【 ORIGINAL ARTICLE 】

Diabetes Mellitus Prevents an Improvement in the Serum Albumin Level During Interferon-free Sofosbuvir-based Therapy for Chronic Hepatitis C Patients: A Multi-institutional Joint Study

Akira Kawano[1], Hirohisa Shigematsu[1], Koichiro Miki[1], Yasunori Ichiki[2], Chie Morita[3],
Kimihiko Yanagita[4], Kazuhiro Takahashi[5], Kazufumi Dohmen[6], Hideyuki Nomura[7],
Hiromi Ishibashi[8] and Shinji Shimoda[9]

Abstract:

Objective Interferon-free regimens of direct-acting antiviral agents have improved the treatment response for chronic hepatitis C virus (HCV) infection, and improvement in the serum albumin level during interferon-free therapy has been reported. The aim of this study was to identify the factors that influence the improvement in the serum albumin level in patients receiving interferon-free antiviral therapy.

Methods This retrospective, multicenter study consisted of 471 Japanese patients with chronic hepatitis and compensated liver cirrhosis infected with HCV who completed 12-week interferon-free sofosbuvir (SOF)-based therapy [SOF plus ledipasvir for genotype 1 (n=276) and SOF with ribavirin for genotype 2 (n=195)]. We evaluated the changes in the serum albumin level from baseline to the end of treatment (ΔAlb).

Results When compared with the normal-albumin group (baseline serum albumin >35 g/L, n=406), the low-albumin group (baseline serum albumin ≤35 g/L, n=65) showed a significant increase in the mean ΔAlb (5.5 g/L vs. 1.0 g/L, p<0.001). In the low-albumin group, a multivariate logistic regression analysis extracted diabetes mellitus as a negative predictive factor of median ΔAlb >5.0 g/L (odds ratio: 0.19, 95% confidence interval: 0.048-0.79, p=0.020). In the low-albumin group, the mean ΔAlb was significantly lower in the diabetic patients (n=14) than in the non-diabetic patients (n=51) (3.9 g/L and 5.7 g/L, p=0.049).

Conclusion Interferon-free SOF-based therapy significantly improved the serum albumin in the low-albumin group patients with chronic HCV infection. However, the improvement in the serum albumin level was significantly lower in the diabetic patients than in the non-diabetic patients.

Key words: Child-Pugh score, direct-acting antiviral drugs, interferon-free, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis

(Intern Med 57: 1533-1542, 2018)
(DOI: 10.2169/internalmedicine.9857-17)

Introduction

Hepatitis C virus (HCV) infection is a major cause of liver cirrhosis, hepatocellular carcinoma (HCC) and end-stage liver disease (1). For the past two decades, chronic HCV infection has been treated with interferon (IFN)-based therapy in combination with ribavirin (RBV), which is asso-

[1]Department of Internal Medicine, Kitakyushu Municipal Medical Center, Japan, [2]Department of Internal Medicine, Japan Community Health care Organization Kyushu Hospital, Japan, [3]Department of Internal Medicine, JR Kyushu Hospital, Japan, [4]Department of Internal Medicine, Saiseikai Karatsu Hospital, Japan, [5]Department of Medicine, Hamanomachi Hospital, Japan, [6]Department of Internal Medicine, Chihaya Hospital, Japan, [7]The Center for Liver Disease, Shin-Kokura Hospital, Japan, [8]Department of Hepato-Biliary-Pancreatic Medicine, Fukuoka Sanno Hospital, Japan and [9]Department of Medicine and Biosystemic Science, Graduate School of Medical Sciences, Kyushu University, Japan

ciated with frequent and sometimes severe adverse events (2, 3).

Recently, a number of direct-acting antivirals (DAAs) have been developed that selectively target HCV proteins: nonstructural (NS) 3/4A, a protease essential for cleaving the nonstructural portion of the HCV polyprotein; NS5A, a phosphoprotein required for HCV replication; and NS5B, an RNA-dependent RNA polymerase required for the synthesis of HCV RNA (4). The advent of all-oral, IFN-free regimens of DAAs has radically improved the sustained virological response rate for chronic HCV infection (over 90%), and the regimens are well-tolerated because of the low rate of adverse events (5-8).

IFN-free regimens of sofosbuvir (SOF, an NS5B polymerase inhibitor)-based therapies are recommended worldwide for the treatment of HCV infection (9-11). In Japan, ledipasvir (LDV, NS5A inhibitor) and SOF for genotype 1 and SOF with RBV for genotype 2 HCV infection have been approved for the treatment of chronic hepatitis and compensated liver cirrhosis (Child-Pugh score 5 and 6) by the Japanese Ministry of Health, Labor and Welfare (12, 13).

Serum albumin is an important index of liver protein synthesis and is also a key factor of the Child-Pugh score that strongly influences the prognosis of liver cirrhosis (14). Recently, improvements in the serum albumin level in HCV-infected patients treated with IFN-free DAA combination therapies have been reported, especially in patients with a serum albumin level below 35 g/L (15-19). Improvements in the serum albumin level lead to an improvement in the Child-Pugh score (15, 16, 18-21). An improved Child-Pugh score can be associated with decreased hepatic decompensation, de-listing of liver transplant candidates and a reduced liver-related mortality (19, 22). However, to what extent serum albumin can be improved in patients receiving IFN-free regimens of DAAs is unclear. In addition, little is known about factors that influence the improvement in the serum albumin level during IFN-free antiviral therapy.

