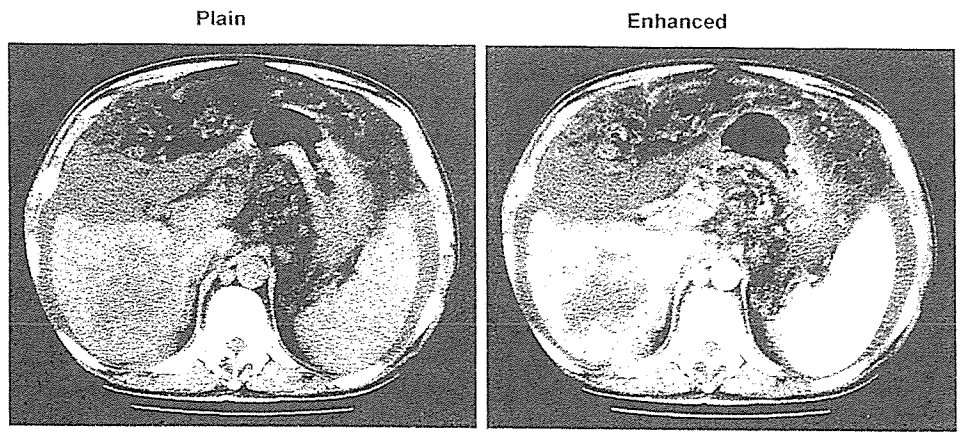

Fig. 4. By computed tomography, the liver tumor appeared as a low-density area showing rim enhancement after contrast injection.

rated ascites was clear in appearance and appeared transudate in nature. Cytology revealed no tumor cells, and bacteriology was negative. Laboratory tests showed anemia and hypoproteinemia, with a hemoglobin level of 9.8 g/dL; total proteins, 5.6 g/dL; albumin, 2.5 g/dL; serum bilirubin, 2.3 mg/dL; aspartate aminotransferase, 32 IU/L; alanine aminotransferase, 13 IU/L; alkaline phosphatase, 577 IU/L (normal 109–344); γ-glutamyl transpeptidase, 137 IU/L. As for tumor markers, alpha-fetoprotein, PIVKA-II, carcinoembryonic antigen and CA19-9 were normal. Elevation was observed for SCC-related antigen (3.2 ng/mL [<1.5]) and cytokeratin fragment 21.1 (CYFRA21.1) (8.4 ng/mL [<2.8]). After admission, the liver tumor grew to 13 cm in diameter, and a 3-cm new tumor appeared in segment 3. In parallel with rapid growth of the tumor, the ascites increased further, and marked jaundice appeared. The patient was managed conservatively for right upper quadrant pain. His condition progressively deteriorated, and he died 8 months after the initial diagnosis.

2.2. *Pathologic features*

The histology of the tumor biopsy showed sarcomatoid spindle cell component and multiple foci of squamous cells with nuclear atypia and large eosinophilic keratinizing cytoplasm, which were compatible with SCC lesions (Fig. 5). Areas of transition from SCC to the sarcomatoid tissue were visible. SCC cells and spindle-shaped cells were both positive for monoclonal antibody MNF116 directed to human cytokeratins 5, 6, 8 and 17 and specific for epithelial lesions (Dako Cytomation Co., Ltd., Kyoto, Japan). Neither SCC cells nor spindle cells showed reactivity to anti-vimentin monoclonal antibody Vim3B4 specific for mesenchymal cells and anti-human hepatocyte monoclonal antibody OCH1E5 (Dako Cytomation Co., Ltd.). Thus, the diagnosis reached was liver SCC with a sarcomatoid change.

At autopsy, the liver appeared cirrhotic with a finely nodular surface. Cut surfaces of the liver revealed a large whitish mass arising primarily from the right liver lobe extending into the left lobe and the right kidney, the largest of which measured 14 cm in diameter (Fig. 6). Tumor thrombi were observed in the portal veins adjacent to the main tumor. No cystic changes were present in the liver, and the hepatic and bile ducts were normal. Lymph node metastasis was seen at the hepatic hilus, and tumor deposits due to direct spread were present in the intestinal serosa. In an attempt to demonstrate a possible alternative primary site of the tumor other than liver, we undertook a careful examination of the whole body, but no tumor was found elsewhere. Microscopically, the main liver tumor showed appearances similar to those seen in the previous biopsy sections. The vast majority (90%) had sarcomatoid features with keratinizing SCC lesions embedded in it. Thorough examination identified, albeit extremely infrequently, an SCC island which contained a few carcinoma cells with Alcian blue-positive mucin-like substance (Fig. 7). However, glandular formation was not evident. The

