of liver disease and the development of HCC.[19,47,51] It is not effective in all patients, however, and discontinuation of treatment should be considered if improvement is not seen in ALT levels (≤40 IU/L) or AFP levels (≤10 ng/mL) within 6 months.

IFN therapy for decompensated cirrhosis

Patients with decompensated cirrhosis are at high risk of death due to liver failure, and liver transplant is the most effective treatment in suitable cases. However, post-transplant recurrence of hepatitis C causes allograft failure in approximately 30% of recipients within 5 years, so in overseas countries, pretransplant IFN therapy is administered with the aim of HCV eradication or suppression.[161,162] Several studies have demonstrated the efficacy of Peg-IFN (± ribavirin) therapy in patients with HCV genotype 2.[163–165] Patients with decompensated cirrhosis are at high risk of thrombocytopenia, anemia, infections and liver decompensation, however, and treatment discontinuation due to severe cytopenias is common. Serious bacterial infections associated with IFN therapy have been reported to be more common in patients with patients with Child–Pugh C than in Child–Pugh A/B disease.[166]

Treatment of patients with thrombocytopenia

In patients with marked thrombocytopenia associated with hypersplenism, it is difficult to introduce Peg-IFN or ribavirin combination therapy. Measures such as splenectomy or partial splenic embolization (PSE) are employed to increase the platelet count before commencing IFN therapy.[167–169] In Japan, mainly in patients with Child–Pugh A disease, Peg-IFN (± ribavirin) therapy is commenced following splenectomy or PSE. An increase in the platelet count is seen in almost all patients following either procedure, and high SVR rates are seen in patients with HCV genotype 2. However, postoperative complications including overwhelming post-splenectomy infection, portal vein thrombosis and hepatic dysfunction have been reported following both splenectomy and PSE.[168–170] The thrombopoietin receptor agonist, eltrombopag, has been developed overseas as an oral agent that increases platelet counts,[171] but it is not yet available for clinical use in Japan.

Recommendations:

1 *In patients with compensated cirrhosis (Child–Pugh class A) associated with HCV, aggressive IFN therapy should be commenced with the aims of preventing hepatocellular carcinogenesis and liver failure. This patient group requires careful observation during treatment due to the high incidence of adverse reactions such as cytopenias.*
2 *Patients with compensated cirrhosis associated with HCV should be given Peg-IFN + ribavirin combination therapy, irrespective of genotype or viral load. The standard dose is 1.0 μg/kg/week for Peg-IFN-α-2b and 90 μg/week for Peg-IFN-α-2a. The usual treatment period is 48 weeks, although consideration should be given to response-guided therapy and the discontinuation criteria for chronic hepatitis C.*
3 *Patients with compensated cirrhosis associated with HCV genotype 1 and a lower viral load, or genotype 2, not suited to combination therapy with ribavirin, should be administered HLBI or IFN-β monotherapy. HLBI therapy commences with HLBI 6 MU consecutive daily for 2 weeks, then 3–6 MU three times weekly. IFN-β therapy is usually commenced with 6 MU daily for a week, followed by 3 MU daily for 5 weeks, then 3 MU three times a week from treatment week 7. For both HLBI and IFN-β, if HCV RNA becomes undetectable before treatment week 12, the treatment period should be extended to 48–72 weeks.*
4 *If HCV RNA does not become undetectable before treatment week 12 with Peg-IFN + ribavirin combination therapy or IFN monotherapy in patients with compensated cirrhosis associated with HCV, long-term HLBI therapy at a dose of 3 MU three times weekly should be commenced with the aim of inhibiting hepatocellular carcinogenesis. Treatment should be discontinued if improvement is not seen in ALT levels (≤40 IU/L) or AFP levels (≤10 ng/mL) within 6 months.*
5 *The efficacy of IFN therapy is low in patients with decompensated cirrhosis associated with HCV (Child–Pugh class B and C). In particular, patients with Child–Pugh class C do not tolerate IFN therapy well, and serious adverse reactions such as cytopenias and infections have been reported, so IFN therapy is not recommended in this patient group.*
6 *If IFN therapy is being considered in a patient with compensated HCV cirrhosis associated with a platelet count <50 000/μL, one option is to perform splenectomy or PSE before commencing IFN therapy.*

3.9 Management of patients with normal ALT levels

In a study of Peg-IFN + ribavirin combination therapy and hepatocellular carcinogenesis in 809 patients with chronic hepatitis C and normal pretreatment ALT levels (male/female, 269/540; average age, 57 ± 11

years; genotype 1/2, 550/247; mean observation period, 36.2 ± 16.5 months), in the group with platelet counts ≥150 000/μL ($n = 586$) no significant difference was seen in the incidence of HCC according to therapeutic effect, with 1.5% of non-responders developing HCC within 3 years. In the group with platelet counts <150 000/μL ($n = 323$), however, the cumulative incidence of HCC was high at 10.1% in non-responders, with no cases of HCC among the responders or relapsers. These results demonstrated that Peg-IFN + ribavirin therapy significantly inhibits hepatocellular carcinogenesis ($P < 0.001$).[172] The efficacy of Peg-IFN + ribavirin combination therapy is similar in patients with normal and elevated ALT levels.[173,174]

Accordingly, antiviral therapy should be considered even in patients with ALT levels ≤30 IU/mL if their platelet count is <150 000/μL. On the other hand, antiviral therapy does not need to be commenced immediately in patients with an ALT level ≤30 IU/mL and a platelet count ≥150 000/μL, and follow-up while waiting for the next generation DAAs is a reasonable option. ALT levels may rise during the follow-up period, however, and treatment is indicated if the patient has a strong desire to commence antiviral therapy. At present, the available evidence regarding patients with normal ALT levels is mainly related to Peg-IFN + ribavirin combination therapy, although high therapeutic efficacy can also be anticipated with telaprevir + Peg-IFN + ribavirin combination therapy in this patient group.

