the treatment, monocyte-derived DCs were generated from PBMC obtained from the patients according to methods reported previously [21]. As controls, monocyte-derived DCs were simultaneously generated from healthy donors. As responder cells in mixed lymphocyte reactions (MLR), naive $CD4^+$ T cells were isolated from PBMC of irrelevant healthy donors by using a naive $CD4^+$ T cell enrichment kit (Stemcell Technologies, Vancouver, BC). Allogeneic MLR with DC was performed as reported previously [21]. In order to compare the ability of DC among patients, we determined the MLR ratio between patients and controls as counts per minute (cpm) of [^{3}H]thymidine incorporated into CD4 T cells at the T cell/DC ratio of 10:1.

Statistical analyses

To analyze the relationship between clinical and immunological data at the baseline and virological response, univariate analysis using the Mann–Whitney *U* test or chi-squared test and multivariate analysis using logistic regression analysis were performed. The significance of trends in values was determined with the Mantel–Haenszel chi-square test. Differences of continuous variables between groups were compared by two-way analysis of variance (ANOVA). A two-railed *P* value less than 0.05 was considered significant. These statistical analyses were performed with SPSS version 15.0 (SPSS Inc. Chicago, IL, USA).

Results

Outcome of the PEG-IFNα2b and ribavirin therapy

In 67 patients who had been treated for 48 weeks, 29 (43%) achieved SVR, 18 (27%) were TR, 17 (25%) were NR, and 3 (4%) were unknown (Fig. 1). The clinical backgrounds of these patients are summarized in Table 1. Among these cohorts, 32 patients were c-EVR and were further categorized into 24 SVR (EVR-SVR group) and 8 TR (EVR-TR group). Of the other 35 patients who were not c-EVR, 5 were SVR, 10 were TR, 17 were NR and 3 were unknown. Details of the therapeutic response in the current study are shown in Fig. 1.

Higher platelet counts and Treg increase are involved in SVR in patients who underwent PEG-IFNα2b and ribavirin therapy

In order to clarify whether the frequency and function of immune cells are involved in the outcomes of the combination therapy, we first compared these parameters between SVR and non-SVR groups. Representative dot plots of the immune cell populations are shown in Fig. 2. The identification and enumeration of immune cells were determined by FACS. The pretreatment percentages of DC in SVR were higher than those in the non-SVR group. However, those of PDC, NK cells, Th1, Th2, Treg, and DC function as judged by MLR were not different between them (Fig. 3).

As for the changes of DC subsets during the therapy, in the SVR group, the frequencies of PDC increased after the

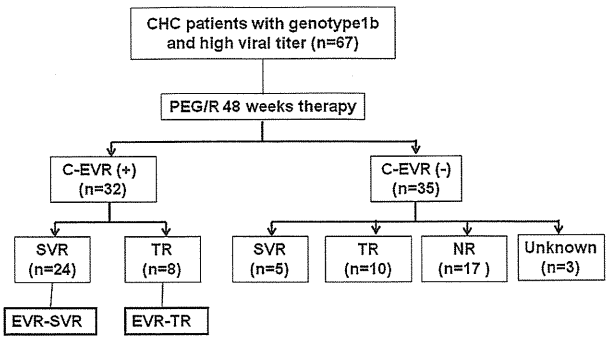


Fig. 1 Detailed outcomes of chronic hepatitis C patients treated with 48-week PEG-IFNα2b and ribavirin combination therapy. In 67 patients who had been treated for 48 weeks, 29 achieved SVR, 18 were TR, 17 were NR, and 3 were unknown. The complete early virological responders (c-EVR) were defined as those who show a reduction in HCV RNA quantity to an undetectable level by qualitative PCR at week 12 of the therapy. According to this criterion, 32 patients were c-EVR and were further categorized into 24 SVR (EVR-SVR) and 8 TR (EVR-TR). Of the other 35 patients who were not c-EVR, 5 were SVR, 10 were TR, 17 were NR, and 3 were unknown. *SVR* sustained virological responder, *TR* transient responder, *NR* non-responder

Table 1 Demographics and clinical backgrounds of the subjects

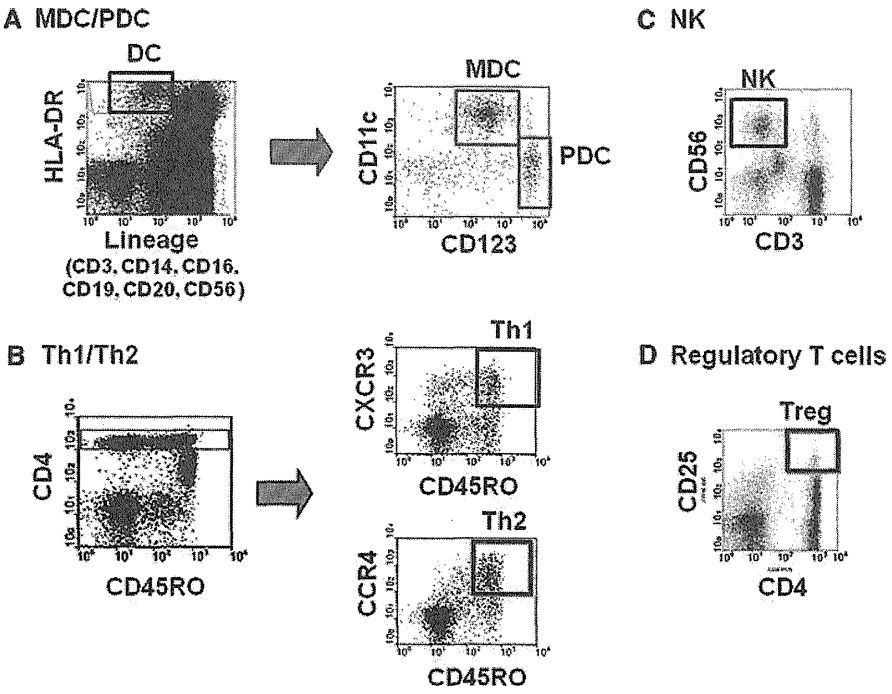
Factors	Value	Range
Number	67	
Age (years)	51.0 ± 10.3	(24–67)
Gender (M/F)	44/23	
HCV RNA (KIU)[a]	2415	
Activity: A0/1/2/3[b]	0/35/30/1	
Fibrosis: F0/1/2/3/4[b]	2/27/27/9/1	
WBC (/ml)	5229 ± 1299	(2960–9400)
Neutro (/ml)	2663 ± 826	(1077–4516)
Hb (g/dl)	14.6 ± 1.2	(12.0–18.0)
Platelets ($\times 10^4$/mm^3)	16.6 ± 4.6	(5.0–31.0)
ALT (IU/l)	83.1 ± 53.9	(14–269)
T. chol (mg/dl)	172 ± 29	(118–238)
Cr (mg/ml)	0.8 ± 0.2	(0.4–1.3)

All results are expressed as mean ± SD and range

T. chol total serum cholesterol, *Cr* creatinine

[a] Amplicore HCV monitor

[b] Ishak's histological scores



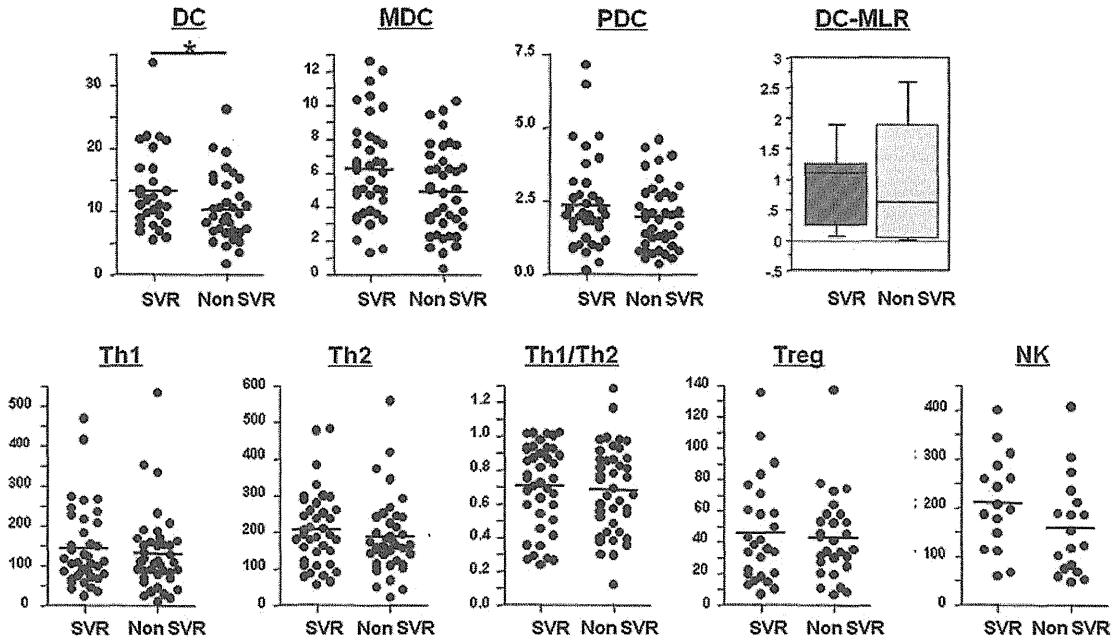


Fig. 2 Phenotypic identification of blood cells by flow cytometry. Representative analyses of myeloid and plasmacytoid dendritic cells (MDC and PDC), type 1 and type 2 helper T cells (Th1 and Th2), natural killer (NK) cells, and regulatory T cells are shown. The combination of surface molecules for the identification of cells is described in "Materials and methods"