The objective of this study was to evaluate the improvement in the serum albumin level in patients receiving all-oral, IFN-free SOF-based therapy and to identify the factors that influence this improvement.

Materials and Methods

Patients

This retrospective multicenter study consisted of 486 consecutive Japanese patients with chronic hepatitis and compensated liver cirrhosis chronically infected with genotype 1 or 2 HCV who started 12-week all-oral, IFN-free SOF-based therapy from May 2015 until January 2017. The inclusion criteria were (1) chronic hepatitis or compensated liver cirrhosis (Child-Pugh score 5 or 6) and (2) serum alanine aminotransferase (ALT) level ≥31 IU/L and/or platelet (PLT) count <150×10^9/L according to the Japanese treatment guideline (23). The exclusion criteria were 1) co-infection of

hepatitis B virus or human immunodeficiency virus; 2) other causes of liver disease (autoimmune hepatitis, or primary biliary cholangitis); 3) excessive active alcohol consumption (a daily intake of more than 40 g of ethanol) or drug abuse; 4) active cancer or hematological malignancy at entry; 5) treatment with immunosuppressive agents prior to enrollment; 6) very poorly controlled heart diseases, pulmonary disorders or severe impairment of the renal function or 7) pregnancy in progress or planned during the study period of either partner. All patients underwent an ultrasound examination to exclude HCC within the last six months before therapy. During antiviral treatment, 15 patients were excluded from the analysis [treatment discontinuation (n=3), severe adverse events (n=1), accidental events during treatment (n=2), development of HCC during or at the end of the treatment (EOT) (n=9)]. None of the patients experienced viral breakthrough. We ultimately selected 471 patients who completed 12-week therapy without severe adverse or accidental events (Fig. 1).

Clinical and laboratory assessments

Blood samples were obtained at baseline; weeks 1, 2, 4, 6, 8 and 12 of treatment; and weeks 4, 8 and 12 after EOT. Clinical parameters were measured by standard laboratory techniques at a commercial laboratory (SRL Laboratory, Tokyo, Japan). Serum HCV RNA level was measured by a real-time reverse transcription polymerase chain reaction assay (COBAS TaqMan HCV test v2.0; Roche Diagnostics, Tokyo, Japan), with a detectability of ≥15 IU/mL and a linear dynamic range of 1.2-7.8 log IU/mL. The HCV genotype was determined by sequence determination in the 5' non-structural region of the HCV genome followed by a phylogenetic analysis (24). The FIB-4 index was calculated using the following formula: age (years)×aspartate aminotransferase (IU/L)/[PLT count (10^9/L)×ALT (IU/l)$^{1/2}$] (25). The aspartate aminotransferase-to-PLT ratio index (APRI) was calculated using the following formula: [(aspartate aminotransferase level / upper limit of normal) / PLT count (10^9/L)]×100 (26). The Child-Pugh score at baseline and EOT were assessed (14). Liver cirrhosis was defined by a liver biopsy showing a METAVIR score of F4 (27) or APRI >2 (28) coupled with an ultrasound examination with signs of cirrhosis (spleen size >12 cm, portal vein >16 mm or nodules within the hepatic parenchyma). We evaluated the changes in the serum albumin level from baseline to the end of treatment (ΔAlb).

Anti-viral treatment

All patients received 12-week IFN-free SOF-based therapy. Patients infected with genotype 1 HCV received a fixed-dose combination tablet (Harvoni; Gilead Science, Tokyo, Japan) containing 90 mg of LDV and 400 mg of SOF, administered orally once-daily. Patients infected with genotype 2 HCV received 400 mg of SOF (Sovaldi; Gilead Science) orally once-daily and RBV (Rebetol; MSD, Tokyo, Japan or Copegus; Chugai Pharmaceutical, Tokyo, Japan)
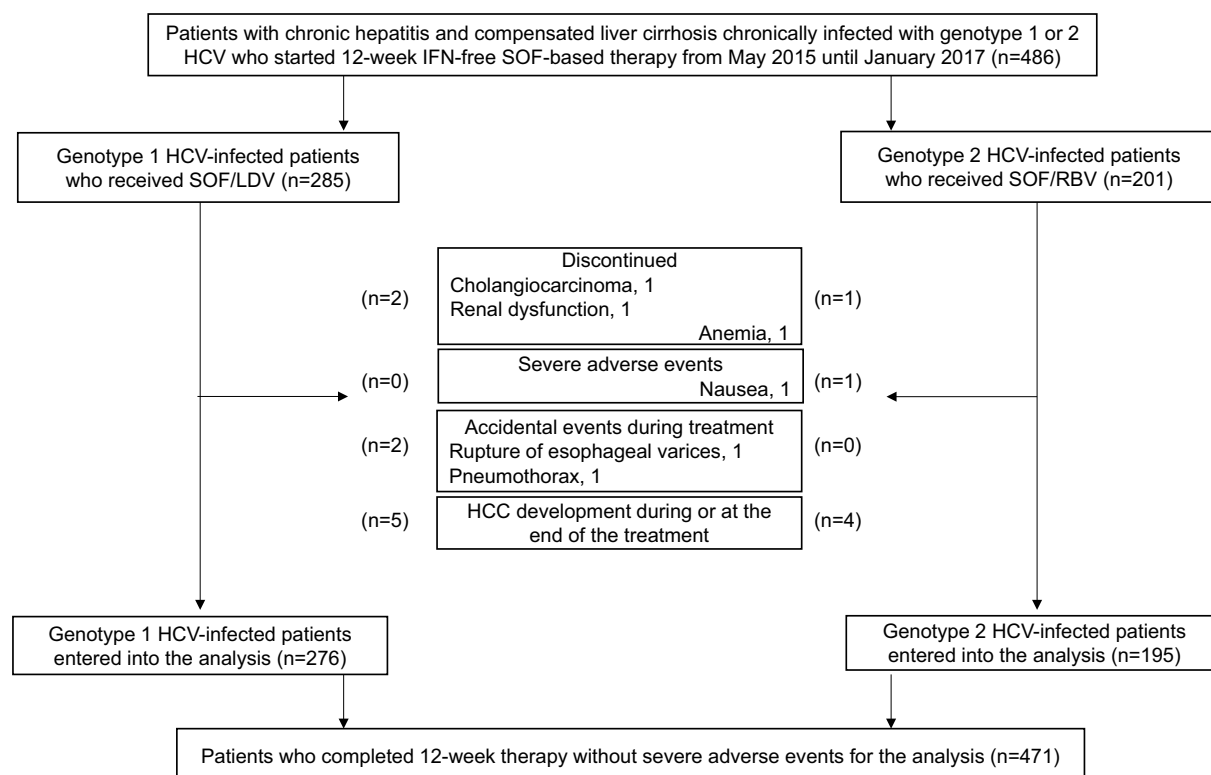
Figure 1.   Patient acquisition flow diagram. HCV: hepatitis C virus, IFN: interferon, SOF: sofosbu-vir, LDV: ledipasvir, RBV: ribavirin, HCC: hepatocellular carcinoma