Recommendation:

Antiviral therapy for patients with normal ALT levels (ALT, ≤30 IU/mL) can be administered in the same way as for patients with elevated ALT levels. Aggressive therapy is particularly desirable in patients with platelet counts <150 000/μL.

4. PROTECTIVE THERAPY

THE AIM OF protective therapy is not HCV clearance, but rather to reduce inflammation and inhibit the progression of fibrotic change in the hepatic tissue. The indications for protective therapy in patients with chronic hepatitis C are: patients with abnormal ALT and AST levels unable to undergo IFN or other antiviral therapy; patients who failed to achieve viral clearance with antiviral therapy; and patients who do not wish to undergo antiviral therapy. UDCA and SNMC are the protective therapies that have been scientifically shown to be useful.

UDCA

Ursodeoxycholic acid is a bile acid formulation, approved for use in doses of 600–900 mg daily by national medical insurance. The main mechanism of action of UDCA in hepatitis is a hepatocytoprotective effect. Other postulated mechanisms of action include protection of the hepatocyte cell membrane by substitution of UDCA for other cytotoxic bile acids, antioxidative stress affects, immunoregulatory effects and anti-apoptotic effects.[175]

Improvement of liver function is seen from UDCA doses of 150 mg/day.[176,177] In a Japanese nationwide multicenter double-blind trial, significantly greater improvement was seen in AST, ALT and γ-glutamyl transpeptidase levels in the groups administered 600 and 900 mg/day than in those given 150 mg/day.[176] Accordingly, the UDCA dose for the treatment of chronic hepatitis C is generally 600 or 900 mg/day. Adverse reactions are mainly gastrointestinal symptoms such as epigastric discomfort, diarrhea and constipation, but these are generally mild. A retrospective study of inhibition of hepatocellular carcinogenesis by UDCA reported that it significantly reduced the incidence of HCC.[178]

SNMC

The main constituent of SNMC is glycyrrhizin, a compound extracted from the liquorice root. The mechanisms of action of SNMC in the treatment of hepatic dysfunction are derived from anti-inflammatory effects related to the steroid-like properties of glycyrrhizin, and hepatocyte cell membrane protective effects. These actions are considered to lead to improved ALT levels. In a Japanese double-blind trial of SNMC 40 mL daily for 1 month, significant improvement in AST and ALT levels was seen in the SNMC group in comparison with the placebo group.[179,180] Doses are 40–100 mL daily or alternate daily, although Japanese dosage comparison trials found significantly greater improvement in ALT levels with 100 mL than with 40 mL.[181,182] In another study, long-term administration of SNMC significantly inhibited progression to liver cirrhosis in comparison with the control group.[183] Adverse reactions to SNMC include hypokalemia and hypertension.

Studies of inhibition of hepatocellular carcinogenesis by SNMC found that the incidence of HCC was significantly lower in the treatment group than in the control group.[183,184] SNMC therapy has also been found to significantly reduce the incidence of HCC in non-responders to IFN therapy.[185,186]

UDCA + SNMC combination therapy

An RCT comparing SNMC monotherapy and UDCA + SNMC combination therapy found significantly greater improvement in ALT levels in the combination therapy group.[187] This combination is useful in reducing inflammation.

Recommendation:

Oral UDCA and i.v. SNMC, or both in combination, are recommended as protective therapy in patients with chronic hepatitis C.

5. THERAPEUTIC PHLEBOTOMY

IRON METABOLISM PLAYS an important role in patients with chronic hepatitis C. Iron is an essential metal, and a constituent of important proteins, including Hb. When iron is present in excess, however, cytotoxic hydroxyl radicals are produced, causing oxidative stress. Therapeutic phlebotomy was devised as a supportive therapy for patients with chronic hepatitis C because oxidative stress associated with iron overload is a factor in progression of liver disease. Restriction of dietary iron is also important in the management of patients undergoing iron reduction therapy. As for protective therapy, therapeutic phlebotomy is indicated in patients with chronic hepatitis C with abnormal ALT and AST levels unable to undergo IFN or other antiviral therapy, patients who failed to achieve viral clearance with antiviral therapy and patients who do not wish to undergo antiviral therapy.

In 1994, a Japanese study reported that therapeutic phlebotomy lowered ALT levels in patients with chronic hepatitis C.[188] A Japanese multicenter RCT also confirmed improvement in ALT levels with therapeutic phlebotomy.[189] Other studies have reported a 50% decrease in ALT levels in 80% of patients, and normalization of ALT levels in 40–70% of patients.[190,191] Histological studies have reported inhibition of progression,[192] and even improvement,[193] of histological changes. Long-term therapeutic phlebotomy has been reported to significantly inhibit hepatocellular carcinogenesis.[190]

In general, therapeutic phlebotomy involves removal of 200–400 mL blood at 1–2-week intervals with the aim of reducing the serum ferritin level to ≤20 ng/mL. If the Hb level drops below 9–10 g/dL, phlebotomies are discontinued to allow recovery of hematopoietic function. After the target has been reached, therapeutic phlebotomies are performed as appropriate with reference to ferritin and Hb levels. Adverse reactions are rare, involving bradycardia and hypotension associated with the vagal reflex.

An additive effect is seen when therapeutic phlebotomy is performed in conjunction with UDCA or SNMC therapy. Greater reduction in ALT levels was seen with UDCA in combination with therapeutic phlebotomy than with UDCA monotherapy.[194] In patients on SNMC therapy, further reduction in ALT levels was seen with the addition of small volume phlebotomies.[195] The combination of therapeutic phlebotomy with another therapy with a different mode of action provides additional improvement in ALT levels.

Recommendations:

Therapeutic phlebotomy is a useful therapeutic modality in patients with chronic hepatitis C. Its use in combination with a protective therapy, oral UDCA or i.v. SNMC should also be considered.

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Original article

Age and total ribavirin dose are independent predictors of relapse after interferon therapy in chronic hepatitis C revealed by data mining analysis

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Background: This study aimed to define factors associated with relapse among responders to pegylated interferon (PEG-IFN) plus ribavirin (RBV) therapy in chronic hepatitis C.