Fig. 3 Comparison of pretreatment frequency of blood cells and allostimulatory capacity of monocyte-derived dendritic cells between SVR and non-SVR patients who had been treated with 48-week PEG-IFNα2b and ribavirin therapy. The frequencies of MDC, PDC, Th1 and Th2 cells, Th1/Th2 ratio, NK cells, regulatory T cells, and allogeneic MLR were compared between SVR and non-SVR patients. The MLR ratio between patients and controls was determined from the counts per minute (cpm) of [^{3}H]thymidine incorporated into $CD4^+$ T cells at T cell/DC ratio of 10:1. $^*P < 0.05$ by Mann–Whitney U test

beginning of therapy and showed a peak at week 12 of therapy (T12W), which subsided to the end-of-treatment (EOT). Such a PDC increase at the early phase was not observed in the non-SVR group (Fig. 4a). In contrast, the MDC frequency remained at a similar level throughout the therapy, regardless of viral response (data not shown). Alternatively, in the SVR group, the percentages of Treg ($CD4^+CD25^{high+}$ cells) increased through the therapy,

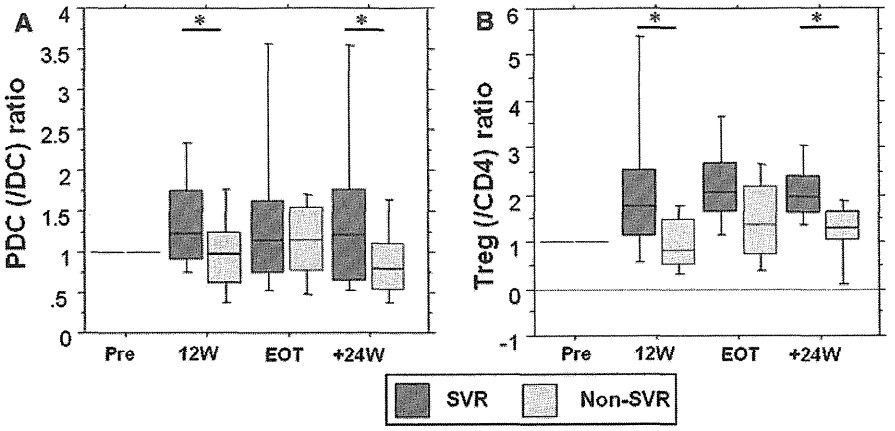


Fig. 4 Changes in frequencies of plasmacytoid dendritic cells and regulatory T cells during and after 48-week PEG-IFNα2b and ribavirin therapy in SVR and non-SVR patients. The ratios of frequencies of PDC (**a**) and Tregs (**b**) at each time point to the pretreatment values were compared between SVR and non-SVR patients. *Boxes* represent lower and upper quartiles, *solid line* within each *box* the median value, *whiskers* the minimum and maximum values. $*P < 0.05$ by Mann–Whitney *U* test. *EOT* end-of-treatment (at 48 weeks of the therapy), *+24W* 24 weeks after the completion of therapy

with cell levels being higher than those in the non-SVR group (Fig. 4b). The other cells, including Th1, Th2, and NK cells, did not differ between the groups (data not shown). Univariate and multivariate analyses were performed to assess the significance of various factors, including demographic, biochemical, virological, immunological parameters, and drug adherence. The allostimulatory capacity of DC after the completion of therapy, whose significance was demonstrated in the previous paper [21], was not included in this study because the numbers of patients examined for it were limited. In univariate analyses, platelet counts, histological activity and fibrosis, dose of PEG-IFNα2b, and attainment of c-EVR were found to be significant in SVR (Table 2). As for immunological markers, pretreatment DC frequency, PDC frequency, their ratio at T12W, and Treg frequency ratio at T12W are significant (Table 3). Based on these parameters, multivariate analysis revealed that platelet counts and Treg frequency at T12W were independent factors involved in SVR (Table 4). These results show that higher platelet counts and Treg increment may be related to SVR in 48 weeks of PEG-IFNα and ribavirin treatment.

Table 2 Univariate analyses of clinical factors involved in SVR

Factors	SVR	Non-SVR
N	29	38
Age (years)	48.0 ± 11.8	53.3 ± 8.6
Gender (M/F)	20/9	24/14
WBC (/mm^3)	5361 ± 1314	5127 ± 1295
Neutro (/mm^3)	2969 ± 861	2461 ± 753
Hb (g/dl)	14.6 ± 1.2	14.5 ± 1.2
Platelets ($\times 10^4$/mm^3)	18.2 ± 4.4*	15.2 ± 4.4
ALT (IU/l)	72 ± 54	92 ± 53
HCV RNA (KIU/ml)	2103	2654
Activity: 0–1/2–3/n.d.	29/0/0$^{\#}$	27/10/1
Fibrosis: 0–2/3–4/n.d.	20/9/0*	15/22/1
PEG-IFN dose (μg/kg/day)	1.43 ± 0.14$^{\#}$	1.31 ± 0.22
Ribavirin dose (mg/kg/day)	10.6 ± 1.5	9.9 ± 1.4
c-EVR: +/−	24/5$^{\#}$	8/27

Mann–Whitney *U* test, chi-square test

n.d. not determined

$*P < 0.05$, $^{\#}P < 0.01$

Higher platelet counts and PDC increase are independent factors involved in SVR after attainment of c-EVR

Next, we examined the above-mentioned immunological parameters in patients who attained c-EVR, as they were considered to be comparable with respect to the virological response to the therapy. Among 32 patients in the c-EVR group, 24 developed to SVR (EVR-SVR) and the remaining 8 to TR (EVR-TR) (Fig. 1). Univariate analysis disclosed that lower age is a characteristic of the EVR-SVR patients compared with those in the EVR-TR group (Table 5). As for immunological markers, pretreatment DC frequency, PDC frequency, and PDC ratio at T12W were higher in EVR-SVR patients than those in EVR-TR (Table 6). The pretreatment percentages of MDC, PDC, Th1, Th2, NK cells, and Tregs and those at any all points during the therapy did not differ between EVR-SVR and EVR-TR patients (data not shown). Multivariate analyses revealed that higher platelet counts and PDC increase at T12W were independent factors involved in EVR-SVR (Table 7). These results indicate that the dynamics of PDC

Table 3 Univariate analyses of immunological factors involved in SVR

Factors	SVR	Non-SVR	*P* value
N	29	38	
DC pre (/μl)	13.3 ± 6.5	10.3 ± 5.4	0.038
PDC-12W (/DC)	0.23 ± 0.09	0.18 ± 0.07	0.017
PDC-12W (/DC) ratio	1.42 ± 0.72	1.04 ± 0.63	0.028
Treg-12W (/CD4) ratio	2.49 ± 2.62	1.03 ± 0.64	0.016

Mann–Whitney *U* test, chi-square test

Only the factors that are of significance are shown

DC pre DC number before therapy, *PDC-12W (/DC)* PDC frequency in DC at T12W, *PDC-12W (/DC) ratio* the ratio of PDC frequency in DC at T12W to the pretreatment value, *Treg-12W (/CD4) ratio* the ratio of regulatory T cell frequency in CD4 at T12W to the pretreatment value

Table 4 Multivariate analyses of clinical and Immunological factors involved in SVR

Factors	Category	Odds ratio	95% CI	*P* value
Platelets		0.531	0.322–0.875	0.013
Treg-12W (/CD4) ratio	<1.2/>1.2	0.026	0.001–0.750	0.033

Logistic regression analysis, stepwise method

Table 5 Univariate analyses of clinical factors involved in SVR after the attainment of c-EVR in 48 weeks of therapy

Factors	EVR-SVR	EVR-TR
N	24	8
Age (years)	46.9 ± 12.3*	57.6 ± 6.5
Gender (M/F)	17/7	6/2
WBC (/mm^3)	5442 ± 1382	5211 ± 805
Neutro (/mm^3)	2975 ± 890	2587 ± 759
Hb (g/dl)	14.7 ± 1.1	15.1 ± 1.2
Platelets (×10^4/mm^3)	18.7 ± 4.5	15.0 ± 3.8
ALT (IU/l)	69 ± 56	91 ± 61
HCV RNA (KIU/ml)	1723	1296
Activity: 0–1/2–3/n.d.	24/0/0	6/2/0
Fibrosis: 0–2/3–4/n.d.	16/8/0	5/3/0
PEG-IFN dose (μg/kg/day)	1.43 ± 0.15	1.39 ± 0.23
Ribavirin dose (mg/kg/day)	10.8 ± 1.5	10.1 ± 2.1

Mann–Whitney *U* test, chi-square test

n.d. not determined, *EVR-SVR* SVR patients who attained complete EVR at T12W, *EVR-TR* TR patients who attained complete EVR at T12W

*$P < 0.05$

frequency during therapy serve as an independent immunological predictor for SVR in patients who attained c-EVR with PEG-IFNα and ribavirin therapy.

