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Original Article

Simple formula to predict response to peginterferon alpha2b and ribavirin combination therapy in genotype 1 chronic hepatitis C patients with high viral loads

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Aim: We advocate a simple formula which can conveniently predict the outcome of Peg-interferon (IFN) alpha2b and ribavirin (RBV) combination therapy for genotype 1 chronic hepatitis C (CH-C) with high viral load.

Methods: A total of 338 (group A: 230, Group B: 108) genotype 1 CH-C patients treated with Peg-IFN alfa-2b and RBV were enrolled. Clinical parameters differing significantly between sustained virological responders (SVRs) and non-SVRs in group A were categorized, then a simple formula to predict SVR was constructed and re-evaluated in group B. Another formula containing hepatitis C virus amino acid mutations/substitutions also was constructed.

Results: In group A, gender and HCV RNA load <1000 KIU were significant predictors of SVR by multivariate logistic regression analysis. A simple formula was constructed (formula A): male gender (point 2) + HCV RNA load <1000 KIU (3) + platelet counts ≥15 × 10^4 /mm^3 (1) + age <60 (1). In group A, score (0–1) predicted SVR rate 23.8% (2–4): 48.1% and (5–7): 70.2%. According to this formula, score (0–1) predicted SVR rate 7.1% (2–4): 38.6%, and (5–7): 70.3% in group B. Information on HCV amino acid mutations/substitutions seemed to add some accuracy.

Conclusions: This simple formula can be used to roughly determine, at the patients' first/second visit, the probability of response to Peg-IFN alpha2b and RBV combination therapy for genotype 1 CH-C with high viral load.

Key words: chronic hepatitis C, genotype 1b, peginterferon and ribavirin combination therapy, predictive formula.

INTRODUCTION

WITH THE ADVANCES in antiviral therapy for chronic hepatitis C (CH-C), around 50% of genotype 1 patients with high hepatitis C virus (HCV) RNA loads can now be cured by peginterferon (Peg-IFN)/ribavirin (RBV) combination therapy.[1,2] However, in Japan the majority of patients with CH-C are relatively old[3,4] and IFN based antiviral therapy sometimes cannot be completed because of adverse effects,[5] which suggests to us the need to identify before treatment the patients highly likely or unlikely to be cured by the combination therapy.

A simple and convenient formula to predict the likelihood of cure before starting treatment is recommended to establish effective Peg-IFN and RBV combination therapy for genotype 1 CH-C, because a substantial proportion of CH-C patients are not followed by experts in clinical hepatology without antiviral therapy.

A previous paper reported that a logistic regression model, including mutations in the interferon sensitivity determining region (ISDR) in the nonstructural protein

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5A (NS5A) region of HCV, T helper type 1/T helper type 2 balance, body weight and neutrophil count, is useful for predicting accurately the likelihood of SVR before starting therapy.[6] Recently, prediction of SVR has been achieved using another formula containing the on-treatment laboratory data.[7] Although these formulae are superior in accuracy, the necessity for laboratory work or complicated calculations hamper their use in clinical practice.

In this study, we set out to construct a simple formula, based on pretreatment clinical data, which can be used to determine conveniently at the patients' first visit the probability of response to Peg-IFN and RBV combination therapy for genotype 1 CH-C with high viral load.

SUBJECTS AND METHODS

Patients

THIS STUDY WAS conducted at University Hospital of Kyoto Prefectural University of Medicine, Kyoto, Osaka University, Osaka, Ehime University, Ehime, Japan and related hospitals. Enrollment of the patients was started in January 2006 and ended in July 2008, and the follow up study was completed in January 2009. Among the patients with genotype 1 CH-C who had high viral loads (Amplicor HCV RNA kit, version 2.0; Roche Diagnostics, Tokyo) and completed the course of Peg-IFN alpha2b and RBV combination therapy for 48 weeks, 370 patients, aged 23 to 73 years, were enrolled. Two hundred and thirty patients were randomly assigned to group A. Among the remaining140 patients, 108 patients whose amino acid substitution of HCV core 70 and mutations in the ISDR were determined were assigned to group B.

Patients with decompensated liver disease, co-infection with hepatitis B virus or human immunodeficiency virus, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis and Wilson's disease were excluded by liver biopsy before treatment or by appropriate serological/biochemical data. Patients with uncontrollable hypertension or diabetes mellitus and those with a history of heavy alcohol drinking also were excluded.

Study design

All patients received weekly injections of PEG-IFN-α-2b (PEG-INTRON; Shering-Plough, Kenilworth, NJ) of 1.5 μg/kg.bw and oral administration of RBV (Rebetol; Shering-Plough) of 600 to 1000 mg/day. The amount of RBV was adjusted based on the body weight; (600 mg for <60 kg.bw, 800 mg for ≥60 kg.bw and <80 kg.bw, 1000 mg for ≥80 kg.bw. The dose of PEG-IFN-α-2b was decreased by 50% when platelet counts was below 8×10^4 /mm^3 or the neutrophil counts was below 750 /mm^3. The dose of RBV was lowered by 200 mg/day when the hemoglobin concentration fell below 10 g/dL. The full dose regimen was re-started when the adverse events improved. Written informed consent was obtained from all patients before treatment and this study was approved in 2005 by the ethical committee of the university.

Determination of HCV core amino acids 70 and the interferon sensitivity determining region (ISDR)

Frozen serum samples obtained before the commencement of therapy were stored at −80 °C for the analyses of amino acid substitutions in HCV core 70 and mutations in ISDR in NS5A. The sequences corresponding to amino acids 1–191 (HCV core) and amino acids 2209–2248 (ISDR) were analyzed by direct sequencing, as described by Akuta *et al.*[8,9] and Enomoto *et al.*[10] Briefly, after total RNA was extracted from the sera and converted into cDNA, first and second round polymerase chain reactions (PCRs) were performed. Primers used in the PCR were as follows. (i) For the core region: the first-round PCR was performed with CC11 (sense, 5′-GCC ATA DTD GTC TGC GGA ATG-3′) and e14 (antisense, 5′-GGA GCA GTC CTT CGT GAC ATG-3′) primers, and the second-round PCR with CC9 (sense, 5′-GCT AGC CGA GTA GTG TT-3′) and e14 (antisense) primers. (ii) For the ISDR in NS5A: the first-round PCR was performed with ISDR1 (sense, 5′-ATG CCC ATG CCA GGT TCC AG-3′) and ISDR2 (antisense, 5′-AGC TCC GCC AAG GCA GAA GA-3′) primers, and the second round PCR with ISDR3 (sense, 5′-ACC GGA TGT GGC AGT GCT CA-3′) and ISDR4 (antisense, 5′-GTA ATC CGG GCG TGC CCA TA-3′) primers (hemi-nested PCR). The amplicons were sequenced and the sequences were compared with the consensus sequence of genotype 1b (HCV-J).[11] Amino acids 70 were arginine in the wild type and glutamine/histidine in the mutant.

Statistical analysis

All data analyses were conducted using the Statistical Package (SPSS). Individual characteristics between groups were evaluated by means of the Mann-Whitney *U*-test. Variables exhibiting statistical significance ($P < 0.05$) in the univariate analysis were subjected to multivariate logistic regression analysis. Multivariate logistic regression analysis with stepwise method was used to investigate the multivariate association of SVR

Table 1 Clinical background of the 230 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (group A). Data are compared between SVR and non-SVR patients by Mann-Whitney *U*-test

	SVR	Non-SVR	*P* value
Gender (male/female)	75/38	56/61	0.005
Age	54 (25–73)	57 (27–73)	0.010
HCV RNA (KIU/mL)	1 500 (100–>5 000)	2 000 (139–>5 000)	0.015
Hb (g/dL)	14.5 (10.7–18.1)	14.1 (11.9–20.2)	0.011
PLT($\times 10^4$/μL)	18.1 (6.2–36.6)	16.1 (7.1–30.1)	0.001
WBC (/μL)	5 200 (2 300–11 000)	4 900 (2 600–11 000)	0.077
Neutrophil (/μL)	2 652 (1 071–7 040)	2 511 (524–6 457)	0.424
ALT (IU/L)	71 (15–740)	60.5 (17–298)	0.143
LDH (IU/L)	194.5 (122–425)	193.5 (113–472)	0.956
ALP (IU/L)	248 (82–620)	261 (55–897)	0.575
γGTP (IU/L)	43 (5–282)	45 (10–501)	0.151
T-Chol (mg/dL)	170 (82–294)	174 (101–249)	0.422
TG (mg/dL)	89 (45–296)	96 (37–395)	0.260
Ferritin (mg/dL)	140.0 (7.3–1491.1)	164.3 (19.0–949.8)	0.161
Hyaluronate (ng/dL)	55 (9–555)	63 (9–694)	0.197

P-value <0.05 was considered to be statistically significant.

with clinical background. All *P*-values of $P < 0.05$ by the two-tailed test were considered statistically significant.