orally twice-daily. RBV was given at a daily dose of 600-1,000 mg based on body weight (600 mg for patients weighing <60 kg, 800 mg for those weighing 60-80 kg, and 1,000 mg for those weighing >80 kg). For the patients who could not tolerate the starting dose of RBV, the dose was reduced as necessary based on the hemoglobin levels or other adverse events. No dose adjustment was allowed for SOF or LDV. Patients were asked to visit the hospital regularly for the monitoring of treatment effects and adverse events throughout the treatment period.

A diagnosis of diabetes mellitus

The definition of diabetes mellitus in this study was a high plasma glucose level [(1) a fasting plasma glucose (FPG) level of ≥126 mg/dL; (2) a 2-h plasma glucose level of ≥200 mg/dL in the 75-g oral glucose tolerance test; or (3) a casual plasma glucose level of ≥200 mg/dL] and/or a hemoglobin A1c (HbA1c) level ≥6.5% (29). In cases of liver cirrhosis, in which the HbA1c might be low, the plasma glucose level was used for the diagnosis of diabetes mellitus. The patients who were already diagnosed with diabetes mellitus at the start of antiviral therapy and had received intervention with medical treatment, including diet therapy, were also regarded as having diabetes mellitus.

Endpoints

The primary endpoint was the change in the serum albumin level from baseline to EOT. The secondary endpoint was the factors that influenced that improvement.

Statistical analyses

Statistical analyses were conducted using the SPSS statistics software program, version 22.0 (IBM SPSS, Chicago, USA). Continuous data were expressed as the mean with the standard deviation, and categorical variables were reported as frequencies and percentages. For the comparison of characteristics between two groups, categorical variables were analyzed using the chi-squared test or Fisher's exact test, and continuous variables were analyzed using Mann-Whitney U test and Student's t-test. To compare continuous variables before and after treatment, a paired t-test or Wilcoxon signed-rank test was used. Using the patient characteristics at baseline, univariate and multivariable logistic regression analyses were performed to identify the independent predictive factors that influence the improvement in the serum albumin level. Variables with p<0.05 in univariate tests were used as candidate factors for a multivariable logistic regression analysis. The results are expressed as the odds ratio (OR) and 95% confidence interval (CI). All p values were derived from two-tailed tests, and p values of <0.05 were regarded as statistically significant in all analyses.

Ethics

This 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 not obtained because this study utilized

Table 1.   Baseline Characteristics of the Patients.