Table 6 Univariate analyses of immunological factors involved in SVR after the attainment of c-EVR in 48 weeks of therapy

Factors	Category	EVR-SVR	EVR-TR	*P* value
N		24	8	
DC pre (/μl)		13.5 ± 6.8	8.9 ± 4.5	0.030
PDC-12W (/DC) ratio	<0.8/>0.8	3/21	4/4	0.047

Mann–Whitney *U* test, chi-square test

Only the factors that are of significance are shown

DC pre, PDC-12 (/DC) ratio: see Table 3

Table 7 Multivariate analyses of clinical and immunological factors involved in SVR after the attainment of c-EVR in 48 weeks of therapy

Factors	Category	Odds ratio	95% CI	*P* value
Platelets		0.627	0.402–0.978	0.040
PDC-12W (/DC)	<0.18/≥0.18	0.028	0.001–0.787	0.036
PDC-12W (/DC) ratio	<0.8/≥0.8	0.032	0.002–0.673	0.027

Logistic regression analysis, stepwise method

PDC-12W (/DC), PDC-12W(/DC) ratio: see Table 3

Discussion

In this study, we demonstrated that the increase of Treg frequency during therapy is involved in SVR, and that of PDC is in SVR patients who attained c-EVR in 48 weeks of PEG-IFNα and ribavirin therapy. Of particular importance is that such significance is independent of viral dynamics (c-EVR), host factors (fibrosis, gender), and drug adherence.

Regulatory T cells (Treg) are immune suppressors that are supposed to alleviate HCV-induced liver inflammation. In chronic HCV infection, the increment of Tregs has been reported by several investigators, including us, although the underlying mechanisms were unspecified [20, 22]. The increase of Treg in SVR patients observed herein seems to be inconsistent with the previous reports regarding Treg as a tolerance inducer in chronic hepatitis C patients. Several controversial reports have been published with regard to the involvement of Tregs in the efficacy of PEG-IFNα and ribavirin therapy for chronic hepatitis C. Soldevila et al. [23] showed that the pretreatment frequency of Treg is higher in patients with non-response (NR) than those in the non-NR groups. Akiyama et al. [24] reported that Tregs in PBMC increased in SVR patients at earlier time points, while Tregs in liver-infiltrating lymphocytes decreased. By contrast, another group disclosed that frequency, phenotype, and function of Tregs are comparable regardless of the outcomes of PEG-IFNα and ribavirin therapy [25].

The current observation raises the possibility that the reduction of HCV load and/or liver inflammation correlates with the increment of Treg frequency, or vice versa. Recently, it was reported that liver inflammation caused by HCV induces PD-L1 on hepatocytes, which then suppress Treg proliferation in liver [26]. If such a scenario is operative as well in PEG-IFNα and ribavirin therapy, alleviation of liver inflammation may reduce PD-L1 expression on hepatocytes, thereby stimulating Treg proliferation. However, most of the TR patients, who were categorized as being in the non-SVR group, displayed normalized serum ALT levels and negative HCV RNA during treatment, of which conditions are equivalent with the SVR patients. Thus, it is still uncertain whether or not such mechanisms are applicable to the present results.

The other possibility is that phenotypically determined Tregs in this study partly consist of activated T cells. It is well known that $CD127^-$ and $FOXP3^+$ are reliable markers of Tregs [27]. In order to examine whether or not the increment of Treg frequency in this study is a contamination of activated T cells, we determined Tregs as $CD4^+CD25^{high}FOXP3^+CD127^-$ cells instead of $CD4^+CD25^{high}$ cells in some patients. In the comparison of the ratio of $CD4^+CD25^{high}FOXP3^+CD127^-$ cell frequency between the SVR and non-SVR groups at T12W, similar results were obtained with those of $CD4^+CD25^{high}$ cells (SVR vs. non-SVR, 10 patients in each group, 2.50 ± 1.20 vs. 1.54 ± 0.53, $P < 0.05$ by Mann–Whitney U test). These results suggest that the analytical results of $CD4^+CD25^{high}$ T cells reflect those of $FOXP3^+$ Tregs. Further investigation is needed to show that such Tregs are functionally suppressive and to see if the change of frequency parallels with suppressor capacity or not.

According to the AASLD practice guidelines for the treatment of chronic hepatitis C, a combination of PEG/R for 48 weeks is recommended for patients who attained c-EVR at week 12 of therapy [17]. However, in some cohorts with large numbers of patients, approximately 30% of them eventually relapse after cessation of the therapy [5]. The factors involved in post-therapeutic relapse have not been fully explored. We and others have reported that liver fibrosis, female gender, late virological response, and dosage of ribavirin (drug adherence) are critically involved in relapse [19, 28, 29]. It is well known that platelet counts in patients with chronic liver disease are well correlated with the degree of fibrosis. In the present study, multivariate analyses revealed that platelet counts but not fibrosis stage are involved in SVR. The reasons for such discrepant contributions to SVR are not clear; however, it demonstrates that the degree of fibrosis is involved in the therapeutic response in this cohort. In addition, the current study showed that the changes of PDC frequency are also somewhat involved in virological relapse in patients that once attained c-EVR.

Plasmacytoid DCs (PDC) play crucial roles in antivirus immune responses by producing IFN-β and -α [30]. In the previous study by us [14], the increment of PDC is observed in patients with SVR, of which change is more significant in those with c-EVR. No concrete explanation is available for the mechanisms of PDC increase in SVR patients. One of the possibilities is that the PDC increase is a consequence of better response to exogenous IFN-α in patients who have a higher chance of attaining SVR. IFN-α is reported to act as a regulatory factor on $CD11c^-$ DCs to sustain their viability and to inhibit gaining the ability to stimulate Th2 development [31]. Such a possibility is supported by the findings that higher induction of IFN-stimulated genes (ISGs) in hepatocytes after PEG-IFNα and ribavirin therapy, but not higher ISG levels before therapy, is critically involved in successful outcome [32]. Thus, patients who respond well to IFN-α, as demonstrated by better PDC survival during the treatment, are likely to have better chances to eradicate HCV.

Another possible reason for the PDC increase in the periphery of SVR patients is that PDC alter their localization during the treatment. Mengshol et al. [33] reported that PDC and myeloid DC (MDC) are accumulated in inflamed liver through the interactions of chemokines and their receptors. Of particular interest is that the expression of such chemokine receptors on DCs decreased in SVR patients, but not in non-SVR ones [33]. Therefore, it is plausible that PDC may migrate from the liver to periphery/lymphoid tissue after being unleashed from chemokines in the liver. In support for this, it is reported that IFN-α alters the profiles of chemokine receptors on DC, resulting in changes of the DC migrating ability [34].

Recently, numerous other factors were reported to be involved in therapeutic response in chronic hepatitis C patients, such as mutations of HCV genome (core region) [35] or host genetic variation (single nucleotide polymorphisms near the IL28B gene) [36]. In the current study, we were unable to analyze such factors because of the limited numbers of patients. A prospective study is warranted to analyze the involvement of such factors in relation to immune cell markers, in the outcomes of SOC, or the treatment with direct-acting antiviral agents.

In summary, we demonstrated that the increase of Treg frequency is an independent factor involved in SVR in 48 weeks of SOC for chronic hepatitis C patients. In addition, the increase of PDC gains similar significance in SVR patients who attained c-EVR. The assessment of the dynamics of such cells during therapy could offer some clues to identify potential relapsers and give them a better chance of attaining SVR by rescheduling the therapy.

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Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Efficacy of pegylated interferon plus ribavirin combination therapy for hepatitis C patients with normal ALT levels: a matched case–control study

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Abstract
Background The antiviral effect of pegylated interferon (Peg-IFN) plus ribavirin combination therapy in chronic hepatitis C (CHC) patients with normal alanine aminotransferase (ALT) levels (N-ALT) has been reported to be equivalent to that for patients with elevated ALT levels (E-ALT). However, the actual antiviral effect in N-ALT patients remains obscure because efficacy can be overestimated in patients with an advantageous background.
Methods In this study, 386 patients were extracted, for a matched case–control study, from 1320 CHC patients treated with Peg-IFN alpha-2b plus ribavirin combination therapy; 193 N-ALT patients [116 with hepatitis C virus genotype 1 (HCV-1), 77 with HCV genotype 2 (HCV-2)] were matched with 193 E-ALT patients by a propensity score method using the variables of age, sex, IFN treatment history, body mass index, and platelet counts.
Results On multivariate analysis for sustained virological response (SVR) in N-ALT patients, younger age, low HCV RNA level at baseline, and HCV-2 were significant factors. The matched case–control study showed that the SVR rates of N-ALT patients were equivalent to those of E-ALT patients; at 49 and 40% in the HCV-1 group ($P = 0.146$), and 78 and 81% in the HCV-2 group ($P = 0.691$). However, in N-ALT patients with non-SVR, approximately 40% showed ALT elevation at 24 weeks post-treatment.
Conclusion Our findings indicate that the antiviral effect of Peg-IFN plus ribavirin therapy in N-ALT patients is comparable to that for E-ALT patients irrespective of their advantageous background; however, the application of this therapy for N-ALT patients, especially for those with HCV-1, should be considered carefully.

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Keywords Hepatitis C virus · Normal alanine aminotransferase · Pegylated interferon plus ribavirin combination therapy · Propensity score method · Matched case–control study

Introduction

In patients with hepatitis C virus (HCV) infection, alanine aminotransferase (ALT) levels fluctuate and sometimes biochemical remission is maintained. Approximately 20% of patients with normal ALT levels (N-ALT) show ALT elevation and fibrosis progression within 3–5 years [1–5], and consequently, 70–80% of N-ALT patients have mild to moderate fibrosis on liver biopsy. N-ALT patients have been excluded from conventional interferon (IFN) therapy, because their sustained virological response (SVR) rates on conventional IFN monotherapy have been reported to be only 6–15% [6–9], and ALT levels were noted to increase during or after treatment in 47–62% of the patients. The incidence of ALT flares has raised concerns regarding the risk of conventional IFN therapy compared with a small benefit. However, a large randomized controlled trial has demonstrated that combination therapy with pegylated interferon (Peg-IFN) and ribavirin produced SVR rates in N-ALT patients with chronic hepatitis C (CHC) that were comparable to those of patients with elevated ALT levels (E-ALT) [10]. Thus, such treatment is now being considered for N-ALT patients with CHC [11].