RESULTS

Baseline laboratory data of the patients and construction of a simple and convenient formula to predict the response to peginterferon alpha2b and ribavirin combination therapy

THE BASELINE CHARACTERISTICS of 230 group A patients with genotype 1 CH-C were compared between those with SVR and non-SVR (Table 1). The SVR patients were significantly more often male ($P = 0.005$), younger ($P = 0.010$), had less HCV RNA at baseline ($P = 0.015$), higher hemoglobin concentrations ($P = 0.011$) and higher platelet counts ($P = 0.001$). The other parameters did not differ significantly between the two groups.

Multivariate logistic regression analysis was performed with five items (gender, age, HCV RNA load at baseline, platelet counts and hemoglobin concentration) and the *P*-values were calculated as 0.036, 0.206, 0.101, 0.009 and 0.959, respectively. Because the *P*-value of hemoglobin concentration was 0.959, this item was omitted. Then, four items (gender, age, HCV RNA load at baseline, platelet counts) were analyzed by receiver operating characteristic (ROC) analysis for categorization. The appropriate categories were as follows: gender (male, female), HCV RNA load at baseline (≥1000, <1000 KIU/mL), platelet counts ($\geq 15 \times 10^4$, $< 15 \times 10^4$ /mm^3) and age (≥60, <60 years old).

After categorization, the data were subjected to multivariate logistic regression analysis to investigate the association of SVR with clinical background. As shown in Table 2, the *P*-values were 0.004 and 0.002 for gender and HCV RNA load at baseline, 0.110 and 0.175 for platelet counts and age. Because the *P*-value of HCV

Table 2 Multivariate logistic regression analysis of categorized clinical background, based on SVR and non-SVR, in the 230 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group A). Based on this result, a simple formula (formula A) was constructed

	Odds ratio	(95% CI)	*P* value
Gender (female/male)	2.277	(1.288–4.025)	0.004
HCV RNA			
(1000 KIU/mL ≥/<)	2.579	(1.417–4.693)	0.002
PLT (15 × 104/mL ≥/<)	1.624	(0.895–2.944)	0.110
Age (60 years old ≥/<)	1.510	(0.831–2.743)	0.175

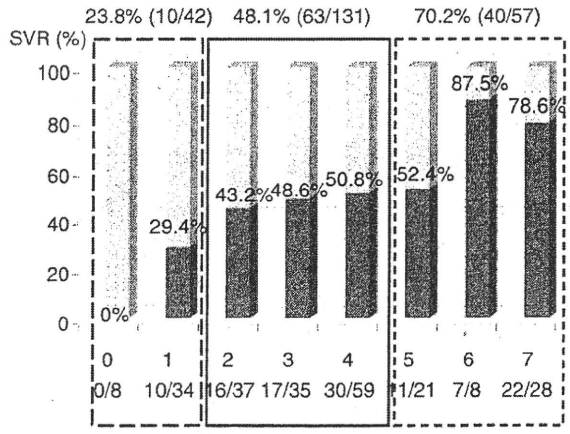

Figure 1 Scoring data according to formula A and the SVR rate in the 230 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group A). Patients were classified into a poorly responsive group (score 0 to 1), a moderately responsive group (score 2 to 4) and a moderately to highly responsive group (score 5 to 7).

RNA load at baseline was 0.002 with the highest Odds ratio (2.579), we set point 3 to HCV RNA load <1000 KIU. Similarly, because the *P*-value of gender was 0.004 with higher Odds ratio (2.277), we set point 2 to male gender. The *P*-values of platelet counts and age were not statistically significant. However, because the Odds ratios of these two items were relatively high (1.624 and 1.510), we set point 1 to platelet counts $\geq 15 \times 10^4$ /mm^3 and age <60. Based on these data, a simple formula was constructed: male gender (point 2) + HCV RNA load <1000 KIU (point 3) + platelet counts $\geq 15 \times 10^4$ /mm^3 (point 1) + age <60 (point 1). This formula was referred to as formula A.

For easy use of formula A in clinical practice, patients in group A could be classified into three groups depending on their response to therapy, that is, poorly responsive (point 0 to 1), moderately responsive (point 2 to 4) and moderately to highly responsive (point 5 to 7) groups (Fig. 1). The SVR rate in the poorly responsive group was 23.8% (10/42), that in moderately responsive group was 48.1% (63/131) and that in moderately to highly responsive group was 70.2% (40/57). To determine the efficacy of formula A, we applied it to group B (Fig. 2). The poorly responsive group (point 0 to 1) showed an SVR rate of 7.1% (1/14), the moderately responsive group (point 2 to 4) 38.6% (22/57) and the moderately to highly responsive group (point 5 to 7) 70.3% (26/37).

Impact of information on amino acid sequences in the ISDR and HCV core on the accuracy of formula A

Because amino acid mutations in the ISDR and substitutions in core region of HCV affect the responsiveness to Peg-IFN/RBV combination therapy,[8–10] we constructed another formula by adding this information, but without liver histology. Because patients with ≥2 amino acid mutations in the ISDR and HCV core amino acid 70 wild type have higher probability to attain SVR,[8–10] we performed multivariate logistic regression analysis with six items (gender, HCV RNA load at baseline, platelet counts, age, amino acid substitutions in ISDR and HCV core amino acid 70) and the *P*-values were calculated to be 0.009, 0.008, 0.143, 0.204, 0.051 and 0.023, respectively (Table 3).

Because the *P*-values of gender and HCV RNA load at baseline were 0.009 and 0.008 with high Odds ratios (3.357 and 3.471), we set point 3 to male gender and HCV RNA load at baseline <1000 KIU. Similarly, because the *P*-values of ISDR mutation and Core 70 mutant/wild type were 0.051 and 0.023 with relatively high Odds ratios (2976 and 3.139), we set point 2 to ≥2 amino acid substitutions in ISDR and HCV core amino acid 70 wild type. The *P*-values of platelet counts and age were not statistically significant. However, because the Odds ratios of these two items were relatively high (2.021 and

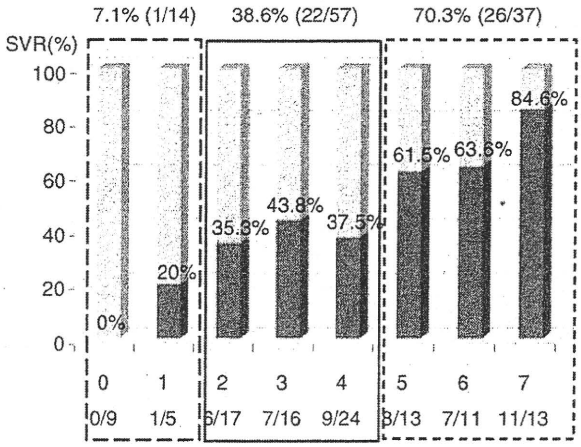

Figure 2 Scoring data according to formula A and the SVR rate in the 108 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group B). Score 0 to 1 represents a poorly responsive group, score 2 to 4 a moderately responsive group and score 5 to 7 a moderately to highly responsive group, which is similar to the data presented in Figure 1.