Methods: A cohort of genotype 1b chronic hepatitis C patients treated with PEG-IFN plus RBV and who had an undetectable HCV RNA by week 12 (n=951) were randomly assigned to model derivation (n=636) or internal validation (n=315) groups. An independent cohort (n=598) were used for an external validation. A decision tree model for relapse was explored using data mining analysis.

Results: The data mining analysis defined five subgroups of patients with variable rates of relapse ranging from 13% to 52%. The reproducibility of the model was confirmed by internal and external validations (r^2=0.79 and 0.83, respectively). Patients with undetectable HCV RNA at week 4 had the lowest risk of relapse (13%), followed by patients <60 years with undetectable HCV RNA at week 5–12 who received ≥3.0 g/kg of body weight of RBV (16%). Older patients with a total RBV dose <3.0 g/kg had the highest risk of relapse (52%). Higher RBV dose beyond 3.0 g/kg was associated with further decrease of relapse rate among patients <60 years (up to 11%) but not among older patients whose relapse rate remained stable around 30%.

Conclusions: Data mining analysis revealed that time to HCV RNA negativity, age and total RBV dose was associated with relapse. To prevent relapse, ≥3.0 g/kg of RBV should be administered. Higher dose of RBV may be beneficial in patients <60 years.

Introduction

The currently recommended therapy for chronic hepatitis C is a combination of pegylated interferon (PEG-IFN) plus ribavirin (RBV) [1]. This therapy is effective in 50% of patients with HCV genotype 1b [2,3]. The most reliable predictor of sustained virological response (SVR) is the response during early weeks of therapy. A satisfactory response to therapy in the early weeks is associated with a high rate of SVR [4–8]. A basic concept of response-guided therapy is to modify the duration of therapy according to the time to HCV RNA negativity. Extended therapy may be given to patients with delayed virological response [9–13]. Modification of duration of therapy or drug dose may also be necessary in patients with early virological

response (EVR), because approximately 20% of these patients experience relapse after the completion of 48 weeks of therapy. Recent reports have revealed that single nucleotide polymorphisms located near the *IL28B* gene are strongly associated with SVR or a null response to PEG-IFN plus RBV therapy [14–16]. However, single nucleotide polymorphisms located near the *IL28B* gene are not associated with relapse after EVR [17]. Identification of risk factors for relapse among patients with virological response may lead to more individualized therapy and improved SVR rate.

Decision tree analysis, a core component of data mining analysis, is a method that explores data to develop predictive models [18]. This method has been originally used in business and recently in medical fields [19–25]. Decision tree analysis was successfully used to build a predictive model of EVR [26] and SVR to PEG-IFN plus RBV combination therapy in chronic hepatitis C [17,27,28]. The results of the analysis are presented as a tree structure, which is easy to understand and use in clinical practice. Patients can be allocated into subgroups by simply following the flowchart form of the decision tree [29].

In the present study, we used decision tree analysis to identify predictors of relapse among patients who achieved EVR to PEG-IFN plus RBV therapy, and to define a more individualized therapeutic strategy beyond response-guided therapy.

Table 1. Background of study population

Characteristic	Value
Age, years	54.9 (10.8)
Gender	-
Male, *n* (%)	557 (59)
Female, *n* (%)	394 (41)
Body mass index, kg/m^2	23.2 (3.3)
Albumin, g/dl	4.1 (1.8)
Creatinine, mg/dl	0.7 (0.2)
AST, IU/l	60.6 (46.2)
ALT, IU/l	80.7 (77.2)
GGT, IU/l	52.0 (60.0)
White blood cell count, cells/μl	4,993 (1,363)
Haemoglobin, g/dl	15.9 (52.6)
Platelets, 10^3/l	174.4 (6.1)
HCV RNA, KIU/ml	1,655 (1,455)
Fibrosis stage	-
F1-2, *n* (%)	626 (66)
F3-4, *n* (%)	98 (10)
NA, *n* (%)	227 (24)
Time to HCV RNA negativity 4/8/12 weeks	-
4 Weeks, *n* (%)	233 (24)
8 Weeks, *n* (%)	386 (41)
12 Weeks, *n* (%)	332 (35)
Treatment duration, weeks	42 (13)
Total RBV dose, g/kg body weight	3.1 (1.3)
Total PEG-IFN dose, μg/kg body weight	62.5 (38.6)
Outcome	-
Relapse, *n* (%)	238 (25)
SVR, *n* (%)	713 (75)

Total *n*=951. Data are expressed as mean (SD) unless otherwise indicated. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; NA, not available; PEG-IFN, pegylated interferon; RBV, ribavirin; SVR: sustained virological response.

Methods

Patients

This is a multicentre retrospective cohort study involving Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University, Yamanashi University, Osaka City University, and their related hospitals. The inclusion criteria were chronic hepatitis C patients treated with PEG-IFN-α2b plus RBV, genotype 1b, pretreatment HCV RNA titre >100 KIU/ml as confirmed by quantitative PCR; Cobas Amplicor HCV Monitor version 2.0; Roche Diagnostic Systems, Pleasanton, CA, USA), an undetectable HCV RNA level within week 12 after the start of therapy, no coinfection with HBV or HIV, and no other causes of liver disease. Patients were treated with PEG-IFN-α2b (1.5 μg/kg) subcutaneously every week plus a daily weight-adjusted RBV dose (600 mg for patients weighing <60 kg, 800 mg for patients weighing 60–80 kg and 1,000 mg for patients weighing >80 kg). Dose reduction or discontinuation of PEG-IFN and RBV was considered based on the recommendations of the package inserts and the discretion of physicians at each university and hospital. The standard duration of therapy was set at 48 weeks, but extension of duration was allowed and implemented at the discretion of each physician. The duration of therapy was extended beyond 48 weeks in 118 patients (mean duration was 56.3 weeks, ranging from 49 to 72 weeks). Although the exact reason for the prolonged treatment in each case was not available, one reason may be that each physician tried to achieve high adherence of RBV by extending the duration of therapy. Another reason may be the late time point of HCV RNA negativity even within early virological response. Among 118 patients, time to HCV RNA negativity was between 9 to 12 weeks in 56% of patients.