Comparison of the characteristics of N-ALT and E-ALT patients has shown that the mean age of N-ALT patients was lower than that of E-ALT patients, and females and HCV genotype 2 patients were predominant among N-ALT patients [4, 7, 12–17]. In the American Association for the Study of Liver Disease guideline, the pretreatment predictors of achieving SVR with Peg-IFN plus ribavirin combination therapy for CHC patients are HCV genotype 2 or 3 infection, low viral load (<600 KIU/ml), female gender, and age less than 40 years [11]. Considering these characteristics, N-ALT patients with CHC can be said to have an advantageous background, and their response to antiviral therapy, including Peg-IFN plus ribavirin combination therapy, can be overestimated. Therefore, patient background, especially factors affecting the treatment efficacy of the combination therapy, needs to be matched between study groups in order to compare the treatment efficacies in N-ALT patients with CHC and E-ALT patients with CHC accurately. In this study, we evaluated, by a matched case–control study approach, whether the antiviral efficacy in N-ALT patients with CHC, reported to be equal to that in E-ALT patients with CHC, could be obtained without their advantageous background, and whether the factors contributing to SVR in N-ALT patients were the same as those in E-ALT patients. In addition, ALT flares after treatment in N-ALT patients without SVR were examined.

Patients and methods

Patient selection and study design

The subjects were 1320 consecutive CHC patients, 1015 with HCV genotype 1 (HCV-1) and 305 with HCV genotype 2 (HCV-2) who had undergone combination therapy with Peg-IFN alpha-2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) and ribavirin (REBETOL; Schering-Plough) at standard doses for 48 weeks (patients with HCV-1) or for 24 weeks (patients with HCV-2) at 30 medical institutions participating in the Osaka Liver Forum between December 2004 and December 2007. Peg-IFN alpha-2b and ribavirin dosages were based on body weight according to the manufacturer's instructions and were modified based on the manufacturer's instructions according to the severity of adverse hematologic effects. In the 1 month preceding treatment, none of the patients had received any IFN formulations or other types of drugs for liver supporting therapy. Before starting treatment, all patients had positive anti-HCV and a detectable level of HCV RNA according to a polymerase chain reaction (PCR)-based assay (COBAS Amplicor HCV Monitor Test v2.0; Roche Diagnostics, Branchburg, NJ, USA). None of the patients showed evidence of dual infection with hepatitis B virus or human immunodeficiency virus, or other forms of liver diseases such as alcoholic liver disorder, autoimmune hepatitis, or drug-induced liver injury.

In this study, a normal serum ALT level was defined as ALT $\leq$30 IU/l at the start of the combination therapy, as, in the guidelines for treatment of hepatitis C in N-ALT patients in Japan, ALT levels of $\leq$30 IU/l are regarded as an indicator of no or little inflammation in the liver, and patients whose ALT levels are $\leq$30 IU/l are recommended to be followed without antiviral therapy, especially if the platelet count is $\geq 15 \times 10^4/mm^3$.

Among the 1320 consecutive CHC patients, the antiviral effect in 193 N-ALT patients (116 with HCV-1, 77 with HCV-2) was compared with that in 193 E-ALT patients (116 with HCV-1, 77 with HCV-2) who were matched by a propensity score method based on age, sex, IFN treatment history, body mass index (BMI), and platelet counts. BMI was calculated as weight (kg)/[height (m)]^2.

HCV RNA was determined at week 4, week 12, end of treatment (EOT), and 24 weeks after EOT. HCV RNA was also determined at week 24 for HCV-1 patients. HCV RNA was monitored by the PCR Amplicor method with a detection limit of 50 IU/ml. (COBAS Amplicor HCV v2.0;

Roche Diagnostics). Complete early virological response (cEVR) and end-of-treatment response (ETR) were defined as undetectable HCV RNA at week 12 and EOT, respectively.

Written informed consent was obtained from each patient, and the study protocol was reviewed and approved according to the ethical guidelines of the 2004 Declaration of Helsinki by institutional review boards at the respective sites.

Propensity score

Propensity score methods are used to create balanced covariates and reduce selection bias in a matched case–control study. Propensity scores were calculated using a multivariate logistic regression model that had ALT levels as a dependent variable and other covariates as independent variables, and the model was utilized for matching between the N-ALT patients with CHC (the case group) and the E-ALT patients with CHC (the control group). Data analyses were conducted using SAS, version 9.2 (SAS Institute, Cary, NC, USA).

Statistical analysis

Continuous variables are reported as the mean with standard deviation (SD) or median levels, while categorical variables are shown as the count and proportion. Statistical significance was assessed by Student's t test (mean), the Mann–Whitney U test (median), and the χ^2 test for independent samples, and the paired t test for paired samples. For all tests, two-sided P values were calculated, and the results were considered statistically significant if $P < 0.05$. Variables that achieved statistical significance on univariate analysis were subjected to multivariate logistic regression analysis. Stepwise and multivariate logistic regression models were used to explore the independent factors that could be used to predict SVR. Statistical analysis was performed using the SPSS program for Windows, version 15.0 J (SPSS, Chicago, IL, USA).

Results

Baseline characteristics of all CHC patients according to HCV genotype and ALT levels before matching

The baseline characteristics of 1320 patients at the commencement of combination therapy with Peg-IFN and ribavirin are shown in Table 1, according to HCV genotype and ALT levels before matching. Of the 116 N-ALT patients with HCV-1 there were 36 males and 80 females (69%), with a mean age of 54 ± 11 years. Eighty-five (73%) were IFN-naïve. In terms of liver histology, 66 (73%) patients had

Table 1 Demographic characteristics of patients with normal ALT and patients with elevated ALT

	HCV genotype 1			HCV genotype 2		
	Normal ALT ($n = 116$)	Elevated ALT ($n = 899$)	P value	Normal ALT ($n = 77$)	Elevated ALT ($n = 228$)	P value
Sex: male/female	36/80	512/387	<0.001	32/45	121/107	0.081
Age (years)	54 ± 11	56 ± 10	0.136	51 ± 13	52 ± 13	0.423
Body mass index (kg/m^2)	22.9 ± 3.1	23.3 ± 3.2	0.131	23.0 ± 2.9	23.3 ± 3.2	0.424
Past IFN therapy: naïve/experienced (relapser/non-responder)[a]	85/31 (18/5)	547/352 (131/154)	0.011	58/19 (9/4)	175/53 (21/10)	0.876
Histology (METAVIR)[b]						
Activity: 0–1/2–3	66/25	296/338	<0.001	43/6	69/94	<0.001
Fibrosis: 0–1/2–4	67/24	330/304	<0.001	42/7	101/62	0.002
HCV RNA (KIU/ml)[c]	1800	1700	0.793	2200	1100	<0.001
White blood cell (/mm^3)	5220 ± 1507	5137 ± 1582	0.595	5538 ± 1687	5338 ± 1725	0.377
Neutrophil (/mm^3)	2770 ± 1074	2595 ± 1078	0.108	3017 ± 1180	2688 ± 1230	0.047
Hemoglobin (g/dl)	13.6 ± 1.5	14.2 ± 1.4	<0.001	13.8 ± 1.6	14.2 ± 1.4	0.071
Platelet ($\times 10^4$/mm^3)	19.9 ± 5.7	16.2 ± 5.3	<0.001	20.5 ± 4.5	17.8 ± 5.8	<0.001
ALT (IU/l)	24 ± 5	88 ± 62	<0.001	22 ± 5	97 ± 67	<0.001

ALT alanine aminotransferase, *HCV* hepatitis C virus, *IFN* interferon

[a] Status was unknown in 8 patients in the normal ALT group and 67 in the elevated ALT group with HCV genotype 1; and in 6 in the normal ALT group and 22 in the elevated ALT group with HCV genotype 2

[b] Data missing in 25 patients in the normal ALT group and in 265 in the elevated ALT group with HCV genotype 1; and in 28 in the normal ALT group and in 65 in the elevated ALT group with HCV genotype 2

[c] Values are expressed as medians

mild activity (activity, 0–1) and 67 (74%) had mild fibrosis (fibrosis, 0–1) by the METAVIR system. Mean white blood cell counts, hemoglobin levels, and platelet counts were 5220 ± 1570 /mm^3, 13.6 ± 1.5 g/dl, and 19.9 ± 5.7 × 10^4/mm^3. In 899 E-ALT patients compared to N-ALT patients, the proportions of female and IFN-naïve patients were significantly lower, at 43% ($P < 0.001$) and 61% ($P = 0.011$), respectively. Higher scores for activity ($P < 0.001$) and fibrosis ($P < 0.001$) were observed in E-ALT patients. E-ALT patients had higher hemoglobin levels and lower platelet counts than N-ALT patients, at 14.2 ± 1.5 g/dl ($P < 0.001$) and 16.2 ± 5.3 × 10^4/mm^3 ($P < 0.001$), respectively. Mean ALT levels were 24 ± 5 IU/l in N-ALT patients and 88 ± 62 IU/l in E-ALT patients ($P < 0.001$).