Table 3 Multivariate logistic regression analysis, based on SVR and non-SVR in the 108 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group B). Based on this result, formula B was constructed

	Odds ratio	(95% CI)	*P* value
Gender (female/male)	3.357	(1.346–8.375)	0.009
HCV RNA			
(1000 KJU/mL ≥/<)	3.471	(1.390–8.666)	0.008
PLT ($15 \times 10^4/\mu L$ ≥/<)	2.021	(0.895–2.944)	0.143
Age (60 years old ≥/<)	1.929	(0.700–5.316)	0.204
ISDR mutation (0.1/≥2)	2.976	(0.995–8.904)	0.051
Core 70 mutant/wild type	3.139	(1.172–8.406)	0.023

1.929), we set point 1 to platelet counts $\geq 15 \times 10^4$ /mm^3 and age <60. Based on these data, formula B was constructed: male gender (point 3) + HCV RNA load at baseline <1000 KIU (point 3) + platelet counts $\geq 15 \times 10^4$ /mm^3 (point 1) + age <60 (point 1) + ≥2 amino acid substitutions in ISDR (point 2) + HCV core amino acid 70 wild type (point 2). In group B, a total score of 0 to 3 could be categorized as the poorly responsive group (SVR ratio: 4.8% [1/21]), that of 4 to 7 the moderately responsive group (SVR ratio: 43.6% [27/62]) and that of 8 to 12 the moderately to highly responsive group (SVR ratio: 84% [21/25]) (Fig. 3).

DISCUSSION

IN THIS STUDY, we constructed a formula to predict the efficacy of Peg-IFN/RBV combination therapy:

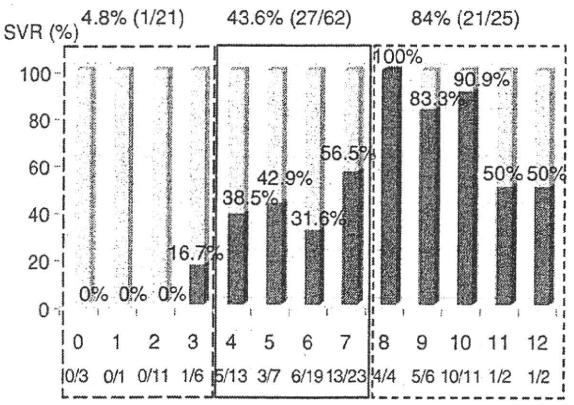

Figure 3 Scoring data according to formula B and the SVR rate in the 108 patients with chronic hepatitis C with high viral loads and treated with PEG-IFN and RBV combination therapy (Group B). Score 0 to 3 represents a poorly responsive group, score 4 to 7 a moderately responsive group and score 8 to 12 a highly responsive group.

male gender (point 2) + HCV RNA load at baseline <1000 KIU (point 3) + platelet counts $\geq 15 \times 10^4$ /mm^3 (point 1) + age <60 (1 point). This simple formula (formula A) could distinguish a poorly responsive group (score [0–1]), a moderately responsive group (score [2–4]) and a moderately to highly responsive group (score [5–7]) (Fig. 1). Thus, formula A may be used by general physicians easily to roughly guess the probability of response to Peg-IFN and RBV combination therapy at the patient's first visit. Another formula (formula B) was constructed by adding the information of amino acid substitutions in the HCV genome. Although examination of amino acid substitutions in the HCV genome is not covered by the public health insurance in Japan, formula B distinguished a poorly responsive group (score [0–3]), a moderately responsive group (score [4–7]) and a highly responsive group (score [8–12]) (Fig. 3).

In Peg-IFN and RBV combination therapy for CH-C with a high viral load, the interval between the start of therapy and disappearance of HCV RNA from the serum is widely accepted as the most reliable marker to predict outcome,[12] and response-guided therapy is recommended. According to nationwide registration trials in Japan, in patients with a rapid virological response (RVR), demonstrating disappearance of HCV RNA within the first four weeks, the SVR rate was expected to be 76% to 100%, and in patients with an early virological response (EVR), showing the disappearance of HCV RNA in the first 5 to 12 weeks, the SVR rate was expected to be 71% to 73%.[13,14] In contrast, in patients with a late virological response (LVR), demonstrating clearance of HCV RNA between weeks 13 to 24, the expected SVR rate was as low as 29 to 36%. However, in clinical practice, most patients are happy to know the probability of SVR at the first or second visit, or at least before starting therapy. In this regard, formula A we advocate may be useful for a wide range of physicians.

According to formula B which included the substitutions of amino acids in the ISDR and HCV core, the predicted SVR rate also was classified into three groups, and with increased accuracy (Fig. 3). Recently, a strong association between interleukin 28B (IL28B) gene polymorphism and the response to PEG-IFN and RBV combination therapy was reported for CH-C patients.[15–17] Because determination of IL28B gene polymorphism as well as the amino acid sequences of the ISDR or HCV core is not covered by the public health insurance in Japan, it is difficult to advocate a formula containing these factors for a wide range of Japanese general physicians.

In patients with CH-C, liver biopsy is recommended to determine the treatment.[12] Because liver biopsy is not required for IFN-based antiviral therapy in Japanese public health insurance, a proportion of the patients refuse liver biopsy but are willing to be treated by Peg-IFN and RBV combination therapy. In this regard, formula A is useful in providing information concerning the likely efficacy of treatment at the first or second visit.

We constructed a simple formula to predict the outcome of treatment of genotype 1 CH-C with high viral load with Peg-IFN and RBV for 48 weeks. Recently, response-guided therapy recommended prolonged therapy up to 72 weeks for patients with LVR.[18–21] A larger study is required to establish a better formula to be utilized readily by the general physicians.

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Review Article

Predictors of Virological Response to a Combination Therapy with Pegylated Interferon Plus Ribavirin Including Virus and Host Factors

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A combination therapy with pegylated interferon (PEG-IFN) plus ribavirin (RBV) has made it possible to achieve a sustained virological response (SVR) of 50% in refractory cases with genotype 1b and high levels of plasma HCVRNA. Several factors including virus mutation and host factors such as age, gender, fibrosis of the liver, lipid metabolism, innate immunity, and single nucleotide polymorphism (SNPs) are reported to be correlated to therapeutic effects. However, it is difficult to determine which factor is the most important predictor for an individual patient. Data mining analysis is useful for combining all these together to predict the therapeutic effects. It is important to analyze blood tests and to predict therapeutic effects prior to initiating treatment. Since new anti-HCV agents are under development, it will be necessary in the future to select the patients who have a high possibility of achieving SVR if treatment is performed with standard regimen.

1. Progress in Virological Response in the Difficult-to-Treat Patients with Genotype 1 Hepatitis C Virus (HCV) Infection and Factors Correlated to the Efficacy

Recently, the average age of the patients with chronic hepatitis C has been increasing in Japan. Incidence of hepatocellular carcinoma (HCC) in the elderly patients with chronic hepatitis C (65 years or older) has demonstrated to be higher than younger ones when adjusted by the stage of hepatic fibrosis [1]. In Japan, refractory cases with genotype 1b and high HCVRNA levels are seen in as high as 70 percent of chronic hepatitis C patients. The outcome of conventional IFN monotherapy has been an SVR response of 3%–5% after 6 months of treatment in genotype 1b and high HCVRNA patients [2, 3], and virus mutation such as interferon sensitivity-determining region (ISDR) is shown to be correlated with the virological response [2]. The association of ISDR mutations and virological response to IFN monotherapy was denied in an Italian study [4]; however, it was confirmed by a Chinese study [5] and an international meta-analysis [6].

However, pegylated IFN (PEG-IFN) extends the duration of therapy and reduces adverse effects, and for this reason, PEG-IFN has become the cornerstone of therapy. Furthermore, by the combination therapy with PEG-IFN and ribavirin (RBV), the rate of SVR has dramatically improved. Even in the patients with genotype 1b and high HCVRNA level, SVR rate reaches as high as 40%–50%, thereby improving the therapeutic effects both in Western countries [7, 8] and in Japan [9, 10].