A total of 951 patients fulfilled the study criteria. The baseline characteristics and representative laboratory test results are listed in Table 1. For analysis, patients were randomly assigned to either the model derivation (636 patients) or internal validation (315 patients) groups. There were no significant differences in the clinical backgrounds between these two groups. For external validation of the model, we collaborated with another multicentre study group consisting of 29 medical centres and hospitals belonging to the National

Hospital Organization (Japan). A dataset collected from 598 patients who were treated with PEG-IFN-α2b plus RBV and had undetectable HCV RNA within week 12 were used for external validation. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

Laboratory tests

Haematological tests, blood chemistry and HCV RNA titre were analysed before therapy and at least once every month during therapy. Rapid virological response (RVR) was defined as an undetectable HCV RNA level at week 4, and complete early virological response (cEVR) was defined as an undetectable HCV RNA level at week 5 through week 12 after the start of therapy. SVR was defined as an undetectable HCV RNA level 24 weeks after the completion of therapy. Detection of HCV RNA level was based on qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor; Roche Diagnostic Systems). A database of pretreatment variables included haematological tests (haemoglobin level, white blood cell count and platelet count), blood chemistry tests (serum levels of creatinine, albumin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, total cholesterol, triglycerides and HCV RNA titre), stage of histological fibrosis and patient characteristics (age, sex and body mass index). Post-treatment variables included time to HCV RNA negativity, calculated total RBV dose (g/kg of body weight), and calculated total PEG-IFN dose (μg/kg of body weight).

Statistical analysis

The Student's *t*-test was used for the univariable comparison of quantitative variables and Fisher's exact test was used for the comparison of qualitative variables. Logistic regression models with backward selection procedures were used for multivariable analysis of factors associated with relapse. IBM SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. For the decision tree analysis [30], the data mining software IBM SPSS Modeler 14 (SPSS Inc.) was used, as reported previously [17,26–28]. The decision tree analysis, the core component of the data mining, belongs to a family of non-parametric regression methods based on binary recursive partitioning of data. In this analysis, the software automatically explored the database to determine optimal split variables to build a decision tree structure. A statistical search algorithm evaluate the model derivation group to determine the optimum variables and cutoff values and to yield the most significant division of patients into two subgroups that were as homogeneous as possible for the probability of relapse. Once patients were divided into 2 subgroups, the analysis was automatically repeated on each subgroup in the same way until either no additional significant variable was detected or the number of patients was <20. Finally all patients were classified into particular subgroups that are homogeneous with respect to the probabilities of relapse.

Results

The decision tree model for the prediction of relapse

The overall rate of relapse was 26% in the model derivation group. The decision tree analysis selected three variables that are associated with relapse: time to HCV RNA negativity, age and total RBV dose (Figure 1). Time to HCV RNA negativity was selected as the best predictor of relapse. The rate of relapse was 13% for patients with RVR compared to 30% for patients with cEVR. Among patients with cEVR, age was selected as the variable of second split. Patients <60 years had a lower probability of relapse (22%) compared with those ≥60 years (41%). The total RBV dose was selected as the third variable of split with an optimal cutoff of 3.0 g/kg of body weight. The rate of relapse was lower in patients who received ≥3.0 g/kg of body weight of RBV compared to patients who received <3.0 g/kg of body weight (among patients <60 years rates were 16% versus 32% and among patients ≥60 years rates were 26% versus 52%, respectively).

According to this decision tree, the patients were divided into five groups with different rates of relapse ranging from 13% to 52%. Patients with RVR had the lowest risk of relapse. Among patients with cEVR, patients <60 years who received ≥3.0 g/kg of body weight of RBV also had a low risk of relapse (16%). By contrast, patients who received <3.0 g/kg of body weight of RBV had higher than the average risk of relapse, especially in patients ≥60 years (52%).

Validation of the decision tree model

The decision tree model was validated using an internal validation group that was not included in the model derivation. The rates of relapse for each subgroup of patients were correlated closely between the model derivation and the internal validation group (r^2=0.79; Figure 2A). When validated using an external validation group, the rates of relapse for each subgroup of patients were again correlated closely between the model derivation and the external validation group. (r^2=0.83; Figure 2B).