Of the 77 N-ALT patients with HCV-2, 32 were males and 45, females (58%). Their mean age was 51 ± 13 years and 58 (75%) were IFN-naïve. In terms of liver histology, 43 (88%) patients had mild activity (activity, 0–1) and 42 (86%) had mild fibrosis (fibrosis, 0–1). Compared to the 228 E-ALT patients, the N-ALT patients had higher HCV RNA levels (median 2200 vs. 1100 KIU/ml, $P < 0.001$). Higher scores for activity ($P < 0.001$) and fibrosis ($P = 0.002$) were observed in E-ALT patients. Neutrophils and platelet counts in N-ALT patients were higher than those in E-ALT patients, at 3017 ± 1180 versus 2688 ± 1230 /mm^3 ($P = 0.047$) and 20.5 ± 4.5 × 10^4 versus 17.8 ± 5.8 × 10^4/mm^3 ($P < 0.001$), respectively. Mean ALT levels were 22 ± 5 IU/l in N-ALT patients and 97 ± 67 IU/l in E-ALT patients ($P < 0.001$).

Prognostic factors for SVR in the N-ALT patients

For all N-ALT patients (HCV-1, 116; HCV-2, 77), univariate analysis for factors associated with achieving SVR was performed for the following variables: sex, age, BMI, history of past IFN therapy, histology, baseline HCV RNA level, HCV genotype, white blood cell count, neutrophil count, hemoglobin level, platelet count, and ALT level (Table 2). The results indicated that age, fibrosis, baseline HCV RNA level, and HCV genotype contributed to SVR. Next, multivariate logistic regression analysis was performed for all N-ALT patients ($n = 193$), using these factors except for fibrosis, as there were many missing samples. The multivariate analysis showed that younger age [by 10-year increase: odds ratio (OR) 0.552; 95% confidence interval (CI) 0.404–0.756; $P < 0.001$] and lower baseline HCV RNA level (by 100-KIU/ml increase: OR 0.976; 95% CI 0.954–0.998; $P = 0.037$), as well as HCV genotype (genotype 2 vs. genotype 1: OR 3.724; 95% CI 1.859–7.463; $P < 0.001$) were independently associated with SVR (Table 3).

Table 2 Factors associated with SVR in patients with normal ALT—univariate analysis

Factor	SVR ($n = 117$)	Non-SVR ($n = 76$)	P value
Sex: male/female	43/74	25/51	0.645
Age (years)	50 ± 13	57 ± 9	<0.001
Body mass index (kg/m^2)	22.8 ± 3.3	23.1 ± 2.6	0.511
Past IFN therapy: naïve/experienced	88/29	55/21	0.737
Histology (METAVIR)[a]			
Activity: 0–1/2–3	67/14	42/17	0.148
Fibrosis: 0–1/2–4	69/12	40/19	0.022
HCV genotype: 1/2	57/60	59/17	<0.001
HCV RNA (KIU/ml)[b]	1700	2100	0.040
White blood cell (/mm^3)	5461 ± 1426	5170 ± 1798	0.213
Neutrophil (/mm^3)	2968 ± 1167	2709 ± 1032	0.126
Hemoglobin (g/dl)	13.7 ± 1.4	13.7 ± 1.6	0.970
Platelet (×10^4/mm^3)	20.4 ± 4.8	19.8 ± 5.8	0.388
ALT (IU/l)	23 ± 5	24 ± 5	0.384

SVR sustained virological response, *ALT* alanine aminotransferase, *IFN* interferon, *HCV* hepatitis C virus

[a] Data missing in 36 patients in the SVR group and in 17 in the non-SVR group

[b] Values are expressed as medians

Table 3 Factors associated with SVR in patients with normal ALT—multivariate analysis

Factor	Category	Odds ratio	95% CI	P value
Age	By 10 years	0.552	0.404–0.756	<0.001
HCV genotype	1/2	3.724	1.859–7.463	<0.001
HCV RNA	By 100 KIU/ml	0.976	0.954–0.998	0.037

The number of patients used for this multivariate analysis was 193 (SVR, $n = 117$; non-SVR, $n = 76$)

SVR sustained virological response, *ALT* alanine aminotransferase, *CI* confidence interval, *HCV* hepatitis C virus

Comparison of patient characteristics between patients with normal ALT and those with elevated ALT matched by a propensity score method

The baseline characteristics of CHC patients matched by a propensity score method at the commencement of combination therapy with Peg-IFN and ribavirin were compared between N-ALT patients and E-ALT patients (see Table 4). There were 116 CHC patients with HCV-1 in each of the groups of N-ALT and E-ALT patients. The two groups were well matched by propensity score methods and there was no significant difference, except in ALT values (mean value, N-ALT, 24 ± 5 IU/l vs. E-ALT, 78 ± 53 IU/l, $P < 0.001$). Similarly, with CHC patients with HCV-2, there were no significant differences, except for ALT levels (mean value, N-ALT, 22 ± 5 IU/l vs. E-ALT, 80 ± 58 IU/l, $P < 0.001$), activity scores [0–1, N-ALT, 88% (43/49) vs. E-ALT, 49% (25/51), $P < 0.001$], and HCV RNA levels (median value, N-ALT, 2200 KIU/ml vs. E-ALT, 1000 KIU/ml, $P < 0.001$).

Treatment efficacy of combination therapy with Peg-IFN and ribavirin in CHC patients

Antiviral effects of the combination therapy with Peg-IFN and ribavirin were evaluated by rapid virological response (RVR), cEVR, ETR, SVR, and relapse rates, as shown in Table 5. Among patients with HCV-1 in the N-ALT and E-ALT patients, respectively, RVR rates were 6% (6/98) and 6% (6/102), cEVR rates were 53% (62/116) and 43% (50/116), and ETR rates were 72% (84/116) and 58% (67/116) ($P = 0.019$). SVR and relapse rates in N-ALT patients were 49% (57/116) and 32% (27/84). These rates in E-ALT patients were 40% (46/116) and 31% (21/67). In the patients with HCV-2, RVR, cEVR, ETR, SVR, and relapse rates were 68% (41/60), 90% (69/77), 96% (74/77), 78% (60/77), and 19% (14/74) for N-ALT patients, and 62% (36/58), 91% (70/77), 91% (70/77), 81% (62/77), and 11% (8/70) for E-ALT patients, respectively. Comparisons between N-ALT and E-ALT patients with HCV-1 or HCV-2 showed no significant differences in RVR, cEVR, ETR, SVR, and relapse rates, except in ETR rates in patients with HCV-1.

Changes in ALT levels during combination therapy and follow-up periods in N-ALT patients with SVR and those with non-SVR

Changes in ALT levels in N-ALT patients during the combination therapy and follow-up periods were evaluated according to the treatment response (Fig. 1). In patients with HCV-1, the mean baseline ALT level in the SVR

Table 4 Comparison of characteristics between patients with normal ALT and patients with elevated ALT matched by a propensity score method

	HCV genotype 1			HCV genotype 2		
	Normal ALT ($n = 116$)	Elevated ALT ($n = 116$)	*P* value	Normal ALT ($n = 77$)	Elevated ALT ($n = 77$)	*P* value
Sex: male/female	36/80	32/84	0.564	32/45	30/47	0.742
Age (years)	54 ± 11	55 ± 11	0.746	51 ± 13	50 ± 13	0.742
Body mass index (kg/m^2)	22.9 ± 3.1	22.6 ± 2.9	0.536	23.0 ± 2.9	22.8 ± 2.9	0.780
Past IFN therapy: naïve/experienced (relapser/non-responder)[a]	85/31 (18/5)	80/36 (13/18)	0.469	58/19 (9/4)	57/20 (7/4)	0.853
Histology (METAVIR)[b]						
Activity: 0–1/2–3	66/25	49/35	0.056	43/6	25/26	<0.001
Fibrosis: 0–1/2–4	67/24	59/25	0.736	42/7	36/15	0.068
HCV RNA (KIU/ml)[c]	1800	1700	0.896	2200	1000	<0.001
White blood cell (/mm^3)	5220 ± 1507	5329 ± 1626	0.569	5538 ± 1687	5530 ± 1780	0.977
Neutrophil (/mm^3)	2770 ± 1074	2702 ± 1094	0.641	3017 ± 1180	2755 ± 1189	0.189
Hemoglobin (g/dl)	13.6 ± 1.5	13.7 ± 1.4	0.542	13.8 ± 1.6	14.0 ± 1.4	0.592
Platelet (×10^4/mm^3)	19.9 ± 5.7	19.4 ± 7.1	0.562	20.5 ± 4.5	20.6 ± 5.5	0.911
ALT (IU/l)	24 ± 5	78 ± 53	<0.001	22 ± 5	80 ± 58	<0.001

ALT alanine aminotransferase, *IFN* interferon, *HCV* hepatitis C virus

[a] Data unknown in 8 patients in the normal ALT group and in 5 in the elevated ALT group with HCV genotype 1; and in 6 in the normal ALT group and 9 in the elevated ALT group with HCV genotype 2

[b] Data missing in 25 patients in the normal ALT group and in 32 in the elevated ALT group with HCV genotype 1; and in 28 in the normal ALT group and 26 in the elevated ALT group with HCV genotype 2

[c] Values are expressed as medians

Table 5 Antiviral effect for patients with normal ALT and those with elevated ALT according to HCV genotype