Characteristics	All patients n=471	Normal-albumin group (Serum albumin>35 g/L) n=406	Low-albumin group (Serum albumin ≤35 g/L) n=65	p
Age (years), mean (SD)	63.2 (13.1)	62.0 (13.1)	70.8 (10.9)	<0.001
Male, n (%)	215 (46)	186 (46)	29 (45)	0.86
Genotype 1, n (%) (vs. Genotype 2)	276 (59)	229 (56)	47 (72)	0.016
Body mass index (kg/m^2), mean (SD)	22.7 (3.5)	22.8 (3.5)	22.6 (3.5)	0.75
HCV RNA level (log IU/mL), mean (SD)	5.9 (0.9)	5.9 (0.9)	5.8 (0.7)	0.20
Total bilirubin (mg/dL), mean (SD)	0.82 (0.32)	0.81 (0.31)	0.89 (0.38)	0.090
Total protein (g/L), mean (SD)	74 (5)	74 (5)	71 (6)	<0.001
Serum albumin (g/L), mean (SD)	40 (4)	41 (3)	33 (2)	<0.001
Aspartate aminotransferase (IU/L), mean (SD)	51 (38)	51 (39)	56 (33)	0.24
Alanine aminotransferase (IU/L), mean (SD)	57 (58)	58 (60)	54 (46)	0.59
γ-glutamyl-transpeptidase (IU/L), mean (SD)	57 (68)	56 (69)	62 (59)	0.50
Serum creatinine (mg/dL), mean (SD)	0.70 (0.17)	0.70 (0.17)	0.71 (0.18)	0.69
eGFR (mL/min/1.73 m^2), mean (SD)	78.2 (17.5)	78.7 (17.5)	74.7 (17.5)	0.086
Total cholesterol (mg/dL), mean (SD)	174 (31)	177 (30)	153 (25)	<0.001
Low-density lipoprotein cholesterol (mg/dL), mean (SD)	97 (27)	101 (27)	74 (20)	<0.001
High-density lipoprotein cholesterol (mg/dL), mean (SD)	58 (17)	58 (18)	57 (16)	0.75
Triglycerides (mg/dL), mean (SD)	105 (54)	105 (55)	103 (52)	0.80
Fasting plasma glucose (mg/dL), mean (SD)	109 (27)	108 (27)	110 (32)	0.59
Hemoglobin A1c (%), mean (SD)	5.9 (0.9)	5.9 (0.8)	5.9 (1.1)	0.91
Serum alpha-fetoprotein level (ng/mL), mean (SD)	9.4 (21.4)	7.4 (12.6)	21.4 (46.3)	<0.001
Prothrombin time (% of normal), mean (SD)	99.2 (16.2)	99.7 (16.6)	96.1 (13.6)	0.097
White blood cell count (×10^6/L), mean (SD)	5,060 (1,615)	5,130 (1,652)	4,634 (1,306)	0.025
Neutrophil count (×10^6/L), mean (SD)	2,739 (1,217)	2,798 (1,253)	2,378 (901)	0.012
Hemoglobin level (g/L), mean (SD)	13.7 (1.5)	13.8 (1.5)	12.9 (1.5)	<0.001
Platelet count (×10^9/L), mean (SD)	160 (61)	165 (60)	127 (59)	<0.001
FIB-4 index, mean (SD)	3.54 (2.87)	3.19 (2.48)	5.71 (4.01)	<0.001
APRI, mean (SD)	1.36 (1.46)	1.27 (1.42)	1.96 (1.57)	<0.001
Cirrhosis, n (%)	120 (25)	88 (22)	32 (49)	<0.001
Diabetes mellitus, n (%)	93 (20)	79 (19)	14 (22)	0.70

Continuous data are expressed as mean values with the standard deviation.

Categorical variables are reported as frequencies and percentages.

SD: standard deviation, HCV: hepatitis C virus, eGFR: estimated glomerular filtration rate, APRI: aspartate aminotransferase-to-platelet ratio index

data provided in the course of normal patient care, and no patient-identifying data were collected.

Results

Patient characteristics

The characteristics of the 471 studied patients (overall and classified by serum albumin level at baseline) are shown in Table 1. Overall, the mean age was 63.2 years old, 46% (n= 215) were male, 59% (n=276) were infected with genotype 1 HCV, 25% (n=120) had cirrhosis, and 20% (n=93) had diabetes mellitus. The mean FPG level was 109 mg/dL [140 mg/dL in the diabetic patients (n=89) and 101 mg/dL in the non-diabetic patients (n=354), p<0.001], and the mean HbA1c level was 5.9% [7.1% in the diabetic patients (n=91) and 5.6% in the non-diabetic patients (n=336), p<0.001]. When classified by the serum albumin level, the patients with serum albumin ≤35 g/L (low-albumin group, n=65) showed a higher age, lower total protein, lower total choles-

terol, lower low-density lipoprotein cholesterol, higher serum alpha-fetoprotein level, lower complete blood count, higher FIB-4 index and higher APRI than those with a serum albumin >35 g/L (normal-albumin group, n=406) (Table 1). This means that the low-albumin group showed marked characteristics of liver cirrhosis.