Multivariable logistic regression analysis for factors associated with relapse

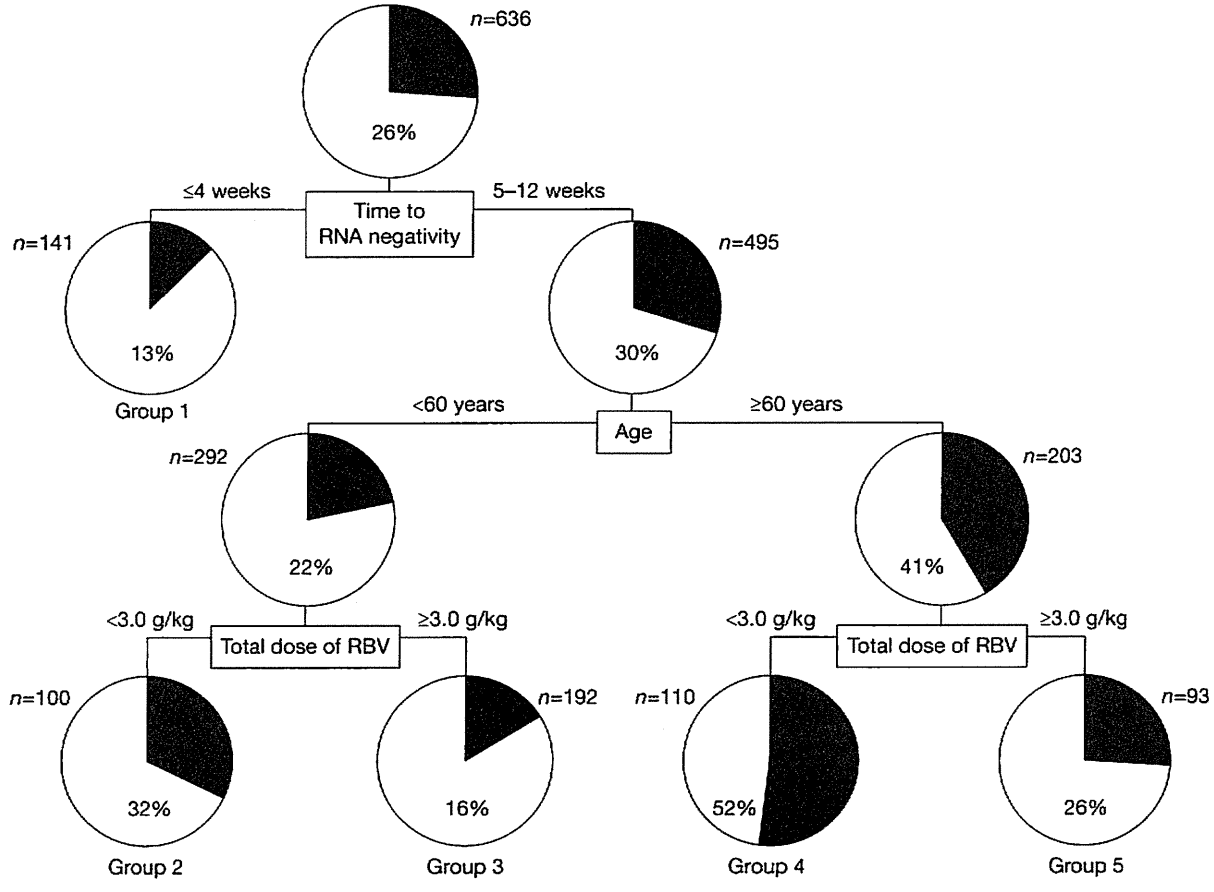
Univariable and multivariable analysis was performed using the combined population of model derivation and internal validation group. Univariable analysis found

that age, sex, serum levels of creatinine, haemoglobin, platelet count, HCV RNA titre, time to HCV RNA negativity, total PEG-IFN dose and total RBV dose were associated with relapse. Duration of therapy was not associated with reduction in relapse rate. Multivariable analysis including these factors showed that age, total RBV dose, serum level of creatinine, and time to HCV RNA negativity were independent predictors of relapse (Table 2). Creatinine was not selected as a splitting variable in data mining analysis probably due to the limitation to stop the analysis when the number of patients was <20. Using the combined population of model derivation and internal validation group, patients in each subgroup of decision tree model were further stratified by creatinine levels and the effect of creatinine level on relapse was analysed. Among patients with RVR, the rate of relapse did not differ between patients with creatinine levels of <0.7 g/dl and ≥0.7 g/dl and were 12% and 12%, respectively. Among patients with cEVR, the rate of relapse was higher in patients with creatinine levels of <0.7 g/dl compared to those with creatinine levels of ≥0.7 g/dl and were 39% versus 23%, respectively, for patients <60 years who received <3.0 g/kg of body weight of RBV, 19% versus 14% for patients <60 years who received ≥3.0 g/kg of body weight of RBV, 58% versus 41% for patients ≥60 years who received <3.0 g/kg of body weight of RBV, and 42% versus 26% for patients ≥60 years who received ≥3.0 g/kg of body weight of RBV.

Effect of age and total RBV dose on relapse among patients with cEVR

The effect of total RBV dose on relapse was analysed among patients with cEVR in a combined group of

Figure 1. The decision-tree model of relapse among patients with rapid virological response or complete early virological response

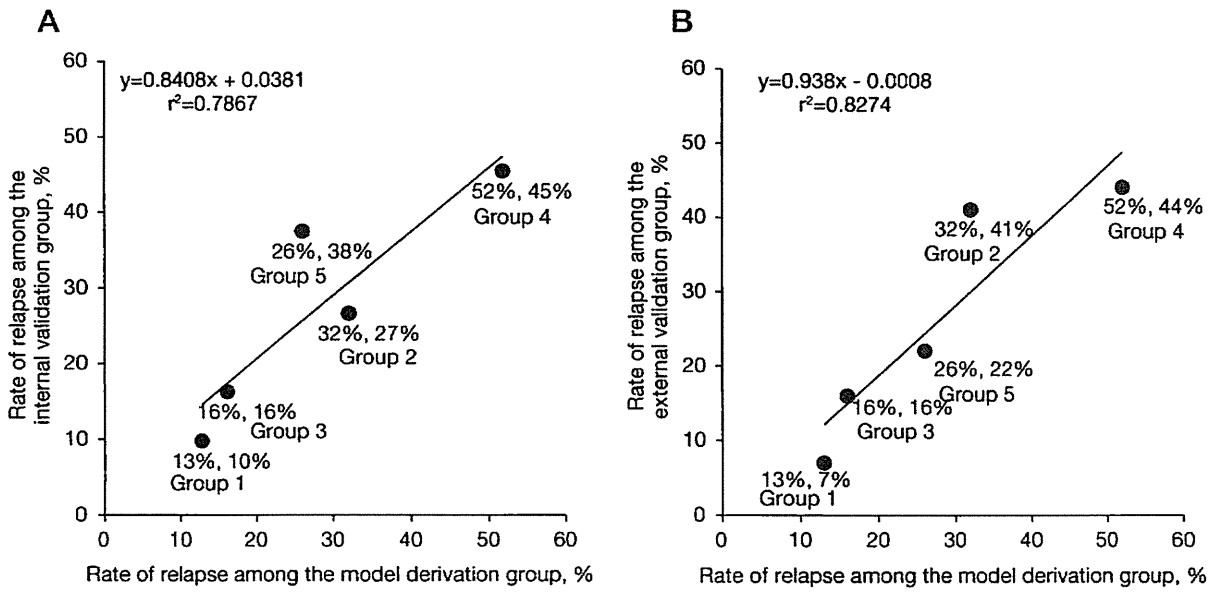


Boxes indicate the factors used for splitting and the cutoff values for the split. Pie charts indicate the rate of relapse for each group of patients after splitting. Terminal groups of patients discriminated by the analysis are numbered from 1 to 5. The rate of relapse was higher than average (>26%) in subgroups 2 and 4, where total ribavirin (RBV) dose was <3 g/kg of body weight.

