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Influence of low-density lipoprotein cholesterol on virological response to telaprevir-based triple therapy for chronic HCV genotype 1b infection

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ABSTRACT

Elevated serum low-density lipoprotein cholesterol (LDL-C) level has been associated with sustained virological response (SVR) by chronic hepatitis C patients treated with pegylated-interferon (PEG-IFN) α and ribavirin (RBV). The aim of this study was to investigate the relation between the baseline LDL-C level and the treatment outcome from telaprevir (TVR)-based triple therapy. This prospective, multi-center study consisted of 241 treatment-experienced patients infected with HCV genotype 1b. All received 12 weeks of TVR in combination with 24 weeks of PEG-IFNα2b and RBV. The SVR rate was 81.3% (196 of 241) by intention-to-treat analysis. Higher LDL-C level was strongly associated with SVR ($P = 1.3 \times 10^{-8}$). The area under the receiver operating characteristic curve for predicting SVR was 0.78 and the cutoff value for the LDL-C level at baseline was 95 mg/dL. In multivariable logistic regression analysis of predictors of SVR, LDL-C ⩾95 mg/dL (odds ratio [OR] 3.60, $P = 0.0238$), α-fetoprotein ⩽5.0 ng/mL (OR 5.06, $P = 0.0060$), prior relapse to PEG-IFNα and RBV (OR 5.71, $P = 0.0008$), and rapid virological response (HCV RNA undetectable at week 4) (OR 5.52, $P = 0.0010$) were extracted as independent predictors of SVR. For prior partial and null responders, the SVR rates of the groups with LDL-C ⩾95 mg/dL were significantly higher than those of the <95 mg/dL groups with IL28B TG/GG and pretreatment platelet count $<150 \times 10^9$/L (both $P < 0.05$). The baseline LDL-C level exerted a potent influence on the SVR of treatment-experienced patients treated with TVR-based triple therapy, especially for prior partial and null responders to PEG-IFNα and RBV.

1. Introduction

Approximately 170 million people worldwide are infected with the hepatitis C virus (HCV) (Lavanchy, 2009), and chronic hepatitis C is the leading cause of hepatocellular carcinoma (HCC) in Japan (Kiyosawa et al., 2004). The number of older aged patients is high in Japan, which gives a preview of the problems the U.S. and Europe will experience as patients in these two areas age and more closely fit the Japanese profile (Bosch et al., 2004). Sustained virological response (SVR) has been associated with a lower risk of HCC (Ogawa et al., 2013a). However, a weakness of the standard of care with pegylated interferon (PEG-IFN) α and ribavirin (RBV) is that it results in a rate of SVR of only about

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40% in patients with HCV genotype 1 (Fried et al., 2002; McHutchison et al., 2009).

Newly developed treatments with direct-acting antivirals (DAAs), nonstructural 3/4A protease inhibitors such as telaprevir (TVR) and boceprevir, have shown promising outcomes in combination with PEG-IFNα/RBV in clinical trials (Jacobson et al., 2011; Zeuzem et al., 2011; Poordad et al., 2012). The SVR rate is improved to over 70% for HCV genotype 1 patients treated with TVR-based triple therapy (Jacobson et al., 2011; Zeuzem et al., 2011; Furusyo et al., 2013). Many factors associated with response to dual therapy do not appear to be significant predictors of response to TVR-based triple therapy from post hoc analyses of phase 3 trials. On the other hand, low fibrosis stage and high serum low-density lipoprotein cholesterol (LDL-C) level are significant pretreatment predictors of SVR for patients given the TVR-based triple therapy (Berg et al., 2011; Serfaty et al., 2012). Although previous studies reported that the β-lipoprotein level is associated with the treatment outcome of patients receiving dual therapy (Sheridan et al., 2009; Harrison et al., 2010; Clark et al., 2012), our recent studies suggest that interleukin-28 (IL28) B polymorphisms and insulin resistance are more significantly correlated with treatment outcome than is the LDL-C level (Furusyo et al., 2011, 2012; Ogawa et al., 2012, 2013c). Unfortunately, little data from clinical practice is available on the association between β-lipoproteins and the treatment outcome of patients given TVR-based triple therapy.

The aim of this study was to investigate the relation between baseline characteristics including serum lipid levels and the treatment outcome from TVR-based triple therapy, in a clinical practice setting.