Changes in the parameters from baseline to EOT

All 471 patients became HCV RNA-negative at EOT, and 466 of these 471 patients (99%) had a sustained virological response 12 (SVR12, HCV RNA-negative 12 weeks after EOT). Changes in parameters from baseline to EOT are shown in Fig. 2. Statistically significant improvements from baseline to EOT were observed in the serum albumin level (39.6 g/L to 41.2 g/L, p<0.001), ALT (56.4 IU/L to 22.4 IU/L, p<0.001), alpha-fetoprotein (10.0 ng/mL to 5.0 ng/mL, p<0.001) and PLT count (157×10^9/L to 179×10^9/L, p<0.001) (Fig. 2A, B, C and D, respectively). When compared with the normal-albumin group (serum albumin at baseline >35 g/ L), the low-albumin group (serum albumin at baseline ≤35
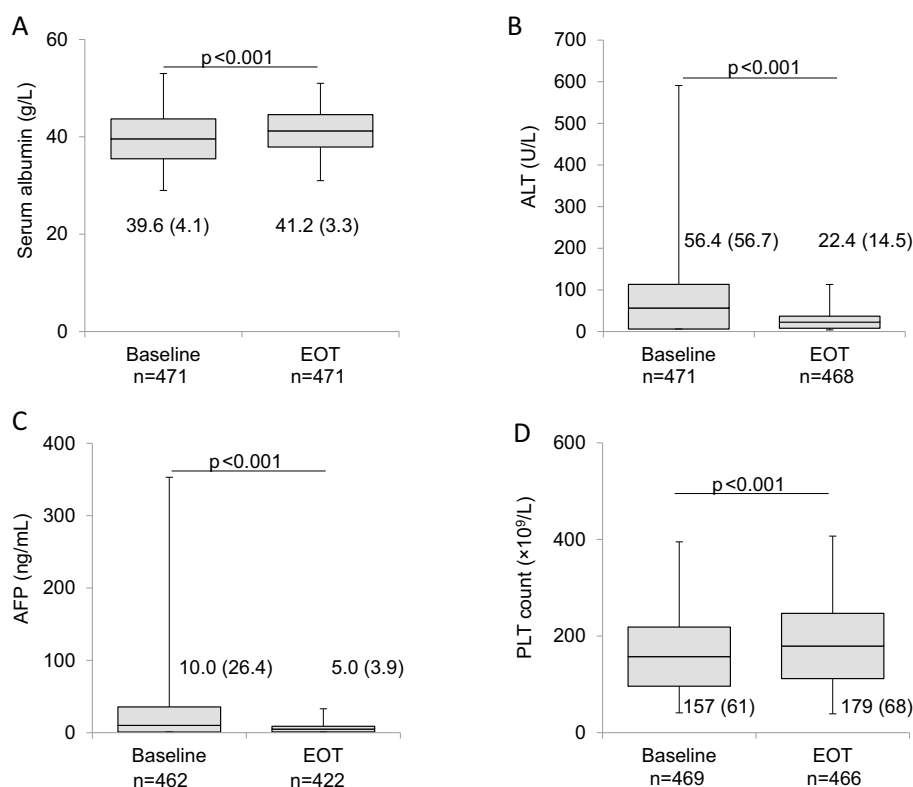
Figure 2.   Changes in the parameters from baseline to the end of the treatment (EOT). Statistically significant improvements from baseline to EOT were observed in (A) serum albumin, (B) alanine aminotransferase (ALT), (C) alpha-fetoprotein (AFP) and (D) platelet count (PLT). The mean value is indicated by the central line, the box outlines the standard deviation for the parameter, and the whiskers show the range of values. All data are expressed as the mean (standard deviation).

g/L) showed a significantly higher mean ΔAlb (5.5 g/L vs. 1.0 g/L, p<0.001) (Fig. 3). The low-albumin group also showed rapid improvement in the mean serum albumin level (32.8 g/L at baseline to 37.0 g/L at week 4, p<0.001) and mean ALT (53.2 IU/L at baseline to 22.4 IU/L at week 4, p<0.001). These improvements continued throughout antiviral therapy (Fig. 4A and B, respectively).

Factors influencing the improvement in the serum albumin

In the low-albumin group, we defined the patients with ΔAlb ≥5 g/L as the good-improvement group (n=41) and those with ΔAlb <5 g/L as the poor-improvement group (n= 24). A univariate analysis extracted triglycerides and diabetes mellitus (p=0.040 and 0.020, respectively) as significant independent predictors of being in the poor-improvement group among those in the low-albumin group, but a multivariate analysis extracted only diabetes mellitus (OR 0.19, 95% CI 0.048-0.79, p=0.020) as such a predictor. FPG and HbA1c were not extracted as predictors of being in the poor improvement group (Table 2).

The characteristics of the patients with diabetes mellitus in the low-albumin group (n=14) were as follows: 5 received oral hypoglycemic drug, 3 received insulin therapy, 6 were in poor control (HbA1c >8.0%), 1 had cardiovascular system complications, and 3 had proteinuria (dipstick analysis ≥

1+). On comparing the diabetic patients (n=14) and non-diabetic patients (n=51) in the low-albumin group, there were no significant differences in the PLT count (104 and 133×10⁹/L, p=0.10), FIB-4 index (6.40 and 5.52, p=0.47), APRI (2.20 and 1.89, p=0.51) or rate of cirrhosis [64% (9/ 14) and 45% (23/51), p=0.20] (Table 3). In the low-albumin group, the mean ΔAlb was significantly lower in the diabetic patients (n=14) than in the non-diabetic patients (n=51) (3.9 g/L and 5.7 g/L, p=0.049) (Fig. 5), and there were more diabetic patients in the poor-improvement group than in the good-improvement group [38% (9/24), 12% (5/41), p= 0.016] (Fig. 6).

Discussion

In this study, we first confirmed the previous reports that viral eradication by all-oral, IFN-free regimens of DAAs leads to a significant improvement in the serum albumin level in patients with chronic HCV infection (17), especially in the low-albumin group (≤35 g/L) (16). Second, we found that the improvement in the serum albumin level was extremely low in patients with diabetes mellitus.