It is important to predict the rate of achieving SVR in the individual patient, before initiating treatment. Both virus- and host-related elements have been reported as factors correlated to therapeutic effects of combination therapy [11–13]. A particular focus has been placed on virus mutations, age, gender, fibrosis of the liver, lipid metabolism, and degree of fatty metamorphosis of the liver.

Among these factors related to PEG-IFN and RBV, innate immunity has been shown to be correlated in virological

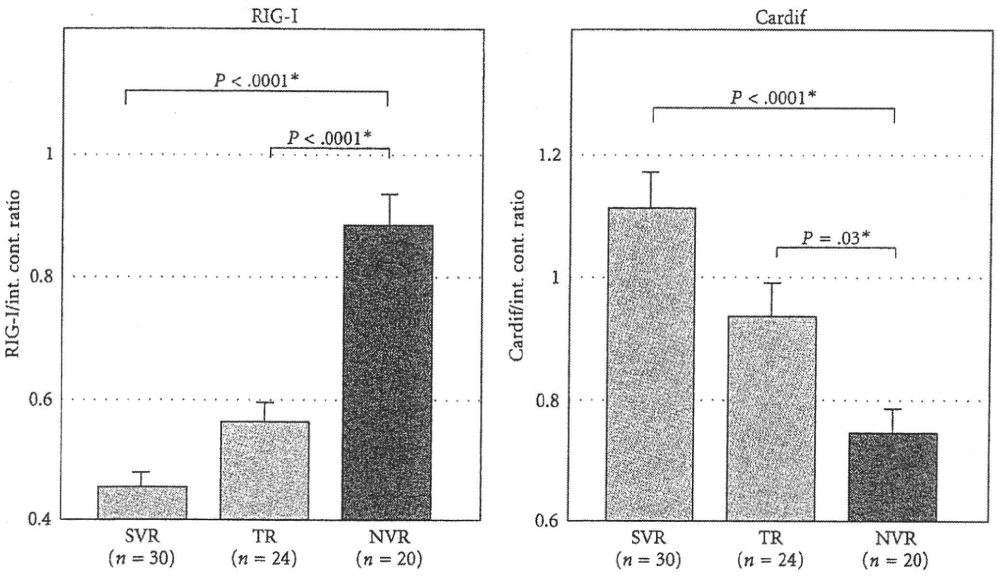

FIGURE 1: Expression of genes correlated to the intrahepatic innate immunity and virological response to PEG-IFN alpha-2b and RBV combination therapy. Open column indicates SVR (n = 30), gray column indicates TR (n = 24), and closed column indicates NVR (n = 20). Error bars indicate the standard error. The P values were analyzed by the Kruskal-Wallis test. Expression of Rig-I was significantly higher in NVR than in SVR patients, and Cardif expression was higher in SVR than in NVR. The figure was cited from [8].

response. Asahina et al. reported that liver biopsies were performed before the PEG-IFN and RBV combination therapy to examine the correlation between the gene expression involved in innate immunity and the therapeutic effects, and in the patients in whom RIG-I expression is high and the expression of Cardif, an adaptor gene, is low, it was found that there are many nonresponders (NVRs) in which HCVRNA does not become negative during the course of treatment [13]. It was therefore revealed that there are many NVRs among the patients in whom the ratio of RIG-I to Cardif in liver tissue is high and that this ratio is low in the SVR patients. Based on these findings, it has become clear that innate immunity is correlated to therapeutic effects (Figure 1).

Furthermore, it was recently discovered that a single nucleotide polymorphism (SNP) of the host gene IL28B is significantly involved in the therapeutic response to the PEG-IFN and RBV combination therapy [14, 15]. The possibility of becoming an NVR is high in cases of the minor allele carriers of IL28B. However, it is not possible to routinely measure an SNP of IL28B in the clinical setting. In this paper, factors which can actually be used in real clinical practice are discussed for the prediction of the efficacy of PEG-IFN and RBV combination therapy.

2. Amount of HCVRNA

In the patients with chronic hepatitis C, it is not possible to directly measure the amount of virus, and the amount of HCVRNA is measured instead. Currently, a real-time PCR method which has an advantage of wide range and high sensitivity is utilized, and measurements can be taken from a single blood sample of a very small amount, that is, 1.2 log copies/ml, to a very large amount, that is, 8 log copies/ml. This method has a higher level of sensitivity than the conventional Amplicor monitor test and can therefore detect HCVRNA even if only a very small amount exists in the plasma. If the amount of HCVRNA in plasma is less than the range of sensitivity of the real-time PCR method, it is recorded as undetectable level. If the indication is "less than 1.2 log copies/ml of HCVRNA", it means that a very small amount of HCVRNA exists in the plasma. Since the indication of the real-time PCR method is based on log counts, a decrease of 1.0 in the numerical value means that the amount of HCVRNA has decreased to 1/10. With the application of this real-time PCR method, it has become possible to measure amounts of HCVRNA up to 8 log copies/ml, and it has also become possible to predict the efficacy before treatment and to monitor appropriately the reactivity during the course of treatment. However, in the patients in whom a PEG-IFN and RBV combination therapy is performed, SVR can be acquired even when the amount of virus prior to the treatment is quite large. It is therefore difficult to predict the virological response solely from the amount of HCVRNA before starting the treatment. Once treatment has commenced, at what week HCVRNA becomes negative is important for the prediction of therapeutic effects, and this serves as a parameter for deciding the duration of treatment [16].

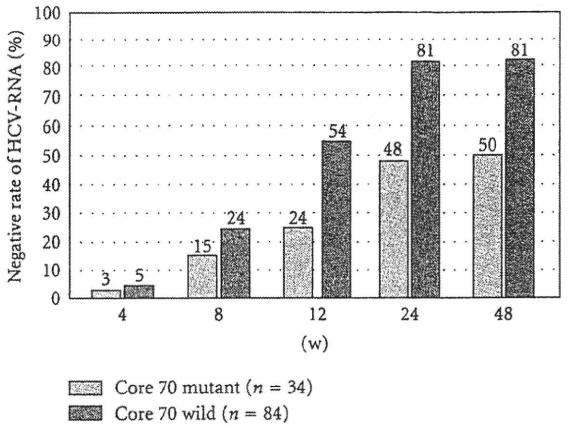

FIGURE 2: Comparison of aa70 mutations in the HCV core region and the rate of HCVRNA becoming negative during the course of treatment. Compared with the wild type, among the patients of aa70 mutations, there were fewer patients in whom HCVRNA had become negative during the course of treatment.

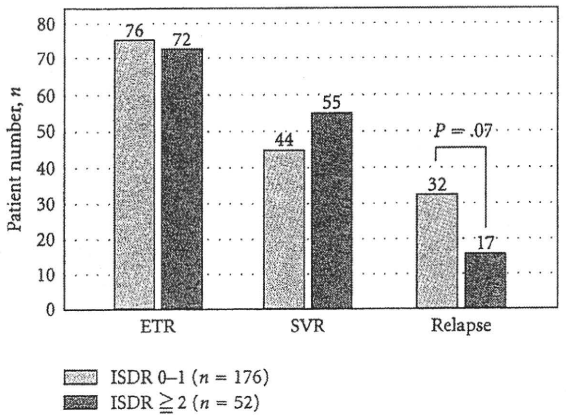

FIGURE 3: Number of ISDR substitutions and the comparison of virological response, SVR, and relapse at the end of the treatment. Compared with the patients with 2 or more sites of substitutions, the rate of SVR was lower and the rate of relapse was higher in the patients in whom there were fewer substitutions in ISDR, that is, 0 or 1 sites.