2. Materials and methods

2.1. Patients

The Kyushu University Liver Disease Study (KULDS) Group consists of Kyushu University Hospital and its affiliated hospitals in the northern Kyushu area of Japan. This prospective study consisted of 241 treatment-experienced patients with chronic HCV infection aged 20 years or older who received TVR in combination with PEG-IFNα2b and RBV. All initiated treatment between December 2011 and May 2012 and completed treatment by the end of November 2012. Exclusion criteria were: (1) positivity for antibody to human immunodeficiency virus or positivity for hepatitis B surface antigen; (2) clinical or biochemical evidence of hepatic decompensation (ascites, bleeding varices, or encephalopathy); (3) other causes of liver disease (autoimmune hepatitis or primary biliary cirrhosis); (4) excessive active alcohol consumption (a daily intake of more than 40 g of ethanol) or drug abuse; (5) suspected HCC at entry; or (6) treatment with statin, ezetimibe or immunosuppressive agents prior to enrollment. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of each participating hospital. Informed consent was obtained from all patients before enrollment.

2.2. Clinical and laboratory assessment

All blood samples were collected after at least a 12 h overnight fast. Clinical parameters were measured by standard laboratory techniques at a commercial laboratory (SRL Laboratory, Tokyo, Japan). Body mass index was calculated as weight in kilograms/height in meters squared. The estimated glomerular filtration rate was calculated based on the Modification of Diet in Renal Disease formula. Aspartate aminotransferase to platelet ratio index (APRI) was calculated, as previously recommended for evaluating severe liver fibrosis (Wai et al., 2003).

Human genomic DNA was extracted from peripheral blood. The allele of the single nucleoside polymorphism (SNP) rs8099917, near the IL28B gene, was determined using the ABI TaqMan allelic discrimination kit (7500 Real Time PCR System; Applied Biosystems, Carlsbad, CA, USA) (Tanaka et al., 2009).

2.3. Assessment of liver fibrosis

Liver biopsy for 149 (61.8%) of the studied patients was done by experienced hepatologists. All antiviral treatment was initiated within 1 month after liver biopsy. The minimum length of the liver biopsy was 15 mm and at least 10 complete portal tracts were necessary for inclusion. The stage of fibrosis of each specimen was assigned according to the METAVIR score (Bedossa and Poynard, 1996).

2.4. Antiviral treatment

All patients received a combination treatment of TVR (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan), PEG-IFNα2b (PEG-Intron; MSD, Tokyo, Japan), and RBV (Rebetol; MSD) for 12 weeks, followed by an additional 12 weeks of PEG-IFNα2b and RBV alone. TVR 750 mg was administrated three times a day at an 8-h interval, after each meal. PEG-IFNα2b was injected subcutaneously once weekly at a dose of 1.5 μg/kg. RBV was given orally at a daily dose of 600–1000 mg based on body weight. The treatment regime and duration are those approved by the Japanese Ministry of Health, Labor, and Welfare. If marked anorexia, an elevation of serum creatinine, or severe anemia developed, the TVR dose could be reduced to 1500 mg/day (750 mg at a 12-h interval, after meals). The method of RBV/TVR dose reduction in the case of anemia was as recently reported (Ogawa et al., 2013b). All treatment was discontinued by patients with a less than $1\log_{10}$ IU/mL HCV RNA decrease from baseline to week 4, with a less than $2\log_{10}$ IU/mL HCV RNA decrease from baseline to week 12.

2.5. HCV RNA level and virological assessment

Clinical follow-up of HCV viremia was done by real-time reverse transcriptase PCR assay (COBAS® TaqMan® HCV assay) (Roche Diagnostics, Tokyo, Japan), with a lower limit of quantitation of 15 IU/mL and an outer limit of quantitation of 6.9×10^7 IU/mL (1.2–7.8 log IU/mL referred to $\log_{10}$ IU/mL). HCV RNA levels were measured at baseline, regularly during treatment, at early discontinuation, and at follow-up visits after the end of treatment. HCV genotype determination was by sequence determination in the 5′-nonstructual region of the HCV genome followed by phylogenetic analysis (Simmonds et al., 1994).

Virological response to the TVR-based triple therapy was categorized as follows: RVR, an undetectable HCV RNA at week 4: SVR, undetectable HCV RNA at week 24 after the end of treatment. Prior treatment response to PEG-IFNα and RBV was categorized as follows: Relapse, relapse of serum HCV RNA by patients whose HCV RNA level was undetectable at the end of treatment or the re-appearance of HCV RNA at any time during treatment after virological response: non-virological response, a decrease in the HCV RNA level of less than $2\log_{10}$ IU/mL at week 12 (null response) or a more than $2\log_{10}$ IU/mL decrease in the HCV RNA level from baseline at week 12 but detectable HCV RNA at weeks 12 and 24 (partial response).