model derivation and internal validation (n=718). The relapse rate decreased with an increase in RBV dose (Figure 3A). When patients were stratified into two groups according to age, the relapse rate decreased with an increase in RBV dose in patients <60 years. The relapse rate was lowest (11%) in patients <60 years who received ≥4.0 g/kg of body weight of RBV. By contrast, among patients ≥60 years, the relapse rate decreased with an increase in RBV dose up to 3.0 g/kg of body weight, but remained relatively stable despite a further increase in the RBV dose beyond 3.0 g/kg of body weight. The rate of relapse was 31% to 33% in patients who received ≥3.0 g/kg of body weight. Patients ≥60 years had higher relapse rate compared with patients <60 years after stratification by RBV dose (P=0.044 for RBV <2.5 g/kg, P=0.009 for RBV 2.5–2.9 g/kg, P=0.150 for RBV 3.0–3.4 g/kg, P=0.036 for RBV 3.5–3.9 g/kg and P=0.006 for RBV ≥4.0 g/kg).

To exclude the effect of the duration of therapy, patients who received 42–54 weeks of therapy were selected (n=544). Again, the relapse rate decreased with an increase in RBV dose in patients <60 years but remained stable despite a further increase in the RBV dose beyond 3.0 g/kg of body weight in patients ≥60 years (Figure 3B); in addition, patients ≥60 years had a higher relapse rate compared with younger patients after stratification by

Figure 2. Internal and external validation of the decision-tree model: subgroup-stratified comparison of the rate of relapse between the model derivation and validation groups



Each patient in the internal and external validation population was allocated to groups 1 to 5 following the flowchart of the decision tree. The rates of relapse were then calculated for each group and a graph was plotted. The rate of relapse in the (A) internal and (B) external validation groups are shown. The rates of relapse are shown as percentages below data points: the value on the left is from the model derivation group and on the right is from the validation group. The rates of relapse in each group of patients correlated closely between the model derivation group and the validation group (correlation coefficient: r^2=0.79 and 0.83, respectively).

Table 2. Multivariable analysis of factors associated with relapse among patients with RVR/cEVR

Factor	OR	95% CI	P-value
No-RVR	4.07	2.57–6.43	<0.0001
Total RBV dose <3.0 g/kg body weight	2.19	1.58 –3.03	<0.0001
Creatinine <0.7 g/dl	1.67	1.22–2.29	0.001
Age ≥60 years	2.37	1.73–3.24	<0.0001

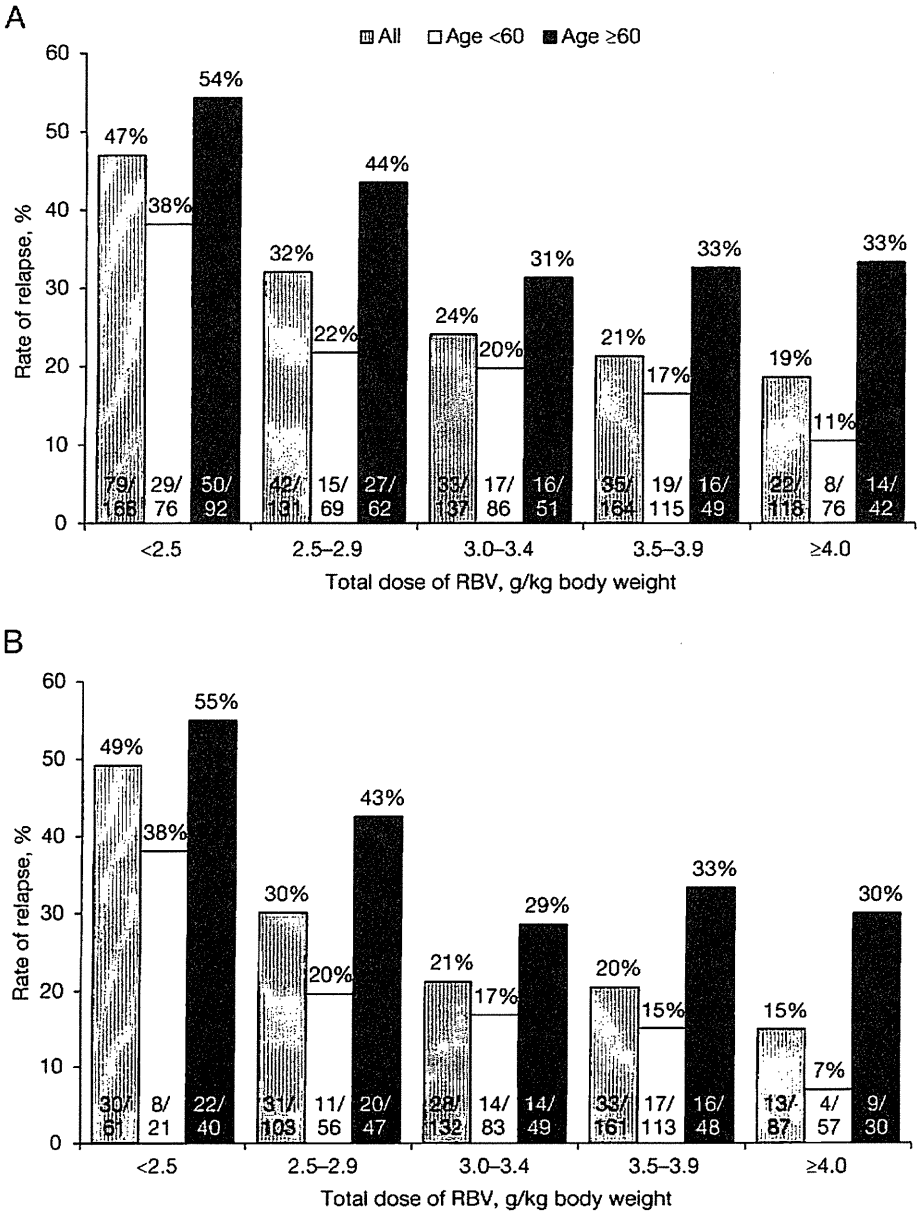
cEVR, complete early virological response (HCV-RNA-positive at week 4, but negative at week 12); RBV, ribavirin; RVR, rapid virological response (HCV-RNA-negative at week 4).