		Normal ALT	Elevated ALT	*P* value
HCV genotype 1		$n = 116$	$n = 116$	
	Undetectable HCV RNA rate			
	At week 4 (RVR)[a]	6% (6/98)	6% (6/102)	1.000
	At week 12 (cEVR)	53% (62/116)	43% (50/116)	0.287
	At week 48 (ETR)	72% (84/116)	58% (67/116)	0.019
	Post-24 weeks (SVR)	49% (57/116)	40% (46/116)	0.146
	Relapse rate	32% (27/84)	31% (21/67)	0.916
HCV genotype 2		$n = 77$	$n = 77$	
	Undetectable HCV RNA rate			
	At week 4 (RVR)[b]	68% (41/60)	62% (36/58)	0.563
	At week 12 (cEVR)	90% (69/77)	91% (70/77)	0.723
	At week 24 (ETR)	96% (74/77)	91% (70/77)	0.191
	Post-24 weeks (SVR)	78% (60/77)	81% (62/77)	0.691
	Relapse rate	19% (14/74)	11% (8/70)	0.212

ALT alanine aminotransferase, *HCV* hepatitis C virus, *RVR* rapid virological response, *cEVR* complete early virological response, *ETR* end-of-treatment response, *SVR* sustained virological response

[a] Data missing in 18 patients in the normal ALT group and in 14 in the elevated ALT group with HCV genotype 1

[b] Data missing in 17 patients in the normal ALT group and in 19 in the elevated ALT group with HCV genotype 2

group ($n = 57$) was similar to that in the non-SVR group ($n = 59$) (mean ± standard error of the mean (SEM): SVR group, 24.5 ± 0.6 IU/l; non-SVR group, 24.2 ± 0.7 IU/l; $P = 0.694$). Transitions of ALT levels were not significantly different between SVR and non-SVR groups during the therapy. However, in the SVR group, the ALT level fell to 15.1 ± 0.7 IU/l at 24 weeks after treatment completion ($P < 0.001$, compared to the baseline level), while in the non-SVR group, higher ALT levels were observed after treatment compared to the baseline level; the ALT level rose to the peak value of 36.2 ± 3.6 IU/l at post-12 weeks ($P = 0.001$), and slightly fell to 31.3 ± 2.6 IU/l at post-24 weeks ($P = 0.007$) (Fig. 1a). In comparison with the SVR group, the non-SVR group showed significant differences in mean ALT levels at post-4, -12, and -24 weeks ($P = 0.002$, <0.001, and <0.001, respectively). At post-48 weeks in the non-SVR group, the ALT level was 30.4 ± 2.9 IU/l, which was still higher than the baseline level ($P = 0.025$).

Similarly, in patients with HCV-2, baseline ALT levels in the SVR group ($n = 60$) and the non-SVR group ($n = 17$) were equivalent (mean ± SEM; SVR, 21.8 ± 0.7 IU/l; non-SVR, 22.5 ± 1.1 IU/l; $P = 0.622$), and there was no significant difference in transitions of the ALT levels during therapy. However, after treatment, in the non-SVR group, ALT levels tended to rise in comparison with those at baseline; they rose to 74.9 ± 26.9 IU/l at post-12 weeks ($P = 0.068$) and fell to 35.7 ± 10.2 IU/l at post-24 weeks ($P = 0.196$). On the other hand, in the SVR group, ALT levels fell significantly, to 16.4 ± 1.3 IU/l at post-12 weeks ($P < 0.001$) and 15.2 ± 1.2 IU/l at post-24 weeks ($P < 0.001$) (Fig. 1b). Comparison of ALT levels between the SVR and non-SVR groups after treatment showed that mean ALT levels in the non-SVR group tended to be high at post-4, -12 and, -24 weeks ($P = 0.045$, 0.051, and 0.066, respectively). At post-48 weeks in the non-SVR group, the ALT level was 32.4 ± 8.9 IU/l, which tended to be high compared with the baseline ALT level, although no significant difference was found ($P = 0.248$).

Next, the ALT levels in N-ALT patients were examined according to the treatment response at 24 weeks after completion of the combination therapy. In HCV-1 patients with SVR, ALT levels remained below the upper limit of normal (ULN) for this study (<30 IU/l) in 55 (98%) patients, and ALT elevation <2 × ULN occurred in only one (2%) patient (ALT 32 IU/l). On the other hand, in patients with non-SVR, ALT levels remained stable in 34 (60%) patients but increased to <2 × ULN in 20 (35%) patients, and to ≥2 × ULN in 3 (5%) patients (ALT 62, 79, and 135 IU/l). Similarly, in HCV-2 patients with SVR, ALT levels remained stable in 56 (95%) patients, and ALT elevation rarely occurred [<2 × ULN, 2 (3%) patients; ≥2 × ULN, one (2%) patient (ALT 68 IU/l)]. In contrast, in patients with non-SVR, ALT levels remained normal in 10 (67%) patients but increased to <2 × ULN in 4 (27%) patients and to ≥2 × ULN in one (6%) patient (ALT 174 IU/l).

Discussion

N-ALT patients with CHC are known to show demographic and virological features associated with higher

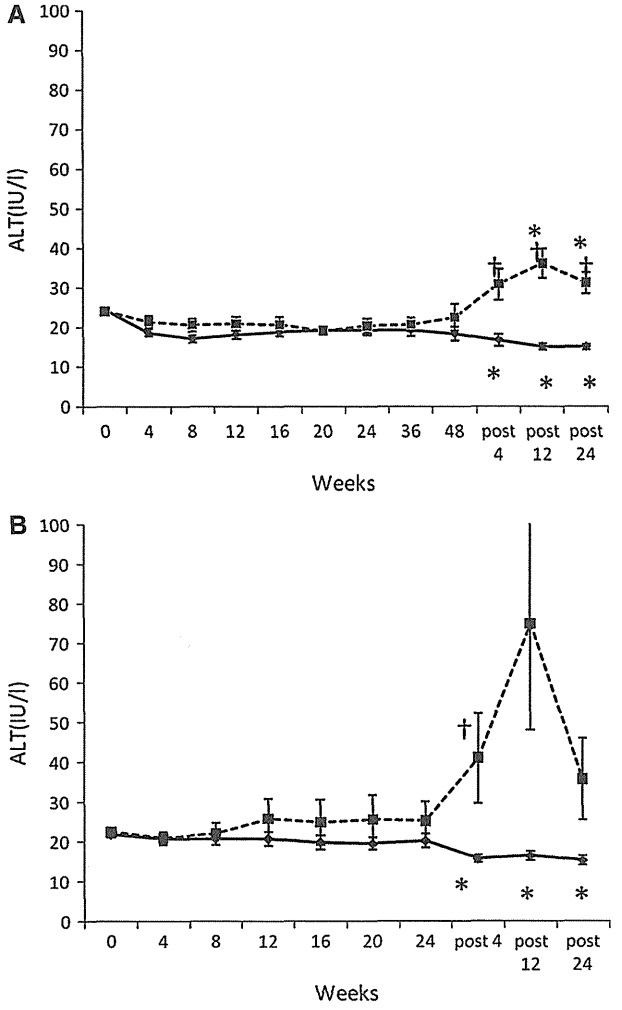

Fig. 1 Changes in serum alanine aminotransferase (*ALT*) levels (±standard error of the mean) according to response in patients with normal ALT levels with chronic hepatitis C treated with pegylated interferon and ribavirin. *Solid lines* show ALT levels in patients with a sustained virological response (SVR), and *dashed lines* show these levels in patients with a non-SVR. *Single-asterisks* denote a statistically significant difference ($P < 0.05$) in mean ALT levels between baseline and each time point of the follow-up period. *Daggers* denote a statistically significant difference between SVR and non-SVR groups. **a** Patients infected with hepatitis C virus genotype 1 (HCV-1). The number of patients was 57 in the SVR group and 59 in the non-SVR group. **b** HCV-2 patients. The number of patients was 60 in the SVR group and 17 in the non-SVR group

response rates to Peg-IFN and ribavirin combination therapy [4, 7, 12–17]. In the present study, N-ALT patients were younger and had higher platelet counts than E-ALT patients, thus giving N-ALT patients an advantage in antiviral efficacy in comparison with E-ALT patients in our cohort. However, the preponderance of females was greater in N-ALT patients with HCV-1 in this study, giving N-ALT patients a disadvantage. Accordingly, a direct comparison was made between these two patient groups after matching E-ALT patients with N-ALT patients using propensity score methods to reduce the bias due to differences in patient backgrounds. As a result, the efficacy of the combination therapy in N-ALT patients was revealed to be still equivalent to that in E-ALT patients, irrespective of their advantageous background. Moreover, in N-ALT patients with HCV-1, not only the ETR rate, but also the SVR rate tended to be higher than these rates in E-ALT patients (49% in N-ALT patients vs. 40% in E-ALT patients). Accordingly, N-ALT patients with HCV-1 can achieve a better treatment response in comparison with E-ALT patients, but further study is needed to clarify this.

In the present study, multivariate logistic regression analysis showed that achieving SVR was strongly influenced by HCV genotype and baseline HCV RNA level in N-ALT patients, which was consistent with findings of multicenter studies with E-ALT patients [18–21]. Therefore, decisions for treatment and the treatment regimen for N-ALT patients can mirror those recommended for E-ALT patients. The results of our multivariate analysis also revealed that patient age influenced the achievement of SVR in N-ALT patients. This offers support for the decision to offer antiviral treatment to younger N-ALT patients.