2.6. Statistical analysis

Statistical analyses were conducted using SPSS Statistics 19.0 (IBM SPSS Inc., Chicago, IL, USA). Baseline continuous data are expressed as median (first–third quartiles) and categorical variables

are reported as frequencies and percentages. Univariate analyses were done using the Chi-square, Fisher's Exact, Mann–Whitney *U* tests, or analysis of variance (ANOVA) as appropriate. Multivariate logistic regression analyses were done using dichotomous variables with $P \leqslant 0.05$ in univariate analysis to identify variables significantly associated with SVR, RVR, and LDL-C $\geqslant$95 mg/dL. The results are expressed as odds ratios (OR) and their 95% confidence interval (CI).

Area under the receiver operating characteristic curve (AUROC) analysis was done to evaluate the relationship between the LDL-C level and SVR. The cutoff values were selected from the receiver operating characteristic (ROC) curve to maximize the total sensitivity and specificity. A *P* value less than 0.05 was regarded as statistically significant in all analyses.

3. Results

3.1. Patient characteristics and predictors of SVR

The baseline characteristics of the 241 patients as classified by the previous treatment outcome are shown in Table 1. All patients were infected with HCV genotype 1b. We were able to determine the allele of the IL28B SNPs (rs8099917) of 236 patients (97.9%). The percentages of patients with the IL28B TT, TG, and GG genotypes were 64.8% (n = 153), 34.3% (n = 81), and 0.8% (n = 2), respectively. The baseline LDL-C level of the prior relapse group was significantly higher than those of the prior partial and null response groups, and the baseline HCV RNA level and the rate of IL28B TG/GG (minor alleles) of the prior partial and null response groups were higher than that of the prior relapse group.

The overall SVR rate was 81.3% (196 of 241) by intention-to-treat analysis. Univariate analysis identified lower age (P = 0.0032), higher serum albumin (P = 0.0014), higher total cholesterol (P = 0.0003), higher LDL-C ($P = 1.3 \times 10^{-8}$), lower α-fetoprotein (P = 0.0042), lower APRI (P = 0.0217), higher hemoglobin (P = 0.0396), higher platelet count (P = 0.0096), no or mild fibrosis stage (P = 0.0079), IL28B TT genotype ($P = 5.5 \times 10^{-9}$), prior relapse ($P = 3.8 \times 10^{-14}$), and RVR ($P = 1.7 \times 10^{-10}$) as significantly associated with SVR (Table 2).

Using the dichotomous variables with the population medians instead of continuous variables, multivariate logistic regression analysis of possible predictors of SVR is shown in Table 3. Fibrosis (METAVIR score) was not included in this model because 38.2% of the values were missing. Significant independent pretreatment predictors were LDL-C $\geqslant$95 mg/dL (OR, 3.81; 95%CI, 1.35–11.99; P = 0.0111), α-fetoprotein $\leqslant$5.0 ng/mL (OR, 6.78; 95%CI, 2.21–24.50; P = 0.0005), IL28B TT genotype (OR, 2.56; 95%CI, 1.10–7.13; P = 0.0357), and prior relapse (OR, 7.28; 95%CI, 2.83–20.14; $P = 6.5 \times 10^{-5}$). By adding the on-treatment variable (RVR) to the analysis, LDL-C $\geqslant$95 mg/dL (OR, 3.60; 95%CI, 1.18–12.24; P = 0.0238), α-fetoprotein $\leqslant$5.0 ng/mL (OR, 5.06; 95%CI, 1.57–19.11; P = 0.0060), prior relapse (OR, 5.71; 95%CI, 2.05–17.02; P = 0.0008), and RVR (OR, 5.52; 95%CI, 1.93–13.84; P = 0.0010) were extracted.

3.2. Usefulness of LDL-C level in the prediction of treatment outcome

ROC analysis was performed to determine the optimal threshold values for the LDL-C level at baseline for predicting SVR by the 241 treatment-experienced patients. The corresponding AUROC was 0.78 and the cutoff value for the LDL-C level at baseline was 95 mg/dL (sensitivity 62.8%, specificity 84.5%, positive predictive value 94.6%, negative predictive value 34.2%). The SVR rates by use of a LDL-C cutoff level of 95 mg/dL of groups stratified by prior treatment efficacy are shown in Fig. 1. We also show the SVR rates by use of a LDL-C cutoff level of 95 mg/dL of groups created by IL28B SNPs and prior treatment efficacy (Fig. 2A) and of groups created by pretreatment platelet count ($\geqslant$150 and <150 $\times 10^9$/L) and

Table 1
Baseline clinical and laboratory findings stratified by previous treatment outcome.