Recently, it has been shown that HCV clearance is accompanied by the rapid down-regulation of various intrahepatic IFN-stimulated genes (30). We noted that ALT quickly normalized during IFN-free antiviral therapy (Fig. 4B), indi-
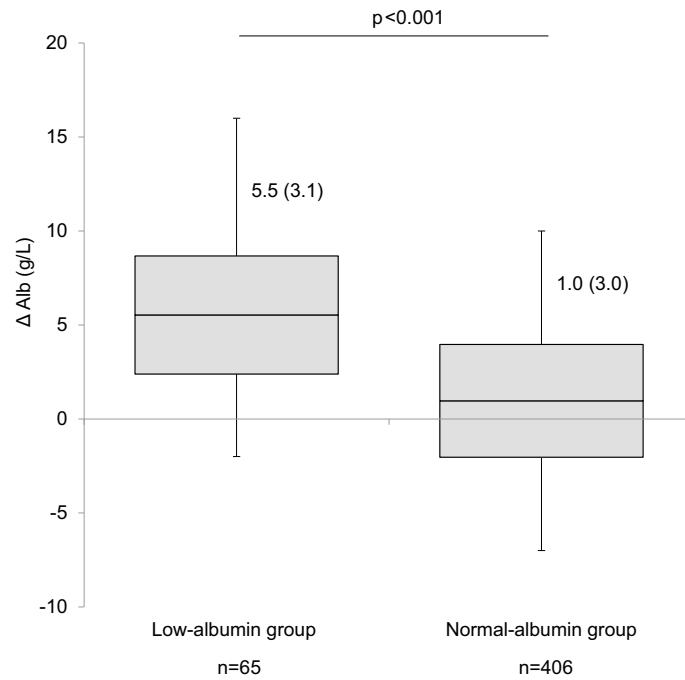
Figure 3. **A comparison of the mean ΔAlb (g/L) between the low- and normal-albumin groups. The low-albumin group (serum albumin at baseline ≤35 g/L) showed a significantly higher mean ΔAlb than the normal-albumin group (serum albumin at baseline >35 g/L) (5.5 g/L vs. 1.0 g/L, p<0.001). The mean value is indicated by the central line, the box outlines the standard deviation for the parameter, and the whiskers show the range of values. All data are expressed as the mean (standard deviation). ΔAlb, changes in the serum albumin level from baseline to the end of treatment**
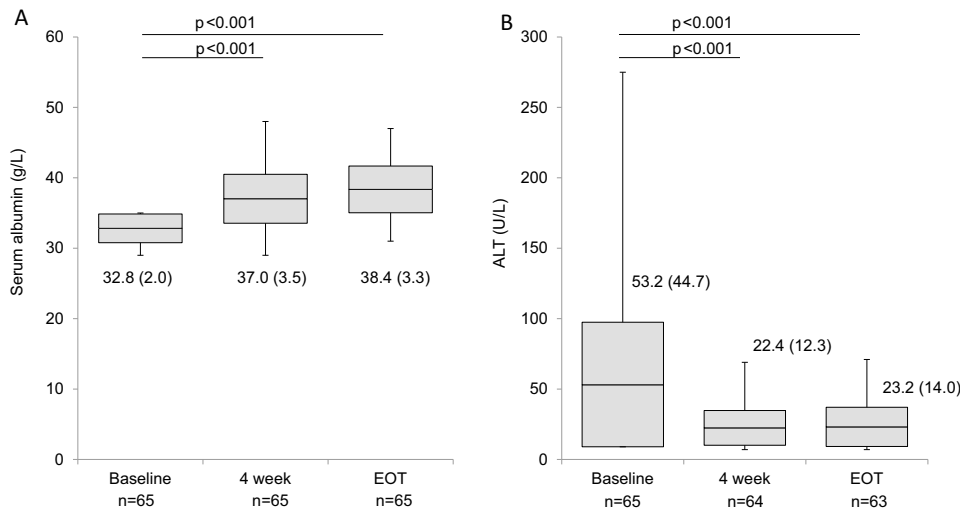

Figure 4. **Changes in the parameters in the low-albumin group from baseline to the end of the treatment (EOT). The low-albumin group showed a rapid improvement in the (A) serum albumin (32.8 g/L at baseline to 37.0 g/L at week 4, p<0.001) and (B) alanine aminotransferase (ALT) (53.2 IU/L at baseline to 22.4 IU/L at week 4, p<0.001). These improvements continued throughout the antiviral therapy period. The mean value is indicated by the central line, the box outlines the standard deviation for the parameter, and the whiskers show the range of values. All data are expressed as the mean (standard deviation).**

cating reduced HCV-induced hepatic inflammation. We also noted a rapid improvement in the serum albumin level (Fig. 4A). These findings support the notion that intrahepatic inflammation contributes to the reduced synthetic capacity of the liver and that suppressing this inflammation by antiviral therapy can restore the liver function, including improving the serum albumin level (16). Liver albumin synthesis in cirrhosis is also determined to a large extent by structural

Table 2. Factors That Influence the Poor Improvement in Serum Albumin in the Low-albumin Group.