RBV dose (P=0.283 for RBV <2.5 g/kg, P=0.017 for RBV 2.5–2.9 g/kg, P=0.127 for RBV 3.0–3.4 g/kg, P=0.011 for RBV 3.5–3.9 g/kg and P=0.009 for RBV ≥4.0 g/kg).

Total dose of RBV was associated with relapse independently of PEG-IFN dose. The cutoff value of 58 zμg/kg of PEG-IFN was selected, which corresponds to the 80% of 1.5 μg/kg dose for 48 weeks. In patients who received <58 μg/kg of body weight of PEG-IFN, the rate of relapse for patients who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 24% and 42%, respectively. In patients who received ≥58 μg/kg of body weight of PEG-IFN, the rate of relapse for patients who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 21% and 38%, respectively.

The data mining analysis procedure did not select further split variables among RVR patients. However,

Figure 3. Correlation between the rate of relapse and total RBV dose among patients with cEVR after stratification by age



Association between the total ribavirin (RBV) dose and the rate of relapse among patients with complete early virological response (cEVR) is shown. **(A)** Higher dose of RBV was associated with reduced rate of relapse. **(B)** These associations were also confirmed in selected patients who received 42–54 weeks of therapy.

when analysed separately, the rate of relapse was also associated with age and total RBV dose among patients with RVR. The rate of relapse for patients who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 5% and 14%, respectively. The rate of relapse for patients <60 and ≥60 years was 9% and 18%, respectively. Collectively, the rate of relapse for patients <60 years who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 2% and 11%, respectively, whereas the rate of relapse for patients ≥60 years who received ≥3.0 g/kg or <3.0 g/kg of body weight of RBV was 12% and 20%, respectively.

Discussion

The result of the present study shows that older age and insufficient dose of RBV are significant and independent risk factors for relapse among patients with cEVR to PEG-IFN plus RBV. Older patients (≥60 years) who received a total RBV dose <3.0 g/kg of body weight had the highest risk of relapse (52%), whereas younger patients who received a total RBV dose ≥3.0 g/kg of body weight had the lowest risk of relapse (16%). The rate of relapse decreased depending on the total RBV dose in younger patients, but remained stable in older patients despite a further increase in the RBV dose beyond 3.0 g/kg of body weight. These findings imply that the target dose of total RBV can be set at 3.0 g/kg of body weight in patients who achieved cEVR, and further increase in RBV dose up to 4.0 g/kg of body weight or greater may be recommended in patients <60 years.

The associations between the drug adherence and virological response had been reported with inconsistent results. In an earlier study, patients who received >80% of the planned dose of PEG-IFN plus RBV for >80% of the planned duration of therapy had a higher rate of SVR compared to those who received a lesser dose (51% versus 34%) [31]. Consistent results were obtained in a study reporting that patients who received >80% of the planned dose of PEG-IFN and RBV within the first 12 weeks of therapy had a higher rate of EVR compared with those who received a lesser dose of both drugs (80% versus 33%) [4]. By contrast, a large-scale multicentre study showed that reducing the PEG-IFN dose during the first 20 weeks reduced SVR; however, reducing RBV did not affect SVR as long as RBV was not prematurely discontinued [32]. The reason for these inconsistencies is unclear. One reason may be the differences in the backgrounds of patients enrolled in the study, and hence the last study was limited to patients with advanced fibrosis and prior non-responders to PEG-IFN therapy. Because the probability of SVR is affected by virological response and relapse after response, the effect of drug dosing should be analysed separately with respect to these two factors.

In the present study, we focused on factors predictive of relapse after early virological response. According to the decision tree model, relapse was less likely in patients with RVR compared with cEVR. Among patients with cEVR, older patients (≥60 years) had a higher risk of relapse compared to younger patients (41% versus 22%). In addition, our results emphasized the effect of RBV dose for the prevention of relapse. In our study, a total RBV dose of ≥3.0 g/kg of body weight was repeatedly associated with a suppressed rate of relapse in the model derivation and validation groups. The rate of relapse in patients <60 years who received an RBV dose of <3.0 versus ≥3.0 g/kg of body weight in the model derivation, internal validation and external validation groups were 32% versus 16%, 27% versus 16%, and 41% versus 16%, respectively. The rate of relapse in patients ≥60 years who received an RBV dose of <3.0 versus ≥3.0 g/kg of body weight in the model derivation, internal validation and external validation groups were 52% versus 26%, 45% versus 38%, and 44% versus 22%, respectively. It has been reported that the rate of relapse is suppressed in 48 weeks of IFN plus RBV combination therapy compared to IFN monotherapy, indicating that RBV contributes to the increase in SVR by reducing relapse [2,3]. Another study, focused on the associations between the drug dose reduction and relapse in patients with virological response, found that maintaining RBV dose ≥12 mg/kg/day during 48 weeks of treatment, which can be translated into a total dose of 4.0 g/kg of body weight, suppressed relapse [33]. Results of the present study are in accordance with this report.