Among patients in our study who achieved SVR with the combination therapy, ALT levels after treatment decreased significantly, as shown in Fig. 1. However, approximately 40% of the non-SVR patients had increased ALT levels of up to $<2 \times$ ULN, and about 5% of patients had increased ALT levels of $\geq 2 \times$ ULN at 24 weeks after completion of the combination therapy, regardless of HCV genotype. When N-ALT patients are commencing the combination therapy, these patients should be told about the possibility of ALT exacerbation [6–9], although it is difficult to know whether this is drug-induced or due to the natural course. It is also difficult to state which patient characteristics make ALT elevation more likely to occur after the treatment.

Taking the findings obtained in the present study together, in N-ALT patients with HCV genotype 2, earlier treatment with Peg-IFN plus ribavirin combination therapy is desirable, as better efficacy was found for younger patients, with an SVR rate of approximately 80% being attained with this combination therapy, and few direct-acting antiviral agents (DAAs) have been developed for genotype 2. On the other hand, N-ALT patients with HCV genotype 1 should consider awaiting the DAAs, because SVR cannot be attained in about half of these patients, and the ALT level rises after treatment in about 40% of patients with non-SVR.

From the aspect of long-term prognosis, we need to verify, by prospective study, that viral eradication is really required for N-ALT patients because the incidence of hepatocellular carcinoma and liver-related mortality in

N-ALT patients has not been clarified. Deuffic-Burban et al. [22] calculated the impact of Peg-IFN plus ribavirin on morbidity and mortality in N-ALT patients using the Markov model and concluded that antiviral therapy in N-ALT patients would decrease morbidity and mortality rates. However, the treatment of N-ALT patients with CHC still remains an area of investigation, particularly with respect to the benefit-to-risk ratio of treatment. To help determine the indications for antiviral therapy in N-ALT patients, the liver histology should be evaluated before treatment. The presence of significant hepatic fibrosis (≥F2 by the METAVIR classification [23]) reflects continuous hepatic inflammation over a period of time and suggests a future risk of liver-related disease progression. Antiviral therapy may be appropriate for these patients. On the other hand, periodic follow up without antiviral therapy is recommended for patients in stages F0-1, because most of such patients show a low risk for progression to cirrhosis and the development of hepatocellular carcinoma [24].

This study had some limitations. First, the factors of viral mutation and host genetic mutation, which have been reported recently to affect the efficacy of Peg-IFN plus ribavirin combination therapy, could not be measured, and evaluation of the serum HCV RNA levels by a real-time PCR method, which is more sensitive to the measurement of serum HCV RNA levels, could not be done in the patients enrolled in this study, because we had few stored patient serum samples. Detailed examinations using the real-time PCR method in patients who are matched based on the factors of viral mutation and host genetic mutation as well as background factors will be needed for further study. Second, we excluded the factor of fibrosis from the multivariate analysis for factors associated with SVR in N-ALT patients, because data for fibrosis were lacking in 53 of the 193 patients in this study. Accordingly, the present study could not demonstrate whether fibrosis was associated with SVR in N-ALT patients. Finally, in this study, we investigated the antiviral efficacy of Peg-IFN plus ribavirin combination therapy for patients with N-ALT at the start of the therapy, not for patients with 'persistently' normal ALT. Accordingly, this study does not show the efficacy of this treatment in patients with persistently normal ALT. However, we believe that the results obtained in this study can be useful for pre-treatment prediction in outpatients who may not be followed by the reason of having normal ALT levels.

We have shown, in this matched case–control study using a propensity score method, that the therapeutic effect of combination therapy with Peg-IFN alpha-2b and ribavirin in N-ALT patients with CHC is comparable to that for E-ALT patients, irrespective of their advantageous background. Further work is needed to verify that HCV eradication can improve the prognosis of N-ALT patients.

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Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Efficacy of re-treatment with pegylated interferon plus ribavirin combination therapy for patients with chronic hepatitis C in Japan

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Abstract
Background It is still not known which patients with chronic hepatitis C who failed to respond to previous pegylated interferon (Peg-IFN) plus ribavirin therapy can benefit from re-treatment.
Methods Seventy-four patients (HCV genotype 1, $n = 56$, genotype 2, $n = 18$) were re-treated with Peg-IFN plus ribavirin.
Results On re-treatment, the sustained virologic response (SVR) rate was 41% for genotype 1 and 56% for genotype 2. With genotype 1, the factors associated with an SVR were previous treatment response and the serum hepatitis C virus (HCV) RNA level at the start of re-treatment. Patients with a $\geq$2-log decrease in HCV RNA at week 12 (partial early virologic response, p-EVR) in previous treatment had significantly higher SVR rates than those without these

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decreases ($p < 0.001$); no patient without a p-EVR in the previous treatment attained an SVR with re-treatment (0/16). All patients with <5 $\log_{10}$ IU/ml of HCV RNA at the start of re-treatment attained an SVR (6/6), while only 33% (15/45) of those patients with ≥5 $\log_{10}$ IU/ml of HCV RNA attained an SVR ($p < 0.01$). Among the patients with relapse in the previous treatment, those who attained an SVR on re-treatment required a longer duration of re-treatment than the duration of the previous treatment (re-treatment, 63.8 ± 13.0 weeks vs. previous treatment, 53.9 ± 13.5 weeks, $p = 0.01$).

Conclusions Re-treatment of genotype 1 patients should be limited to patients with a p-EVR in the previous treatment and a low HCV RNA level at the start of re-treatment. In re-treatment with Peg-IFN plus ribavirin, longer treatment duration can contribute to increasing the anti-viral effect.

Keywords Chronic hepatitis C · Pegylated interferon and ribavirin combination therapy · Re-treatment

Introduction

Pegylated interferon (Peg-IFN) plus ribavirin combination therapy can improve anti-viral efficacy and is currently recommended as first-line therapy for chronic hepatitis C. However, hepatitis C virus (HCV) still persists in approximately half of the genotype 1 patients treated with Peg-IFN plus ribavirin [1–4], and the number of patients who fail to achieve a sustained virologic response (SVR) consequently increases over time.

Recently, the addition of a protease inhibitor to Peg-IFN plus ribavirin combination therapy has been reported to improve the anti-viral effect, but this triple therapy increases side effects, especially severe anemia [5–7]. In Japan, HCV carriers are 10–20 years older than those in the United States and European countries, and patients who are ineligible for triple therapy exist in large numbers due to their potential tendency of having anemia. On the other hand, re-treatment with Peg-IFN plus ribavirin is a possible choice, until triple therapy becomes commercially available, for patients who have failed to show an SVR to previous anti-viral therapy, and for patients who are ineligible for triple therapy. As for re-treatment with Peg-IFN plus ribavirin, there have been only a few studies of patients who failed to show an SVR to previous Peg-IFN plus ribavirin [8–11]. Although re-treatment with Peg-IFN plus ribavirin for patients who failed to respond to previous Peg-IFN plus ribavirin is not recommended in the practice guidelines of the American Association for the Study of the Liver (AASLD) [1], there are some patients who respond to re-treatment. However, it remains obscure in which patients eradication of HCV can be successfully attained by re-treatment with Peg-IFN plus ribavirin.

In the present study, we tried to determine which patients could benefit from re-treatment and to identify the factors associated with an SVR in re-treatment.

Patients and methods

Patients

The present study was a retrospective, multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. This study was conducted with 74 chronic hepatitis C patients (genotype 1, $n = 56$, genotype 2, $n = 18$) who had previously completed Peg-IFN α-2b plus ribavirin combination therapy but had failed to attain an SVR. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcoholic liver disease, autoimmune hepatitis), or coinfection with hepatitis B or anti-human immunodeficiency virus. This study was conducted according to the ethical guidelines of the Declaration of Helsinki amended in 2008, and informed consent was obtained from each patient.

Treatment

For the previous treatment, Peg-IFN α-2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (Rebetol; Schering-Plough) was started between December 2004 and January 2008. For re-treatment with Peg-IFN plus ribavirin, Peg-IFN α-2a (Pegasys; Roche, Basel, Switzerland) plus ribavirin (Copegus; Roche) or Peg-IFN α-2b plus ribavirin was started between February 2006 and January 2009. In principle, as a starting dose, Peg-IFN was given once weekly at a dose of 180 μg of Peg-IFN α-2a and 1.5 μg/kg of Peg-IFN α-2b, and ribavirin was given at a total dose of 600–1000 mg/day based on body weight (for genotype 1, body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg; for genotype 2, body weight <60 kg, 600 mg; >60 kg, 800 mg), according to a standard treatment protocol for Japanese patients.

Dose reduction and discontinuance

Dose modification followed, as a rule, the manufacturer's drug information on the intensity of the hematologic adverse effects. The Peg-IFN α-2a and α-2b doses were reduced to 50% of the assigned dose when the neutrophil count fell below 750/mm^3 or the platelet (Plt) count fell below 8×10^4/mm^3, and the agent was discontinued when the neutrophil count fell below 500/mm^3 or the Plt count fell below 5×10^4/mm^3. Ribavirin was also reduced from 1000 to 600, 800 to 600, or 600 to 400 mg when the

hemoglobin (Hb) was below 10 g/dl, and was discontinued when the Hb was below 8.5 g/dl. Both Peg-IFN and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. No iron supplement or hematopoietic growth factors, such as epoietin alpha or granulocyte–macrophage colony stimulating factor (G-CSF), were administered.