Measuring the rate of viral clearance from serum is helpful for predicting the likelihood of a response to PGEIFN and RBV and useful for determining the optimal duration of therapy if the patients start to receive the treatment [17]. In the AASLD practice guideline, response-guided therapy is highly recommended [18]. In two nationwide registration trials conducted in Japan [9, 10], the SVR rate was high, from 76% to 100%, in patients whose HCVRNA was cleared rapidly from serum by week 4 (rapid virological response; RVR), and 71% to 73% in patients who achieved undetectable HCVRNA from week 5 to week 12 (early virological response; EVR). In contrast, the SVR rate in patients with late clearance of HCVRNA from week 13 to week 24 was low at 29% to 36%. No patients without clearance of HCVRNA by week 24 achieved SVR.

The strategy of extending therapy in patients with delayed virological responses, defined as clearance of HCV-RNA between weeks 12 and 24, was evaluated in five studies [19–23]. These results cannot be compared directly with each other because of the heterogeneous study populations, differences in the baseline characteristics, and the different regimens utilized amongst them. Nevertheless, the results showed a trend toward a higher SVR rate by extending therapy from 48 to 72 weeks in patients with delayed virological response.

3. Viral Mutations in Core and NS5A Region

In the patients with genotype 1b HCV infection, the mutations in aa70 and aa91 in the core region have been shown to correlate with early virological response (EVR) during PEG-IFN and RBV combination treatment [11]. If aa70 in the core region is mutated to anything other than arginine and aa91 to anything other than leucine, it is difficult to achieve EVR, and it is thus highly possible that such cases will become nonresponders. The examination results at our institution including 292 patients with genotype 1b infection demonstrated that, in the cases with mutations in aa70 in the core region, the rate of HCVRNA becoming negative during the course of combination treatment was low compared to the wild type of aa70 (Figure 2). However, core aa70 mutation is shown to have quasispecies detectable by cloning, and 70Q clone was positively selected during combination treatment with PEGIFN and RBV [24].

Furthermore, Enomoto et al. reported that the patients with 4 or more amino acid mutations were observed in interferon sensitivity-determining region (ISDR) within NS5A region [2]; SVR rate is higher than 90% by IFN monotherapy, and SVR is less than 10% in the patients with no mutation in ISDR. It has also been reported that, in PEG-IFN and RBV combination therapy, the number of ISDR mutations is involved in the SVR [12].

We analyzed the relationship between virological response and ISDR mutations in the patients with genotype 1b infection treated by PEG-IFN alpha-2b and BRV combination therapy. In the patients with 0 or 1 ISDR mutation, even if the rate of HCVRNA becoming negative at the end of treatment was the same, the rate of SVR would be lower compared with the patients having 2 or more mutations (Figure 3). This demonstrates that there is a higher incidence of relapse after the end of treatment in the patients with 0 or 1 ISDR mutation.

Enomoto and Maekawa reported that mutations both in NS5A-ISDR (interferon sensitivity-determining region) and core 70Q substitution are associated with no early viral response during PEGIFN and RBV combination therapy [25]. Association of core aa70 substitution and mutations in NS5A region is confirmed to be associated with viral response by PEGIFN and RBV combination therapy in a Japanese multicenter cooperative study [26]. The number of

mutations in the interferon sensitivity-determining region was shown to be associated with the viral response to PEGIFN and RBV combination treatment not only in Japan [27], but also in Tunisia [28].

Recently, a consensus has been established that mutations in aa70 in the core region are important for the prediction of HCVRNA becoming negative during the early course of treatment, and the number of ISDR mutations is important for the prediction of relapse after the end of treatment.

4. Adherence

It has been confirmed that it is important to ensure 80% or more of the scheduled dose of both PEG-IFN and BBV in order to improve the rate of SVR, and together with the duration of treatment, the 80 · 80 · 80 rule has been established. However, Schiffman et al. recently reported that the dose of PEG-IFN in the initial stage of administration is important and that, if a sufficient dose of PEG-IFN is administered, then 60% or more of the RVB dose would be enough [29]. It is therefore of primary importance to ensure the dose of PEG-IFN.

In Japan, the average age of patients with chronic hepatitis C is increasing, and achieving good adherence is difficult in many patients. Consequently, the rate of SVR is low in elderly patients. How to improve the rate of SVR in elderly patients is an important issue. With regard to the dose of RBV, reducing the RBV dose based on the calculation of the total body clearance (CL/F) has been proposed to be useful for decreasing the discontinuation and improving the rate of SVR. Although there is no consensus on an appropriate dose of PEG-IFN in elderly patients, if the initial dose is set lower than the usual dose, discontinuation would be reduced. Thus, it is necessary to investigate whether such an initial dose would improve the rate of SVR.

Recently, the risk of hemolytic anemia was clearly demonstrated to correlate with ITAP gene SNP [30]. The predictive implication should be analyzed prospectively in clinical practice.

5. Host Factors

Zeuzem et al. described the factors related to the less response to interferon-based therapy, and he showed that several host factors such as older age, race, and obesity are responsible factors for the poor response to IFN [31]. Recently, insulin resistance which was examined by homeostasis model assessment index (HOMA-IR) was shown to be associated with a lower rate of SVR, and body mass index (BMI) was not identified as a significant factor for the poor response to PEGIFN and ribavirin combination therapy [32]. Insulin resistance was confirmed as a related factor to the nonresponse to interferon-based treatment [33]. However, Charlton et al. reported that obesity itself is an associated factor for decreased efficacy of interferon-based therapies, and they discussed the possible mechanism [34], and obesity was shown to be associated with the increased enhancement of suppressor of cytokine signaling (SOCS) family in the hepatocytes [35].

6. Data Mining Analysis

Both virus- and host-related factors are correlated to therapeutic effects of PEG-IFN and RBV. One important question is which of these factors should be focused on in order to predict the therapeutic effects in an individual patient. In addition, in each individual patient, the host and virological factors are different. It is therefore difficult to predict the virological response in each case before treatment. Furthermore, although it is important to predict the relapse rate when HCVRNA becomes within an undetectable level in an individual patient, prediction of the rate of SVR including virological and host factors and adherence to the treatment has never been carried out in an individual patient.

A data mining analysis is the process of analyzing a large amount of data by a computer in order to develop an algorithm. Conventional statics have been used to examine certain hypothesis. Data mining is superior in that it can set an algorithm, using a computer, based on a large amount of data without a hypothesis.

We therefore conducted at our institute a data mining analysis of the patients with genotype 1b infection having high levels of HCVRNA to whom a PEG-IFN alpha-2b and RBV combination therapy was administered to investigate whether by the 12th week after the commencement of treatment HCVRNA became negative (EVR) (Figure 4) [36]. The most important factor for the prediction of EVR was the steatosis of the liver: when steatosis was observed in 30% or more of hepatocytes, EVR was found to be difficult to achieve. In the patients in whom steatosis was not severe, the second most important factor was the serum LDL cholesterol value. While the rate of EVR was 57% in the patients in whom this value was 100 mg/ml or above, the rate of EVR was 32% in the patients in whom the LDL cholesterol was less than 100 mg/dl.

The higher the LDL cholesterol value, the earlier the HCVRNA became negative. Among the patients with low LDL cholesterol values, while the rate of EVR was 15% in patients 60 years of age or older, the rate was high in the patients under the age of 60 years old, that is, 49%. Among patients under the age of 60, the rate of EVR was low, that is, 31%, in patients with a blood glucose level of 120 mg/dl or above whereas EVR was achieved in 71% of the patients with a blood glucose level of less than 120 mg/dl (Figure 4).

On the other hand, in the patients with high LDL cholesterol values, the next most important factor was age. While the rate of EVR was 50% in patients 50 years of age or older, EVR was achieved in 77% of the patients under the age of 50. Among patients of 50 years of age or older, the next most important factor was the gamma GTP value. While the rate of EVR was 35% in the patients in whom gamma GTP was 40 IU/L or above, EVR was achieved in 60% of the patients where the value was less than 40 IU/L.