Variables	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Age (years)	0.99 (0.95-1.04)	0.74		
Gender (male:female)	1.13 (0.45-2.83)	0.80		
Genotype 1 (vs. Genotype 2)	1.67 (0.59-4.75)	0.34		
Body mass index (kg/m²)	0.93 (0.82-1.06)	0.29		
Baseline HCV RNA level (log IU/mL)	1.44 (0.72-2.86)	0.30		
Total bilirubin (mg/dL)	0.58 (0.17-1.95)	0.38		
Total protein (g/L)	1.27 (0.62-2.64)	0.52		
Serum albumin (g/L)	0.22 (0.02-2.23)	0.20		
Aspartate aminotransferase (IU/L)	0.99 (0.98-1.01)	0.39		
Alanine aminotransferase (IU/L)	0.99 (0.98-1.01)	0.35		
γ-glutamyl-transpeptidase (IU/L)	1.00 (0.99-1.01)	0.40		
Serum creatinine (mg/dL)	0.89 (0.07-10.66)	0.92		
eGFR (mL/min/1.73 m²)	0.99 (0.97-1.02)	0.83		
Total cholesterol (mg/dL)	1.01 (0.99-1.03)	0.38		
Low-density lipoprotein cholesterol (mg/dL)	0.99 (0.97-1.02)	0.49		
High-density lipoprotein cholesterol (mg/dL)	1.01 (0.98-1.04)	0.61		
Triglycerides (mg/dL)	1.01 (1.00-1.03)	0.040	1.01 (0.99-1.03)	0.070
Fasting plasma glucose (mg/dL)	1.00 (0.99-1.02)	0.99		
Serum alpha-fetoprotein level (ng/mL)	1.01 (0.99-1.03)	0.25		
Prothrombin time (% of normal)	1.02 (0.99-1.06)	0.20		
White blood cell count (×10⁶/L)	0.99 (0.99-1.00)	0.40		
Neutrophil count (×10⁶/L)	0.99 (0.99-1.00)	0.16		
Hemoglobin level (g/L)	0.85 (0.64-1.13)	0.27		
Platelet count (×10⁹/L)	1.02 (0.94-1.10)	0.71		
FIB-4 index	0.97 (0.87-1.08)	0.60		
APRI	0.94 (0.71-1.23)	0.63		
Cirrhosis	0.68 (0.27-1.69)	0.40		
Diabetes mellitus	0.23 (0.07-0.81)	0.020	0.19 (0.048-0.79)	0.020

OR: odds ratio, CI: confidence interval, HCV: hepatitis C virus, APRI: aspartate aminotransferase-to-platelet ratio index, eGFR: estimated glomerular filtration rate

changes caused by liver fibrosis. However, we were able to avoid this influence by excluding patients with advanced liver cirrhosis (Child-Pugh score ≥7) from this study.

We showed that the improvement in the serum albumin level was significantly lower in the patients with diabetes mellitus than in those without. Interestingly, FPG and HbA1c were not extracted as predictors influencing the improvement in the serum albumin level (Table 2). This was probably because the liver damage caused by diabetes mellitus was the main mechanism that impaired the improvement serum albumin, and the levels of FPG and HbA1c at baseline themselves did not affect the serum albumin.

Damage to the liver induced by diabetes mellitus is known as "diabetic liver injury." (31, 32). Factors potentially influencing the mechanisms related to the pathological and functional changes of diabetic liver injury include insulin resistance (33-35), oxidative stress (36, 37), endoplasmic reticulum stress (38-41) and inflammatory cytokines (31, 42, 43). The pathological feature of diabetic liver injury is thought to be non-alcoholic fatty liver disease (NAFLD), which can develop into non-alcoholic steatohepatitis (NASH), cirrhosis and HCC (31). Our hypothesis was that diabetic liver injury remained after HCV eradication and still impaired the improvement in the serum albumin level.

Our results indicated that the control of diabetes mellitus as well as the eradication of HCV was important for improving the clinical course of patients with both chronic HCV infection and diabetes mellitus.

Several limitations associated with the present study warrant mention. First, we assessed only the patients who completed 12-week treatment without severe adverse events or viral breakthrough. In this study, only three patients experienced severe adverse events (renal dysfunction, anemia and nausea), and none experienced viral breakthrough. Although adverse events and viral breakthrough are serious matters, we excluded them in order to analyze simply the change in the parameters caused by HCV eradication. Second, the genotype 2 patients were treated with an RBV-containing regimen. We managed this issue by reducing the dose of RBV as necessary on the basis of the adverse events. As such, only 2 of 210 patients with HCV genotype 2 experienced severe adverse events (anemia and nausea), and those 2 patients were excluded from this analysis (Fig. 1). Third, in this study, we performed a liver biopsy in only 16 of 486 (3%) patients (data not shown), so we were unable to assess

Table 3.   A Comparison of the Diabetic Patients and Non-diabetic Patients in the Low-albumin Group.