Fig. 5. Histology of the tumor biopsy revealed foci of squamous cell carcinoma showing keratinization embedded in the sarcomatoid change (hematoxylin-eosin, original magnification ×20 (A) and ×100 (B)).

lesion was very limited in that this type of carcinoma cells was not found in adjacent sections examined by Alcian blue, mucicarmine and PAS stain. The tumor cells were all negative for cytokeratins 7 and 19, known as markers of the bile duct epithelium. The same histological features with the primary

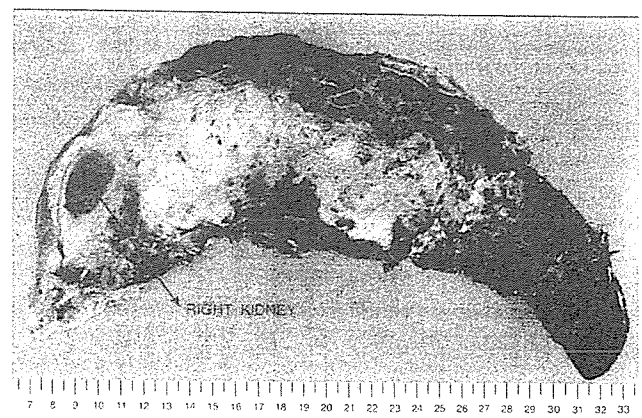

Fig. 6. A cut surface of the autopsied liver. A large whitish tumor arising primarily from the right liver lobe expanded into the left lobe and the right kidney.

Table 1
Summary of reported cases of primary squamous cell carcinoma of the liver

Case no.	Authors	Age (year)/sex	Underlying liver disease	Symptoms	Methods of diagnosis	Treatment	Outcome
1	Bloustein and Silverberg [1]	30/M	SBNHC	Abdominal pain	Exploratory laparotomy	Hepatic resection + chemotherapy and radiotherapy	Died 4 months post-operatively
2	Song et al. [2]	43/M	Hepatolithiasis	Abdominal pain, Jaundice	Exploratory laparotomy	Hepatic resection	Died 6 months post-operatively
3	Gresham and Rue [3]	78/M	SBNHC	Abdominal pain	Post-mortem examination	Symptomatic treatment	Died
4	Lynch et al. [4]	63/M	SBNHC	Abdominal pain, Jaundice	Exploratory laparotomy	Cyst drainage	Died 6 months after admission
5	Arase et al. [5]	63/M	Liver cirrhosis	Abdominal pain	Post-mortem examination	Transarterial infusion of mitomycin C	Died
6	Clements et al. [6]	73/M	Biliary strictures	Jaundice	Post-mortem examination	Symptomatic treatment	Died
7	Roediger and Dymock [7]	51/F	No	Abdominal pain	Exploratory laparotomy	Hepatic resection	Died post-operatively
8	Nieweg et al. [8]	62/F	SBNHC	Abdominal pain	Exploratory laparotomy	Symptomatic treatment	Died 5 months post-operatively
9	Pliskin et al. [9]	82/F	Multiple congenital cysts	No particular symptom	Percutaneous biopsy	Symptomatic treatment	Died
10	Banbury et al. [10]	59/F	SBNHC	Abdominal pain	Exploratory laparotomy	Hepatic resection	No recurrence (over 16mo)
11	Lombardo et al. [11]	59/F	Epidermoid cyst	Abdominal pain	Exploratory laparotomy	Hepatic resection	No recurrence (over 8mo)
12	Weimann et al. [12]	74/F	SBNHC	Abdominal pain	Exploratory laparotomy	Hepatic resection	No recurrence (over 4 years)
13	Shinagawa et al. [13]	56/F	Cystically dilated biliary tract	Jaundice	Bile cytology	Hepatic resection	Died 8 months post-operatively
14	Monteagudo et al. [14]	71/F	SBNHC	Abdominal pain	Percutaneous biopsy	Cystostomy	Died 1 month post-operatively
15	Doctor et al. [15]	68/F	No	Fever	Percutaneous biopsy	Hepatic resection + chemotherapy with cisplatin and 5-fluorouracil	Unknown
16	Vick et al. [16]	51/M	Ciliated foregut cyst	Abdominal pain	Exploratory laparotomy	Hepatic resection	Died 2 month post-operatively

SBNHC, solitary benign nonparasitic hepatic cyst.