Baseline characteristics	Relapse n = 161	Partial response n = 44	Null response n = 36	P value
Age (years)	63 (56–66)	62 (54–67)	63 (58–69)	0.2515
Male sex, n (%)	74 (46.0)	21 (47.7)	17 (47.2)	0.9740
Body weight (kg)	60 (52–67)	56 (50–64)	60 (53–68)	0.6189
Body mass index (kg/m^2)	23.3 (21.4–25.6)	22.1 (20.5–25.1)	23.0 (21.4–24.6)	0.4960
Serum albumin (g/L)	40 (37–43)	40 (38–43)	39 (37–41)	0.3758
Alanine aminotransferase (IU/L)	45 (30–78)	48 (36–88)	58 (49–89)	0.3668
γ-Glutamyl transpeptidase (IU/L)	34 (22–61)	39 (26–79)	64 (31–97)	0.2880
Estimated glomerular filtration rate (mL/min/1.73 m^2)	79 (71–91)	82 (74–95)	81 (73–91)	0.8314
Total cholesterol (mg/dL)	173 (149–199)	166 (152–192)	158 (141–178)	0.0579
Low-density lipoprotein cholesterol (mg/dL)	101 (82–116)	88 (78–109)	80 (69–92)	7.6×10^{-5}
High-density lipoprotein cholesterol (mg/dL)	47 (40–57)	50 (38–59)	51 (40–64)	0.4062
Triglycerides (mg/dL)	95 (72–125)	96 (74–126)	106 (72–147)	0.7946
Fasting plasma glucose (mg/dL)	94 (87–104)	95 (86–101)	97 (89–105)	0.9980
α-Fetoprotein (ng/mL)	5.4 (3.4–10.8)	4.9 (3.0–11.9)	11.8 (5.6–20.3)	0.2872
APRI	0.76 (0.44–1.24)	0.77 (0.43–1.67)	1.05 (0.59–1.76)	0.1070
Hemoglobin (g/L)	137 (128–147)	139 (123–151)	139 (129–153)	0.8973
Platelet count ($\times 10^9$/L)	155 (124–190)	163 (113–203)	134 (115–168)	0.1989
HCV RNA level ($\log_{10}$ IU/mL)	6.4 (5.9–6.8)	6.6 (6.1–6.7)	6.7 (6.2–7.1)	0.0030
Diabetes, n (%)	27 (16.8)	8 (18.2)	6 (16.7)	0.9745
Stage of fibrosis				
F0–2, n (%)	63 (64.3)	17 (65.4)	14 (56.0)	0.7235
F3–4, n (%)	35 (35.7)	9 (34.6)	11 (44.0)	
Not determined, n	63	18	11	
IL28B (rs8099917) genotype				
TT, n (%)	121 (77.1)	23 (53.5)	9 (25.0)	5.5×10^{-8}
TG, n (%)	36 (22.9)	19 (44.2)	26 (72.2)	
GG, n (%)	0	1 (2.3)	1 (2.8)	
Not tested, n	4	1	0	

Data are expressed as number (%) or median (first–third quartiles).
APRI, aspartate aminotransferase to platelet ratio index.

Table 2
Baseline and on-treatment predictors of SVR by chronic hepatitis C patients treated with telaprevir-based triple therapy.

Baseline characteristics	SVR n = 196	Non-SVR n = 45	P value
Age (years)	62 (54–66)	65 (60–69)	0.0032
Male sex, n (%)	94 (48.0)	18 (40.0)	0.3326
Body weight (kg)	60 (52–66)	60 (51–70)	0.6043
Body mass index (kg/m^2)	23.0 (21.0–25.4)	23.4 (21.7–25.6)	0.1963
Serum albumin (g/L)	40 (37–43)	39 (35–40)	0.0014
Alanine aminotransferase (IU/L)	45 (30–80)	58 (48–89)	0.4305
γ-Glutamyl transpeptidase (IU/L)	37 (22–68)	46 (29–90)	0.8248
Estimated glomerular filtration rate (mL/min/1.73 m^2)	81 (72–92)	78 (72–90)	0.6983
Total cholesterol (mg/dL)	171 (152–199)	157 (142–174)	0.0003
Low-density lipoprotein cholesterol (mg/dL)	100 (82–116)	76 (67–88)	1.3×10^{-8}
High-density lipoprotein cholesterol (mg/dL)	48 (40–57)	51 (39–64)	0.4633
Triglycerides (mg/dL)	95 (71–126)	103 (79–127)	0.8486
Fasting plasma glucose (mg/dL)	95 (86–103)	97 (87–105)	0.9431
α-Fetoprotein (ng/mL)	4.8 (3.2–8.9)	13.8 (6.5–30.8)	0.0009
APRI	0.75 (0.44–1.21)	1.25 (0.59–1.82)	0.0217
Hemoglobin (g/L)	138 (129–149)	135 (123–149)	0.0396
Platelet count ($\times 10^9$/L)	157 (126–195)	133 (97–176)	0.0096
HCV RNA level ($\log_{10}$ IU/mL)	6.5 (6.0–6.9)	6.5 (6.2–6.9)	0.0567
Diabetes, n (%)	33 (16.8)	8 (17.8)	0.8801
Stage of fibrosis			
F0–2, n (%)	82 (68.3)	12 (41.4)	0.0079
F3–4, n (%)	38 (31.7)	17 (58.6)	
Not determined, n	76	16	
IL28B (rs8099917) genotype			
TT, n (%)	141 (73.8)	12 (26.7)	5.5×10^{-9}
TG, n (%)	50 (26.2)	31 (68.9)	
GG, n (%)	0	2 (4.4)	
Not tested, n	5	0	
Prior treatment response			
Relapse, n (%)	151 (77.0)	10 (22.2)	3.8×10^{-14}
Partial response, n (%)	33 (16.8)	11 (24.4)	
Null response, n (%)	12 (6.1)	24 (53.3)	
On-treatment parameter			
RVR, n (%)	165 (84.2)	16 (35.6)	1.7×10^{-10}