Virologic assessment and definition of virologic response

The serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 KIU/ml; Roche Diagnostics, Branchburg, NJ, USA) and qualitatively analyzed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/ml). A rapid virologic response (RVR) was defined as undetectable serum HCV RNA level at week 4, a partial early virologic response (p-EVR) was defined as more than a 2-log decrease in HCV RNA level at week 12 compared with the baseline, a complete EVR (c-EVR) was defined as undetectable serum HCV RNA at week 12, a late virologic response (LVR) was defined as detectable serum HCV RNA at week 12 and undetectable at week 24, and an SVR was defined as undetectable serum HCV RNA at 24 weeks after the end of the treatment. Relapse was defined as undetectable serum HCV RNA at the end of the treatment but a detectable amount after the end of the treatment. For both the previous treatment and this re-treatment, patients without a p-EVR or without clearance of HCV RNA at week 24 were considered to be showing non-response (NR) and had to stop treatment. A patient who attained HCV RNA negativity during the re-treatment continued to be treated for 48 or 72 weeks according to response-guided therapy and the decision of the investigator at the participating clinical center.

Statistical analysis

Baseline data of the patients are expressed as mean ± SD or median values. In order to analyze the differences between baseline data or the factors associated with SVR, univariate analysis using the Mann–Whitney *U*-test or the χ^2 test was performed. A two-tailed p value of <0.05 was considered significant. The analysis was conducted with SPSS version 15.0J (SPSS, Chicago, IL, USA).

Results

The baseline characteristics of the patients are summarized in Table 1. Of the 56 genotype 1 patients, 32 were relapsers and 24 showed NR to previous treatment. Among the relapsers, 15 had shown a c-EVR (58%, 15/26) and 29 a p-EVR (100%, 29/29) in the previous treatment. Of the 18 genotype 2 patients, 17 were relapsers and one had shown NR to the previous treatment. Among the relapsers, 5 had shown an RVR (42%, 5/12) in the previous treatment. In the previous treatment, all patients had received Peg-IFN α-2b plus RBV combination therapy. There were no significant differences among the baseline characteristics between the previous treatment and the re-treatment in

Table 1 Baseline characteristics of patients and treatment factors in previous treatment and re-treatment

	Genotype 1						Genotype 2	
	All patients		Previous treatment relapsers		Previous treatment non-responders		All patients	
Number of patients	56		32		24		18	
Sex: male/female	32/24		19/13		13/11		11/7	
	Previous treatment	Re-treatment	Previous treatment	Re-treatment	Previous treatment	Re-treatment	Previous treatment	Re-treatment
Age (years)	57.6 ± 9.2	59.5 ± 9.4	57.8 ± 9.0	59.8 ± 9.4	57.3 ± 9.6	59.0 ± 9.5	57.4 ± 9.0	58.4 ± 1.7
White blood cells (/mm^3)	4909 ± 1404	4670 ± 1566	5117 ± 1276	4756 ± 979	4633 ± 1543	4545 ± 2178	5111 ± 1697	4412 ± 1744
Red blood cells (×10^4/mm^3)	435 ± 40	426 ± 52	444 ± 34	437 ± 36	4243 ± 46	412 ± 67	448 ± 36	447 ± 38
Hemoglobin (g/dl)	13.9 ± 1.2	13.5 ± 1.7	14.1 ± 1.1	13.8 ± 1.3	13.7 ± 1.3	13.1 ± 2.1	14.4 ± 1.2	14.2 ± 1.3
Platelets (×10^4/mm^3)	16.5 ± 6.1	17.5 ± 6.9	18.4 ± 6.6	19.1 ± 6.5	14.1 ± 4.4	15.2 ± 6.9	17.5 ± 6.3	16.2 ± 4.9
AST (IU/l)	58 ± 30	60 ± 45	55 ± 31	56 ± 44	61 ± 28	64 ± 47	52 ± 34	34 ± 13
ALT (IU/l)	74 ± 55	77 ± 74	73 ± 65	79 ± 80	74 ± 40	75 ± 66	65 ± 52	34 ± 18
Serum HCV RNA (KIU/ml)	1600	1100	1600	1100	1600	990	1300	690
Peg-IFN type: α2a/α2b	0/56	24/32	0/32	14/18	0/24	10/14	0/18	4/14

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *HCV* hepatitis C virus, *Peg-IFN* pegylated interferon

Table 2 Factors associated with a sustained virologic response (SVR) in re-treatment with Peg-IFN plus ribavirin

Factor	SVR	Non-SVR	*p* value
Number of patients	23	33	
Age (years)	59.5 ± 7.6	59.5 ± 10.5	0.55
Sex: male/female	16/7	16/17	0.17
White blood cells (/mm^3)	4778 ± 1022	4589 ± 1884	0.29
Neutrophils (/mm^3)	2446 ± 849	2291 ± 1486	0.21
Hemoglobin (g/dl)	13.6 ± 1.3	13.4 ± 1.9	0.73
Platelets (×10^4/mm^3)	18.2 ± 6.3	16.9 ± 7.3	0.28
AST (IU/l)	52 ± 33	65 ± 52	0.46
ALT (IU/l)	75 ± 61	79 ± 82	0.72
Serum HCV RNA: <5log/5log≤	6/15	0/31	<0.01
Peg-IFN type: α2a/α2b	7/16	17/16	0.27
Peg-IFN dose (μg/kg/week)			
α2a	2.64 ± 0.61	2.73 ± 0.72	0.90
α2b	1.18 ± 0.43	1.19 ± 0.34	0.90
Ribavirin dose (mg/kg/day)	8.6 ± 2.9	9.4 ± 2.7	0.28
1st treatment virologic response			
p-EVR; +/−	22/0	14/16	<0.001
Relapse/NR	20/3	12/21	<0.001

p-EVR partial early virologic response, *NR* non-response

terms of peripheral blood cell counts, or the levels of aminotransaminases and serum HCV RNA at the start of treatment.

In genotype 1 patients, the HCV RNA negative rate on re-treatment was 54% (29/54) at week 12 and 71% (40/56) at week 24, and the SVR rate was 41% (23/56). The factors associated with SVR were assessed by univariate analysis for the following variables; age, gender, peripheral blood cell counts, aminotransferases, previous treatment response, serum HCV RNA level, the type of Peg-IFN in re-treatment, and drug adherence (Table 2). As a result, the factors of previous treatment response and serum HCV RNA level at the start of re-treatment were selected as being significant. In examining the efficacy of the re-treatment according to the previous treatment response, the relapsers in the previous treatment had a significantly higher HCV RNA negative rate at weeks 12 and 24 and a significantly higher SVR rate than those with NR in the previous treatment (Fig. 1a). Patients with a p-EVR in the previous treatment showed similar results, while no patient without p-EVR in the previous treatment attained an SVR on re-treatment (0/16) (Fig. 1b). Even among the patients without HCV RNA negativity in the previous treatment, if p-EVR had been attained in the previous treatment, 43% (3/7) of these patients attained an SVR on re-treatment. As for the serum HCV RNA level at the start of re-treatment, all patients with less than 5 $\log_{10}$ IU/ml of HCV RNA attained an SVR (6/6), and 33% (15/45) of those patients with more than 5 $\log_{10}$ IU/ml of HCV RNA attained an SVR ($p < 0.01$).

In examining the efficacy of re-treatment according to treatment duration, among the patients with c-EVR and without RVR on re-treatment, those who were re-treated for 72 weeks tended to attain higher SVR rates than those who were re-treated for 48 weeks (72 weeks, 75%, 9/12, vs. 48 weeks, 25%, 2/8, $p = 0.06$). On the other hand, 43% (3/7) of the patients with an LVR on re-treatment attained an SVR on re-treatment. Among the patients with relapse

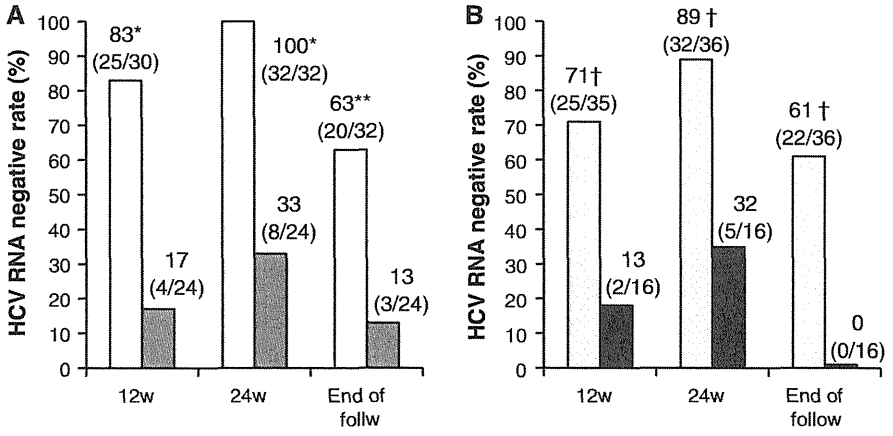

Fig. 1 Virologic response on re-treatment according to previous treatment response. **a** Hepatitis C virus (HCV) RNA negative rate on re-treatment according to relapse or non-response in previous treatment. **b** HCV RNA negative rate on re-treatment according to partial early virologic response (p-EVR) or non-p-EVR in previous treatment. *White bars* patients with relapse in previous treatment. *Dark gray bars* patients with non-response in previous treatment. *Light gray bars* patients with p-EVR in previous treatment. *Black bars* patients with non-p-EVR in previous treatment. *$p < 0.0001$; **$p < 0.01$; compared to non-response. †$p < 0.001$; compared to patients without p-EVR