We therefore compared these factors based on the original data. A univariate comparison of the fatty infiltration of the liver and the rate of EVR demonstrated that the rate of EVR was higher when the steatosis of the liver was less severe (Figure 5(a)). In addition, a comparison of the LDL cholesterol value and the rate of EVR demonstrated a significant correlation, confirming that the higher the LDL cholesterol value, the higher the rate of EVR (Figure 5(b)). Therefore, it was also proposed by the results of univariate analysis of each factor extracted from the data mining analysis that these factors were correlated to the rate of EVR.

From these observations, it is likely to improve the viral response to PEGIFN and ribavirin by reducing steatosis of the liver through daily walking or abstinating alcohol intake or by refraining from high-fat diet.

By utilizing data mining, it is therefore possible to assess virus- and host-related factors together and to predict the virological response in each patient, and thereby clinically useful information can be obtained. The algorithm should be validated including a large number of the patients.

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REVIEW

Recent advances of radiofrequency ablation for early hepatocellular carcinoma

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Key words
Hepatocellular carcinoma, radiofrequency ablation, seeding, transarterial chemoembolization, CLIP, BCLC, JIS, des-gamma-crboxy prothrombin time (DCP), alpha-fetoprotein (AFP).

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Conflict of interest
The author does not have any potentical conflicts of interest to disclose.

Abstract

Hepatocellular carcinoma (HCC) is the third leading cause of death in the malignant neoplastic diseases in the world. Surgical operation is sometimes not indicated because of complicated liver cirrhosis and extrahepatic disorders. Radiofrequency ablation has been developed as a less invasive treatment for HCC since 1999, and long-term outcome has been shown. There are several complications which should be paid attention, and to improve the prognosis, combination treatment with transarterial chemoembolization should be discussed. Overall survival after between RFA and surgical resection should be compared prospectively. Establishment of staging system for treatment allocation of HCC and prevention of HCC recurrence is important issue to be examined.

Introduction

Radiofrequency ablation (RFA) has been utilized as a less invasive and curative treatment for the treatment of hepatocellular carcinoma (HCC), and the methods and procedure have been developed. In some countries, it has been chosen as first line treatment for early stage HCC. Long-term prognosis has been reported and the associated factors for the prognosis after RFA have been shown. Several complications were reported after RFA. The prognosis was compared between patients who were treated by between surgical resection and those treated by RFA. The recent developments and future perspective of RFA is discussed in this review.

Radiofrequency ablation method

Of all therapeutic apparatus compared and evaluated up to now, the RF 3000 generator system (Boston Scientific, Boston, USA) had the most positive therapeutic effects.[1] However, in many articles, an internally cooled single electrode was used.[2] When there was a risk of RFA incurred by the hepatocellular carcinoma (HCC) location, the therapeutic effects were reduced, in particular the complete response rate was low in the vicinity of the gall bladder and the stomach and intestine, as well as the diaphragm, and in the vicinity of large blood vessels.[3] However, it has been reported that, although the therapeutic effects are not reduced when tumors exist in the vicinity of large blood vessels or adjacent to the extrahepatic organs, attention should be paid to the prevention and control of complications.[4] RFA with the use of artificial ascites for HCC adjacent to the diaphragm and to the stomach and intestine produced sufficient therapeutic effects, thereby improving the sonic window.[5]

When performing RFA, the use of a guiding needle with an external insulated sheath was useful because it allowed for precise tumor targeting.[6] The use of laparoscopic RFA has allowed a sufficient therapeutic effectiveness to achieve complete tumor ablation in all cases when the HCC nodule is located with bulging or at subcapsular area, as well as an adequate safety margin, compared to percutaneous RFA.[7] As shown in Fig. 1, extra-hepatic protruding HCC nodule is the most appropriate indication for laparoscopic RFA, and complete necrosis could be achieved after one treatment session under laparoscopic ultrasound guiding. When RFA was performed under laparoscopy, complete necrosis is usually observed.[8]

Assessment of the therapeutic effect of RFA

Although the effect of RFA is, in general, evaluated by dynamic computed tomography (CT) scans taken 1 to 7 days after the procedure, it was possible to assess the therapeutic effect by multidetector row helical CT (MD-CT) immediately after RFA,

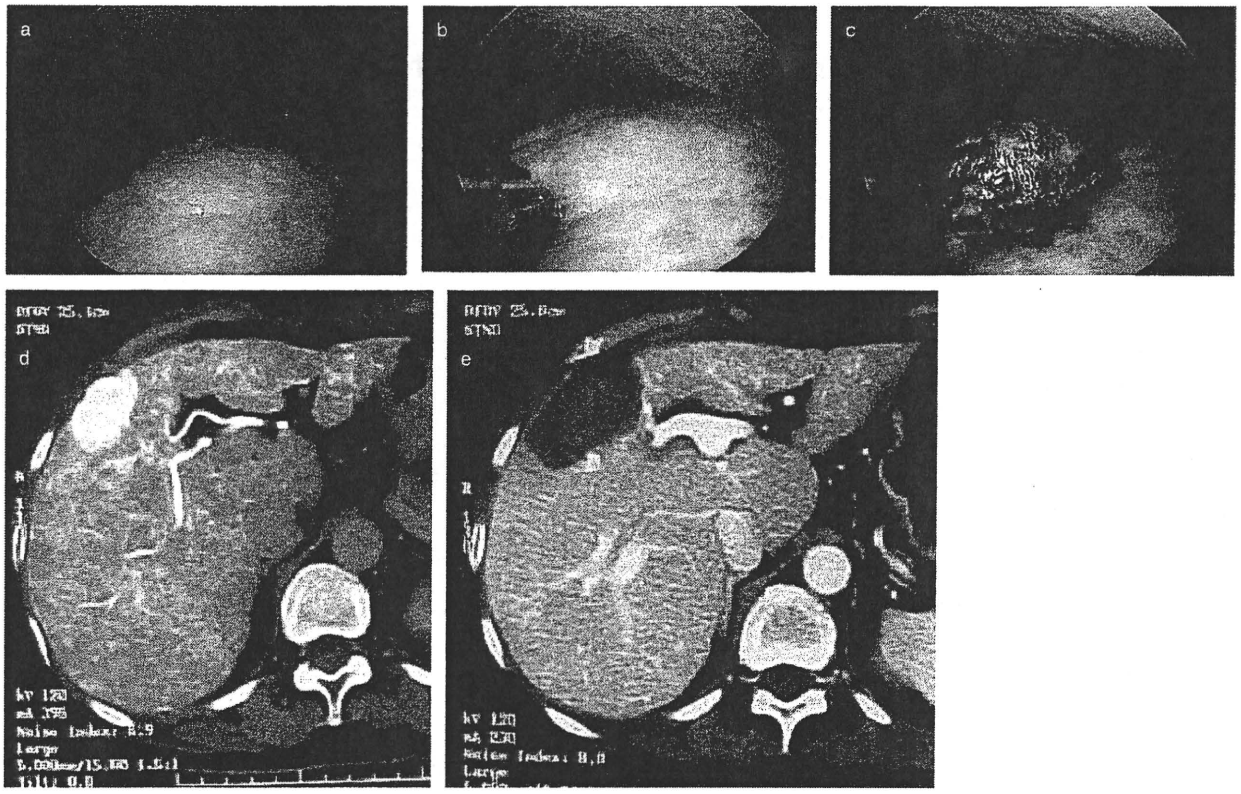

Figure 1 Hepatocellular carcinoma (HCC) nodule protruding from the liver surface is treated by laparoscopic radiofrequency ablation (RFA) under ultrasound guiding. (a) HCC nodule is directly observed under laparoscopy. (b) Under laparoscopic ultrasound guiding, RFA electrode is introduced to accurate position of the nodule, avoiding damage to diaphragm and intrahepatic vessels. (c) The entire HCC nodule was completely ablated by RFA. (d) Computed tomography (CT) scan before the treatment revealed hypervascular nodule with 2.6 cm in diameter at the surface of the liver. (e) After laparoscopic RFA, complete necrosis was confirmed by CT scan.