Data are expressed as number (%) or median (first–third quartiles).
SVR, sustained virological response; APRI, aspartate aminotransferase to platelet ratio index; RVR, rapid virological response (undetectable HCV RNA at week 4).

Table 3
Univariate and multivariate logistic regression analysis of possible predictors of SVR.

Univariate analysis			Multivariate analysis			
			Pretreatment parameters		Pretreatment parameters and RVR	
Pretreatment parameters	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age (years) <65 vs. ⩾65	2.01 (1.04–3.89)	0.0367				
Serum albumin (g/L) <40 vs. ⩾40	1.82 (0.95–3.55)	0.0703				
LDL-C (mg/dL) ⩾95 vs. <95	8.57 (3.85–21.85)	1.1×10^{-8}	3.81 (1.35–11.99)	0.0111	3.60 (1.18–12.24)	0.0238
α-Fetoprotein (ng/mL) <5.0 vs. ⩾5.0	8.04 (3.27–24.27)	7.0×10^{-7}	6.78 (2.21–24.50)	0.0005	5.06 (1.57–19.11)	0.0060
APRI <0.8 vs. ⩾0.8	2.99 (1.50–6.25)	0.0017				
Hemoglobin (g/L) ⩾135 vs. <135	1.63 (0.85–3.15)	0.1416				
Platelet count ($\times 10^9$/L) ⩾150 vs. <150	2.15 (1.12–4.25)	0.0221				
IL28B SNP (rs8099917) TT vs. TG/GG	7.76 (3.81–16.74)	4.8×10^{-9}	2.56 (1.10–7.13)	0.0357		
Prior response Relapse vs. NVR	11.74 (5.58–26.77)	5.6×10^{-12}	7.28 (2.83–20.14)	6.5×10^{-5}	5.71 (2.05–17.02)	0.0008
On-treatment parameter Virological response RVR vs. non-RVR	9.65 (4.76–20.26)	1.7×10^{-10}			5.52 (1.93–13.84)	0.0010

SVR, sustained virological response; OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; APRI, aspartate aminotransferase to platelet ratio index; SNP, single nucleoside polymorphism; NVR, non-virological response (including prior partial and null response); RVR, rapid virological response (undetectable HCV RNA at week 4).

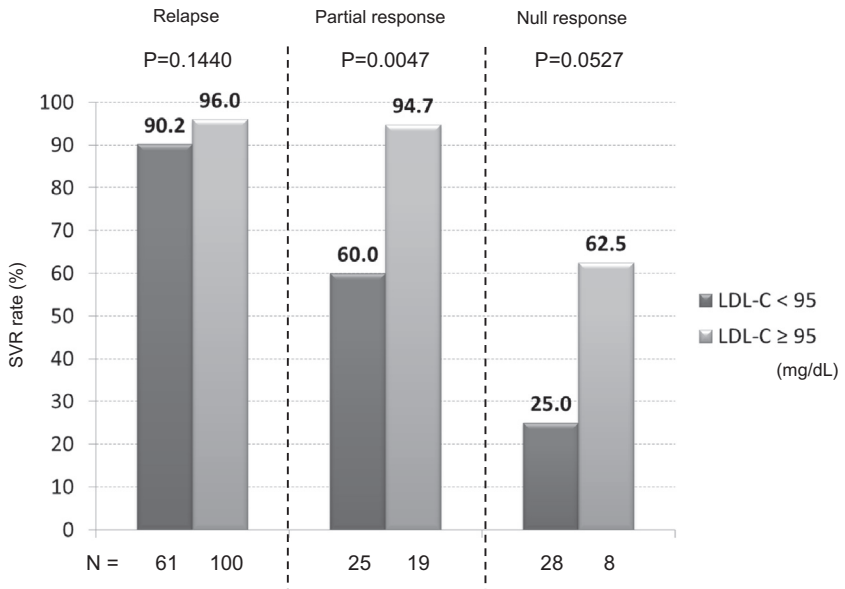

Fig. 1. Sustained virological response (SVR) rates by use of a low-density lipoprotein cholesterol (LDL-C) cutoff level of 95 mg/dL of groups stratified by prior treatment efficacy.