The importance of drug dosing on reduction in relapse is also supported by the findings that extending therapy from 48 to 72 weeks in patients with delayed virological response improved SVR rates by reducing relapse [9–13]. Apart from these clinical studies, in the real world of clinical practice, duration of therapy is extended – even in patients with cEVR – at the physician's discretion. The relationship between duration of therapy or RBV dose, and relapse among patients with cEVR and treated with various lengths of therapy has not been examined. In the combined group of our study, extending the duration of therapy was not associated with a reduction in relapse rate. Rather, the rate of relapse decreased depending on the total RBV dose. These findings suggest that acquiring a sufficient total RBV dose, either within 48 weeks or by extending the duration of therapy, is essential to prevent relapse among patients with cEVR. The limitation of the present study was that the mean duration of therapy was only 56.3 weeks in patients whose duration of therapy was extended beyond 48 weeks. It is probable that extended duration of therapy was not long enough for the prevention of relapse. Further studies with

longer durations of therapy are necessary to confirm the effect of extended duration of therapy on reduction of relapse among patients with cEVR.

Previous reports did not consider the effects of age in setting the optimal dose of RBV. In the present study, the relapse rate decreased with an increase in RBV dose from <2.5 to 3.0–3.5 g/kg of body weight, but remained relatively stable despite a further increase in the RBV dose in older patients. Thus, a total RBV dose ≥3.0 g/kg of body weight should be the target dose for patients ≥60 years with cEVR. By contrast, ≥3.0 g/kg of body weight of RBV was associated with lower risk of relapse in patients <60 with cEVR (16% versus 32%), and a further increase in RBV dose led to a more profound reduction in relapse rates, as low as 11% in patients who received ≥4.0 g/kg of body weight. Thus, a total dose of ≥4.0 g/kg of body weight or even greater should be the target dose in patients <60 years.

In the near future, more potent therapies, such as direct antiviral agents [34,35], may become available. These drugs require RBV and PEG-IFN in combination. However, not all patients may be able to tolerate this triple combination therapy due to adverse drug reactions, such as severe anaemia or skin eruption. In particular, it may be difficult to administer a full dose of triple drugs to older patients. Thus, personalizing the PEG-IFN and RBV combination therapy based on this model may be beneficial to patients who were intolerant to triple combination therapy.

In the present study creatinine was an independent predictor of relapse by multivariable logistic regression analysis. However creatinine was not selected as a splitting variable in decision tree, which may be due to the unique property of data mining analysis. In data mining analysis, limitation is imposed to stop the analysis when the number of patients is <20. This limitation is used to avoid dividing patients into too small subgroups which lead to the generation of rules that only apply to the model derivation population and not reproduced when applied to other populations. This phenomenon is called the over-fitting of the model. Due to this limitation, the variables selected in the data mining analysis are not necessarily identical to the variables that are significant by ordinary multivariable analysis. In a separate analysis, lower level of creatinine was associated with higher rate of relapse in each subgroup of patients with cEVR. The reason for this association is not clear, but lower creatinine level may be related to more efficient clearance of RBV leading to lower serum level of RBV. Further research is needed to confirm this speculation.

A potential limitation of the present study is that data mining analysis has an intrinsic risk of showing relationships that fit to the original dataset, but are not reproducible in different groups. Although internal and external validations showed that our model had high reproducibility, we recognized that further validation on a larger external validation cohort, especially in groups other than Japanese, may be necessary to further verify the reliability of our model.

In conclusion, we built a decision tree model for the prediction of relapse among patients with EVR to PEG-IFN plus RBV. The result of the present study shows that older age and insufficient dose of RBV are significant and independent risk factors for relapse. The target dose of total RBV can be set at 3.0 g/kg of body weight in patients who achieved cEVR. A further increase in RBV dose up to 4.0 g/kg of body weight may be warranted in patients <60 years.

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Disclosure statement

The authors declare no competing interests.

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Model Incorporating the *ITPA* Genotype Identifies Patients at High Risk of Anemia and Treatment Failure With Pegylated-Interferon Plus Ribavirin Therapy for Chronic Hepatitis C

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This study aimed to develop a model for predicting anemia using the inosine triphosphatase (*ITPA*) genotype and to evaluate its relationship with treatment outcome. Patients with genotype 1b chronic hepatitis C (n = 446) treated with peg-interferon alpha and ribavirin (RBV) for 48 weeks were genotyped for the *ITPA* (rs1127354) and *IL28B* (rs8099917) genes. Data mining analysis generated a predictive model for anemia (hemoglobin (Hb) concentration <10 g/dl); the CC genotype of *ITPA*, baseline Hb <14.0 g/dl, and low creatinine clearance (CLcr) were predictors of anemia. The incidence of anemia was highest in patients with Hb <14.0 g/dl and CLcr <90 ml/min (76%), followed by Hb <14.0 g/dl and *ITPA* CC (57%). Patients with Hb ≥14.0 g/dl and *ITPA* AA/CA had the lowest incidence of anemia (17%). Patients with two predictors (high-risk) had a higher incidence of anemia than the others (64% vs. 28%, $P < 0.0001$). At baseline, the *IL28B* genotype was a predictor of a sustained virological response [adjusted odds ratio 9.88 (95% confidence interval 5.01–19.48), $P < 0.0001$]. In patients who achieved an early virological response, the *IL28B* genotype was not associated with a sustained virological response, while a high risk of anemia was a significant negative predictor of a sustained virological response [0.47 (0.24–0.91), $P = 0.026$]. For high-risk patients with an early virological response, giving >80% of the planned RBV dose increased sustained virological responses by 24%. In conclusion, a predictive model incorporating the *ITPA* genotype could identify patients with a high risk of anemia and reduced probability of sustained virological response. ***J. Med. Virol. 85:449–458, 2013.***

KEY WORDS: hemolytic anemia; ribavirin; creatinine clearance; antiviral therapy

INTRODUCTION

Hepatitis C virus (HCV) infection is a leading cause of cirrhosis and hepatocellular carcinoma worldwide [Kim, 2002]. The rate of eradication of HCV by pegylated interferon (PEG-IFN) plus ribavirin (RBV), defined as a sustained virological response, is around 50% in patients with HCV genotype 1 [Manns et al., 2001; Fried et al., 2002]. Failure of treatment is attributable to the lack of a virological response or relapse after completion of therapy. Genome-wide association studies and subsequent cohort studies

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