thereby achieving shorter hospital stays.[9] As well, one report indicates that it was possible to assess the therapeutic effect by contrast-enhanced ultrasonography immediately after RFA.[10] Contrast-enhanced sonography with abdominal virtual sonography was useful in monitoring the therapeutic effect and reducing the CT scan frequency.[11]

Prognosis after radiofrequency ablation

According to a report from a single institution in France, RFA was performed in 235 cases, with up to three HCC ≤ 5 cm in diameter, and achieved complete ablation in 222 cases. 67 cases were judged potentially resectable according to Barcelona Clinical Liver Cancer (BCLC) criteria; in these patients, RFA treatment produced 76% survival at 5 years. The factors contributing to survival were the prothrombin time and serum alpha-feto protein (AFP) levels. Conversely, the factors related to recurrence were multinodular tumors as well as the AFP level. In this report, RFA could be used as an effective first-line treatment in patients with a single nodule of 5 cm or less, a low serum AFP level, and well preserved liver function.[12]

According to a report from Italy, RFA was performed in 218 cases of single nodule HCC, measuring 2 cm or less in diameter, followed by an analysis of the prognosis. The 5-year survival rate was 68.5%, with a low 1.8% incidence of complications. Compared with resection, it was less invasive and could be conducted at a lower cost. It could therefore be considered the treatment of choice for resectable single HCC ≤ 2.0 cm.[13] In Japan, the prognosis of 1000 patients who had undergone RFA was analyzed; the 1, 3, and 5-year survival rates were 94.7%, 77.7% and 54.3%, respectively.[2] According to a report from China, the factors related to the prognosis after RFA were the tumor diameter, the number of tumors, the use of combination therapy with ethanol injection, the margin, and the Child-Pugh score.[14] According to the outcome of RFA treatment for a large single-institution series in Korea, the method had a local recurrence rate of 8.1% at 1 year and 11.8% at 3 years, and patient survival rates were 95.2% at 1 year, 69.5% at 3 years, and 58.0% at 5 years.[15] The five year survival after RFA was similar between Western and Eastern countries (Table 1).

Prognosis after RFA and the staging system

The Cancer of the Liver Italian Program (CLIP) score and BCLC scoring system more accurately predicted the prognosis than the Okuda score in patients with early-intermediate HCC, undergoing

Table 1 5-year overall survival after radiofrequency ablation in the patients with operable HCC nodule

			Overall survival	
Investigator	Diameter of the nodule	Patient number	3 y	5y
N'Kontchou G[16]	≤ 5 cm	235		76%
Livraghi T[17]	≤ 2 cm	218		68.5%
Tateichi R[18]	≤ 3 cm	1000	77.7%	54.3%
Peng ZW[19]	≤ 5 cm	281	57.1%	37.1%
	≤ 3 cm		65.7%	58.6%
Choi D[20]		570	69.5%	58.0%

non-surgical therapy, such as RFA.[21] The results of an analysis in Japan demonstrated that, regardless of the CLIP score, the combination of transarterial chemoembolization (TACE)—RFA had the highest 5-year survival.[22] In Japan, where early-stage HCC is prevalent, the majority of cases are classified into CLIP stage 1 of CLIP scores and, as such, the Japanese integrated staging (JIS) score was proposed as a new early HCC staging system.[23] The results of the validation done in many cases demonstrated that the JIS score yielded a better prediction of the prognosis than the CLIP score.[24] It has also been reported from in Korea that the JIS score is the most appropriate score for predicting the prognosis.[25]

Tumor markers

The tumor marker relevant to the prognosis after RFA is des-gamma-carboxy prothrombin time (DCP) levels; wherein, high DCP levels predicted a poor prognosis after RFA.[16,17] However, the same institution also reported that a comparison of AFP, DCP and AFP-leptin 3 (AFP-L3) demonstrated that AFP-L3 was the most useful indicator of the overall survival and disease-free survival.[18] It was pointed out that the AFP mRNA levels in the blood after RFA are also an objective index of recurrence.[19] On the other hand, blood vascular endothelial growth factor (VEGF) levels have also been reported to be related to the prognosis.[20]

Recurrence

Local tumor recurrence after RFA is 9.0% at 1 year and 17.7% at 3 years; therefore, local recurrence is relevant to the prognosis for survival.[26] Evaluation of the therapeutic effects of RFA by contrast enhanced CT scans or by enhanced magnetic resonance imaging (MRI) here demonstrated that the procedure provides good local control and the recurrence rate is low in cases in which the post-ablation margin was 0.4 cm or more and the tumor size was smaller than 2.5 cm.[27] The overall local recurrence rate after RFA was 12.8% and the tumor diameter of >2.5 cm was a significant independent factor.[28] However, another report indicates that even when local recurrence occurred, it did not adversely affect the survival prognosis.[29] Utilizing the RF 3000 generator system has been reported more positive effects than cool-tip electrode.[30]

On the other hand, the cumulative rate of intrahepatic distant recurrence was reported as 10.4% and 77.0% at 1 and 5 years, respectively. In a multivariate analysis, AFP and DCP values, as well as the safety margin, were significant independent factors.[31] The intra-hepatic distant recurrence was associated with multinodular lesions and hepatitis C virus (HCV), even after curative ablation was achieved.[32] Recurrence at a distant site is an important, poor prognostic factor.[33] Although it is possible to ensure long-term survival by carrying out repeat RFA after recurrence,[34] the more frequently recurrences occur, the higher the risk for subsequent recurrence becomes.[35] Histological grade is relevant to the therapeutic efficiency of RFA and also plays a part in determining survival.[36]

Prognosis and possible measures to improve survival after RFA

Long-term interferon maintenance therapy improved the survival in patients with HCV related HCC after RFA.[37] On the other hand, the administration of lamivudine after RFA for hepatitis B virus (HBV)-related HCC maintained the liver function and was also safe.[38,39] The administration of vitamin K for HCV-related HCC did not produce a chmenopreventative effect.[40] The oral administration of a branched-chain amino acid after RFA made it possible to maintain the serum albumin levels and it was also useful for improving the liver function.[41]

Resection *versus* RFA

With regard to the question of whether surgical resection or RFA is superior, two randomized comparisons have been reported—all from China. In these reports, the life prognoses of single HCCs of 2 cm or less diameter were randomly compared between RFA and resection. It was reported that there would be no difference between the two, or that, for single HCC of 5 cm or less, there was no difference in terms of both disease-free survival and overall survival.[42,43] In Italy, a group of 109 patients who underwent RFA and a group of 91 patients who underwent resection were compared retrospectively; there was no difference in terms of the overall survival and disease-free survival, for HCC of 3 cm or less.[44] Likewise, a retrospective analysis conducted in Korea, compared a group of 55 patients who underwent RFA treatment for single HCC 4 cm or less and well-preserved liver function with a group of 93 patients who underwent resection; the authors concluded that there was no difference in terms of overall survival and recurrence-free survival at 1 year and 3 years after RFA.[45] When laparoscopic RFA was performed on patients with single HCC nodule with Child-Pugh A liver function, RFA and resection had similar survival rates.[46,47]

However, a case control study of resection versus RFA showed that recurrence, tumor diameter, and whether resection or RFA were performed, all affected overall survival. The authors con-

cluded that a resection provided some advantages.[48] Furthermore, with regard to cases of HCC which are not suitable candidates for liver transplantation, a Markov model was used to compare the life-adjusted survival between resection and RFA. The survival rate in the resection group was 5.33 years, while in the RFA group it was 3.91 years. It was concluded therefore, that patients treated by a resection would have a better survival rates.[49] In another study, 79 cases of resection and 79 cases of RFA treated at two different institutions were compared. The result showed that resection would be better than RFA for tumors of 3 cm and larger in diameter with Child A score, but that the overall survival would be the same for surgery and RFA in the case of Child B score.[50]

Comparison between RFA and other ablations

A comparison between microwave coagulation and RFA for HCC, 2 cm or less in tumor diameter demonstrated that RFA was superior because it created a larger necrotic area, resulting in a lower local recurrence rate; this conferred better cumulative survival, while bile duct injury and pleural effusion occurred less frequently.[51]

Another study compared percutaneous ethanol injection (PEI) and RFA. This randomized controlled trial (RCT) conducted in Taiwan demonstrated that RFA required fewer treatment sessions to achieve complete tumor necrosis, and provided better overall survival.[52] Another RCT between PEI and RFA was conducted in Japan. The 4-year survival rates were 74% for RFA versus 57% for PEI, resulting in RFA treatment being associated with a lower risk of death and recurrence. There was no difference in frequency of adverse events.[53] Although it was not RCT, another study compared PEI and RFA and found that local recurrence rates after RFA were lower.[54] An RCT conducted in Italy compared RFA with PEI and found that complete response of RFA after one year was associated with a better outcome, though no survival advantage was observed.[55]

There have been three meta-analyses, based on RCT comparing the effects and complications between RFA and PEI. Each found that RFA had better overall survival, while PEI had a higher local recurrence rate; thus RFA was superior in cancer-free survival rates.[56–58] No difference was observed in the complications between the two.