prior treatment efficacy (Fig. 2B). For patients with prior relapse, the SVR rates of the groups with LDL-C <95 mg/dL were similar to those of the ⩾95 mg/dL groups with any IL28B SNPs and pretreatment platelet count (⩾150 and <150 × 10^9/L). Conversely, for patients with prior partial or null response, the SVR rates of the groups with LDL-C ⩾95 mg/dL were significantly higher than those of the <95 mg/dL groups with IL28B TG/GG and pretreatment lower platelet count (<150 × 10^9/L) (both $P < 0.05$).

3.3. Pretreatment factors associated with RVR

181 of 241 (75.1%) patients achieved RVR. Univariate analysis identified higher LDL-C level ($P = 0.0007$), lower APRI ($P = 0.0349$), higher platelet count ($P = 0.0056$), lower HCV RNA level ($P = 5.7 \times 10^{-5}$), no or mild fibrosis ($P = 0.0230$), IL28B TT genotype ($P = 0.0005$), and prior relapse ($P = 4.4 \times 10^{-8}$) as significantly associated with RVR (Supplementary Table 1). In multivariate logistic regression analysis using the dichotomous variables (Supplementary Table 2), platelet count ⩾150 × 10^9/L (OR, 2.19; 95%CI, 1.13–4.34; $P = 0.0201$), HCV RNA ⩽6.5 log IU/mL (OR, 2.13; 95%CI, 1.05–4.42; $P = 0.0367$), and prior relapse (OR, 3.16; 95%CI, 1.57–6.42; $P = 0.0013$) were significantly associated with RVR. Unlike SVR, LDL-C was not extracted as an independent predictor of RVR.

3.4. Pretreatment factors associated with LDL-C ⩾95 mg/dL

Many factors, including higher serum albumin level ($P = 0.0065$), lower α-fetoprotein ($P = 0.0008$), lower APRI ($P = 0.0031$), higher hemoglobin ($P = 0.0426$), higher platelet count ($P = 0.0006$), no or mild fibrosis ($P = 0.0024$), IL28B TT genotype ($P = 6.2 \times 10^{-8}$), and prior relapse ($P = 2.2 \times 10^{-5}$) were associated with LDL-C ⩾95 mg/dL in the univariate analysis (Supplementary Table 3). In the multivariate logistic regression analysis using the dichotomous variables (Supplementary Table 4), APRI ⩽0.8 (OR, 2.22; 95%CI, 1.03–4.87; $P = 0.0434$), IL28B TT genotype (OR, 5.15; 95%CI, 2.58–10.65; $P = 5.3 \times 10^{-6}$), and prior relapse (OR, 2.11; 95%CI, 1.05–4.26; $P = 0.0355$) were extracted as independently associated with LDL-C ⩾95 mg/dL.

4. Discussion

This prospective, multicenter study is a comprehensive evaluation of the predictors of the treatment efficacy of TVR in combination with PEG-IFNα2b and RBV for chronic HCV genotype 1 infection in treatment-experienced patients. According to previous clinical trials that included treatment-naïve and -experienced patients, TVR-based triple therapy has resulted in significantly improved SVR rates in comparison with dual therapy (Jacobson et al., 2011; Zeuzem et al., 2011). Although host and viral factors associated with the outcome of TVR-based triple therapy have been presented in clinical trials (Berg et al., 2011; Serfaty et al., 2012), little data has been reported in clinical practice. This multicenter study was carried out to address the lack of data, including serum lipid levels, in clinical practice.

Baseline LDL-C ⩾95 mg/dL, α-fetoprotein ⩽5.0 ng/mL, prior response to PEG-IFNα and RBV, and RVR were independent predictors of SVR in multivariate logistic regression analysis. The α-fetoprotein level was the most relevant to advanced fibrosis ($r = 0.43$, $P \leqslant 0.0001$) among all variables. Although LDL-C ⩾95 mg/dL was strongly associated with SVR, it was not extracted as an independent predictor of RVR. A study of treatment-naïve patients had similar findings to ours in terms of the relationship between LDL-C level and virological response (Serfaty et al., 2012). Of note, the LDL-C level had a strong impact on treatment outcome, especially for patients with prior partial and null response, irrespective of IL28B SNPs and platelet count (⩾150 and <150 × 10^9/L). The treatment strategy for patients with prior partial and null response is an important issue in this new era of DAAs, therefore, the LDL-C level provides a potential tool for making decisions as to patient suitability for TVR-based triple therapy.

According to previous studies, a high LDL-C level is an important predictor of SVR by dual therapy for HCV genotype 1 patients (Sheridan et al., 2009; Harrison et al., 2010). Thus, factors involved in the regulation of LDL-C density may affect the interaction of HCV. In the functional mechanism of the relationship between high LDL-C level and preferable treatment outcome, the low-density lipoprotein receptor (LDL-R) is thought to play a significant role. LDL-R is an HCV entry factor, along with CD81, scavenger receptor class B member I, claudin-1, and occluding (Meredith et al., 2012).

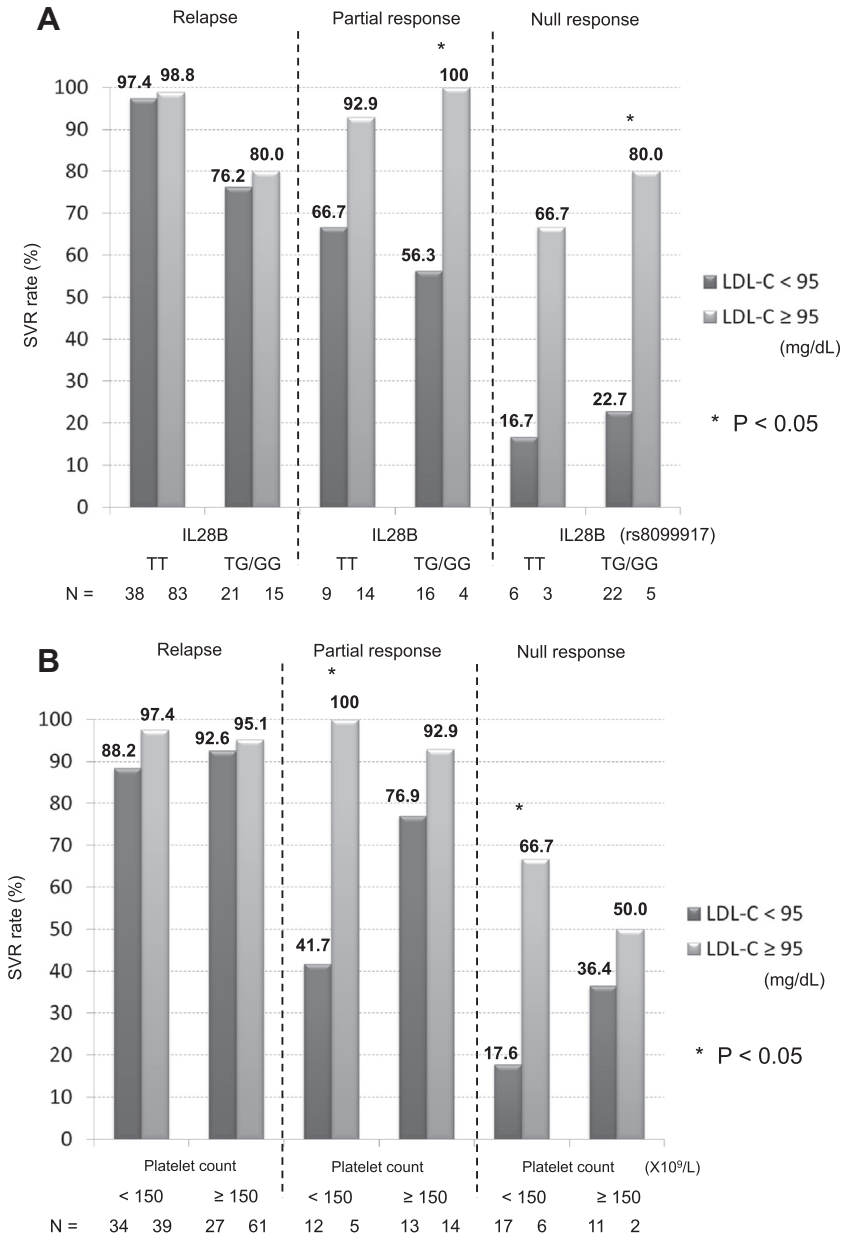

Fig. 2. Sustained virological response (SVR) rates according to a low-density lipoprotein cholesterol (LDL-C) cutoff level of 95 mg/dL among groups matched by IL28B genotype (rs8099917) and prior treatment efficacy (A) and by pretreatment platelet count and prior treatment efficacy (B).