RCT was conducted to identify whether a combination of RFA and PEI would produce a better outcome than RFA alone. For tumors measuring between 3.1 cm and 5 cm in size, RFA + PEI improved patient survival, and overall recurrence was lower with combination treatment.[59]

Combined TACE and RFA treatment

The combination of transarterial embolization (TAE) and RFA or PEI was compared with TACE alone, and it was found that TACE + RFA had a better prognosis.[60] The results of a case-control comparison between RFA combined with TACE and RFA alone demonstrated that there was no difference in cases of single HCC ≤ 5 cm, but that the TACE + RFA combined treatment had a higher survival rate in cases of single HCC > 5 cm or multiple tumors.[61] The combination of TACE and RFA was technically successful in 88% of cases; such patients, complete the therapy after a single treatment session.[62] In addition, the combination of TACE and RFA produced high local control rates.[63] TACE and RFA has been performed for HCC immediately below the diaphragm, and found to be effective.[64] The combination of bland arterial embolization with RFA and a resection has also been compared; the overall survival was found to be similar in patients with single HCC measuring up to 7 cm in diameter.[65]

The extent of necrosis resulting from RFA increases when combined with hepatic arterial balloon occlusion.[66] Furthermore, combined treatment with balloon occlusion after transcatheter arterial infusion chemotherapy (TAI) is effective in expanding the necrotic area.[67] However, some researchers argue that this combination is not necessary because the effects of the combined therapy involving TACE and RFA, and that of RFA alone, for small HCC ≤ 3 cm, are the same.[68]

Complications

Data from 3891 cases were collected in a joint study conducted in Osaka, Japan. Complications were observed in 207 cases (7.9%), with 9 patients dying within 3 months. The causes of death in these cases were: liver failure in 3 cases, rapid progression in 3 cases, biliary injury in 1 case, gastrointestinal bleeding in 1 case, and myocardial infarction in 1 case.[69] Data for 255 cases in China have also been reported, with major complications observed in 31 cases (10%) as follows: 13 cases of liver failure, 10 cases of hydrothorax, 2 cases of tumor seeding, 1 case of upper gastrointestinal bleeding, and one each of intrahepatic abscess, bile duct injury, and cardiac arrest, 5 cases of hyperglycemia, and 11 cases of death due to liver failure.[70] A report from the United States noted that complications had been observed in 7 out of 91 cases as follows: 2 cases of hepatic abscess and one each of skin burn, hemorrhage, myocardial infarction, and liver failure.[71] According to the results of a multicenter survey conducted in Korea, liver abscess (0.66%), peritoneal hemorrhage (0.46%), biloma (0.20%), ground pad burn (0.20%) and pneumothorax (0.20%) were reported as complications.[72]

Liver abscess and bile duct injury

Liver abscess is the most common complication—de Raere *et al.* observed 7 cases out of 350 sessions and a high risk of this complication among patients with a revious bilioenteroc anasomosis.[73] Likewise, Choi *et al.*[74] and Elias *et al.*[75] also reported that liver abscess was seen more often in cases of biliary abnormality, as well as after TACE treatment. In one report, cholangitis and liver abscess occurred simultaneously.[76] Attention should therefore be paid to the fact that the risk for liver abscess complication is high in cases of complicated anastomosis of the bile duct to the intestinal tract.

Biliary stricture was observed in 7 cases after the RFA procedure, with liver abscess as a complication in 3 cases.[77] It was reported that intraductal chilled saline perfusion by endoscope had been effective in preventing bile duct injury.[78]

Bleeding

A total of 4133 RFA treatments were performed in 2154 cases, with hemorrhagic complications occurring in 63 treatments (1.5%)

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as follows: hemoperitoneum (0.7%), hemothorax (0.3%) and hemobilia (0.5%). In addition, there were two deaths due to hemoperitoneum.[79] Poggi *et al.* reported only one case of bleeding that required surgery.[80] Attention has also been focused on bleeding which occurred in one case of subcapsular liver tumor, but there were no complications such as seeding.[81,82]

Intestinal injury

Two cases were reported in which colonic perforation occurred as a complication on the 8th day after RFA. Attention should be paid to the fact that intestinal injury was indolently present.[83,84] Another report indicated the occurrence of duodenopleural fistula formation as a complication.[85]

Hepatic infarction

Hepatic infarction has been observed after RFA; the frequency is 1.8%.[86] The use of internally cooled electrodes is a risk. In addition, portal thrombosis has also been reported to occur.[73,87]

Seeding

A report given by Llovet *et al.* in 2001 on the high rate of seeding after RFA has received much attention. The risk factors included: subcapsular tumor localization, a high degree of poor differentiation, and a high baseline AFP.[88] Since then the risk of seeding after RFA, has been attiributed to subcapsular location, poorly differentiated tumors and high AFP levels.[89,90] However, a discrepancy exists between institutions, with some arguing that, in reality, seeding is exceptionally rare.[91,92] In order to prevent seeding, tract ablation should therefore be properly performed.

Other complications

In another report, pneumothorax occurred after RFA, and so careful attention is required for tumors adjacent to the diaphragm.[93] It has been reported that myoglobinuria occurs as a complication after RFA and that the serum creatinin level rises, making it necessary for attention to be paid thereto.[94] Another case was reported in which hemolysis occurred, thus inducing hemogeobinuria as a complication.[95] There have also been reports that rapid tumor progression occurred after RFA;[96,97] however, the actual frequency was low and it is therefore necessary to investigate whether or not it was indeed a complication associated with RFA.

We have done 1440 sessions of RFA to patients with early stage HCC from July, 1999 to December 2009. The complications have been analyzed as shown in Table 2. The complication rates were 1.8% and 1.9% when the patients were treated by laparoscopic or percutaneous RFA, respectively.

Conclusion

RFA is promising for improving patient survival with early stage of HCC when performed skillfully to avoid serious complication. To prevent the recurrence of HCC is the most important issue for achieving better survival.

Table 2 Complications by laparoscopic or percutaneous RFA in Musashino Red-Cross hospital (from July, 1999 to December, 2009)

	Laparoscopic RFA (n = 107)	Percutaneous RFA (n = 1333)
Bile duct damage	0 (%)	4 (0.4%)
Liver abscess	1 (0.9%)	4 (0.3%)
Inter-costal arterial injury	0 (0%)	3 (0.3%)
Hemothorax	0 (0%)	4 (0.3%)
Hepatic infarction	0 (0%)	3 (0.3%)
Hepatic dysfunction	0 (0%)	2 (0.3%)
Skin burn	0 (0%)	2 (0.2%)
Subcutaneous hematoma	1 (0.9%)	0 (0%)
Peumothorax	0 (0%)	2 (0.1%)
Gastrointestinal perforation	0 (0)	1 (0.1%)
Total	2 (1.8%)	26 (1.9%)

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