Moreover, HCV is thought to enter cells by endocytosis and fusion between the virion envelope and the endosomal membrane (Agnello et al., 1999). As a result of the competition for the binding of LDL-R and endocytosis, LDL-C may be able to block the attachment and entry of HCV. A recent *in vitro* study suggested that another lipoprotein receptor, Niemann–Pick C1-like 1 (NPC1L1), might also play a role in HCV cell entry (Sainz et al., 2012). Ezetimibe, an NPC1L1 antagonist that has been approved worldwide as a cholesterol-lowering treatment, is a strong inhibitor of HCV uptake *in vitro* via a virion cholesterol-dependent step before virion-cell membrane fusion. These findings support β-lipoproteins as competitively inhibiting the infection of hepatocytes with HCV through LDL-R and NPC1L1.

IL28B SNPs have been associated with not only response to IFN treatment but also to β-lipoproteins (Li et al., 2010). The favorable IL28B alleles (rs8099917 TT and rs12979860 CC) have been significantly associated with higher levels of LDL-C and apolipoprotein B, as was also shown in this study. More interesting is that a significant association between the IL28B SNP (rs12979860) and LDL-C was found in only HCV genotype 1 patients, but not in genotypes 3 and 4 (Rojas et al., 2014). Rojas et al. also showed the interaction of lipid metabolism and HCV gene expression *in vivo* and *in vitro*. Although we presented only one IL28B SNP (rs8099917) in this study, we recently reported that rs8099917 and rs12979860 were in linkage disequilibrium in almost all Japanese patients (99.4%, 326 of 328) (Ogawa et al., 2012). According to another Japanese study, only 4 of 416 (1.0%) patients were not in linkage disequilibrium for these two SNPs (Ito et al., 2011). Therefore, our data on the LDL-C levels are probably correlated to the data shown by Rojas et al.

The state of high endogenous IFN activation is considered to be caused by altered lipid profiles, characterized by low LDL-C levels, because a high IFNγ-inducible-protein-10 kDa (IP10) level as a quantifiable serum marker for overall IFN-stimulated gene (ISG) activation was correlated with low LDL-C levels (Sheridan et al., 2012). Further studies of the mechanisms of the interaction

between IL28B and ISGs on lipid pathways are needed, however, the finding that LDL-C is one of the most important pretreatment biochemical and metabolic markers for predicting SVR by patients treated with DAAs is important.

There are several limitations to our study. First, the liver histology data is inadequate because 38% of the data were unavailable. Therefore, it remains unclear whether or not there is significant bias on SVR due to different rates of patients with advanced fibrosis. Instead, our study stratified patients by platelet count to discriminate between likely mild fibrosis ($\geqslant 150 \times 10^9$/L) and advanced fibrosis ($<150 \times 10^9$/L). Notably, the baseline serum LDL-C level had a strong impact on treatment outcome, especially for prior partial and null responders with low platelet count ($<150 \times 10^9$/L). Second, our study consisted of Japanese only. Further studies of other ethnic groups are needed to confirm if our findings are true for other populations. Nevertheless, we provided significant information because our study included patients of older age, different from patient profiles in the U.S. and Europe, but possibly representing the future of these regions. It is not particularly a limitation that our study has a high percentage of patients with IL28B TT (rs8099917) despite the retreatment of chronic HCV infection. IL28B SNPs could improve the diagnostics of the response to antiviral treatment, but these genetic factors are strongly associated with negative predictive value (D'Avolio et al., 2012), therefore, SVR was not always achieved because of the influence on other factors, such as insulin resistance (Ogawa et al., 2012) or RBV plasma concentration (Rendón et al., 2005; Furusyo et al., 2011; D'Avolio et al., 2012), even if patients had preferable IL28B SNPs. Interestingly, recent reports have shown that TVR significantly raises the RBV plasma concentration (Boglione et al., 2013; Karino et al., 2013), although the impact of RBV concentration on treatment outcome in TVR-based triple therapy remains unclear.

In conclusion, treatment with TVR in combination with PEG-IFNα2b and RBV remarkably improves the SVR rate of treatment-experienced patients infected with HCV genotype 1b. In this prospective, multicenter study that was carried out in a clinical practice setting, baseline serum LDL-C level exerted a strong influence on treatment outcome to TVR-based triple therapy regardless of the IL28B polymorphism and platelet count, especially for prior partial and null responders. The LDL-C level may be a helpful tool for making decisions related to the suitability of TVR-based triple therapy for patients with intractable chronic hepatitis C.

5. Disclosures

The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2014.01.004.

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