Characteristics	Diabetes mellitus (+) (n=14)	Diabetes mellitus (-) (n=51)	p
Body mass index (kg/m^2), mean (SD)	22.8 (3.5)	22.6 (3.5)	0.83
Total bilirubin (mg/dL), mean (SD)	0.95 (0.40)	0.87 (0.38)	0.49
Total protein (g/L), mean (SD)	69 (6)	71 (6)	0.28
Serum albumin (g/L), mean (SD)	33 (2)	33 (2)	0.99
Aspartate aminotransferase (IU/L), mean (SD)	60 (42)	56 (30)	0.70
Alanine aminotransferase (IU/L), mean (SD)	62 (69)	51 (37)	0.44
γ-glutamyl-transpeptidase (IU/L), mean (SD)	60 (40)	63 (63)	0.86
eGFR (mL/min/1.73 m^2), mean (SD)	70.4 (15.6)	75.9 (17.9)	0.30
Total cholesterol (mg/dL), mean (SD)	151 (21)	153 (26)	0.85
Low-density lipoprotein cholesterol (mg/dL), mean (SD)	79 (28)	73 (17)	0.31
High-density lipoprotein cholesterol (mg/dL), mean (SD)	54 (17)	58 (15)	0.39
Triglycerides (mg/dL), mean (SD)	115 (67)	100 (47)	0.34
Fasting plasma glucose (mg/dL), mean (SD)	137 (44)	103 (23)	<0.001
Hemoglobin A1c (%), mean (SD)	7.4 (1.4)	5.5 (0.5)	<0.001
Serum alpha-fetoprotein level (ng/mL), mean (SD)	14.1 (8.2)	23.5 (52.1)	0.51
Prothrombin time (% of normal), mean (SD)	95.1 (11.7)	96.4 (14.2)	0.75
White blood cell count (×10^6/L), mean (SD)	4,469 (1,384)	4,681 (1,294)	0.60
Neutrophil count (×10^6/L), mean (SD)	2,609 (934)	2,316 (891)	0.30
Hemoglobin level (g/L), mean (SD)	13.0 (1.8)	12.8 (1.6)	0.77
Platelet count (×10^9/L), mean (SD)	104 (34)	133 (63)	0.10
FIB-4 index, mean (SD)	6.40 (4.01)	5.52 (4.03)	0.47
APRI, mean (SD)	2.20 (1.71)	1.89 (1.54)	0.51
Cirrhosis, n (%)	9 (64)	23 (45)	0.20

Continuous data are expressed as mean values with the standard deviation.

SD: standard deviation, HCV: hepatitis C virus, APRI: aspartate aminotransferase-to-platelet ratio index, eGFR: estimated glomerular filtration rate
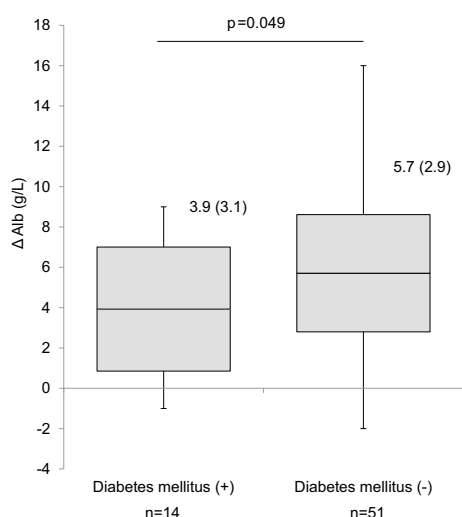

Figure 5.   A comparison of the mean ΔAlb (g/L) in the low-albumin group by the presence of diabetes mellitus. In the low-albumin group, the mean ΔAlb was significantly lower in the diabetic patients (n=14) than in the non-diabetic patients (n=51) (3.9 g/L and 5.7 g/L, p=0.049). The mean value is indicated by the central line, the box outlines the standard deviation for the parameter, and the whiskers show the range of values. All data are expressed as the mean (standard deviation). ΔAlb, changes in the serum albumin level from baseline to the end of treatment.
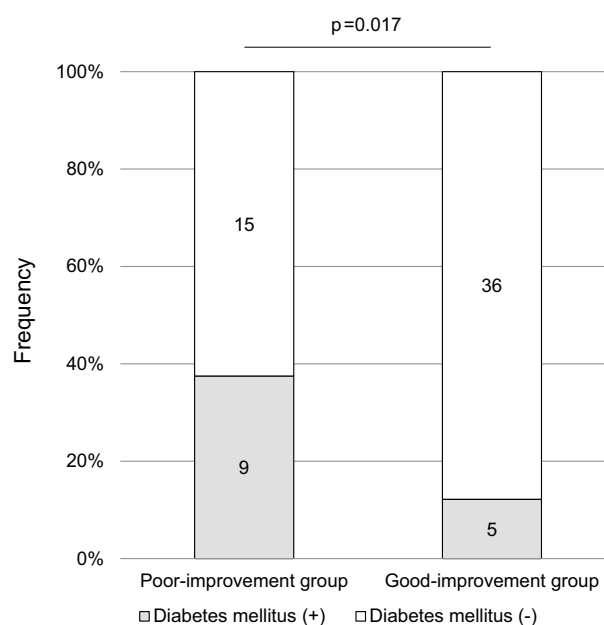

Figure 6.   A comparison of the rate of patients with diabetes mellitus. In the low-albumin group, there were more diabetic patients in the poor-improvement group (median ΔAlb <5 g/L) than in the good-improvement group (median ΔAlb ≥5 g/L) [38% (9/24), 12% (5/41), p=0.016]. ΔAlb, changes in the serum albumin level from baseline to the end of treatment.

the degree of liver steatosis, inflammation or fibrosis directly. However, using the FIB4-index and APRI, we were able to estimate the degree of liver fibrosis. Fourth, we lacked sufficient data on diabetic nephropathy. Proteinuria caused by diabetic nephropathy may influence the serum albumin level and ΔAlb. In this study, in the low-albumin group with diabetes mellitus (n=14), 3 patients had overt proteinuria (dipstick analysis ≥1+). However, there were no significant differences in the ΔAlb between these 3 patients and those without proteinuria (4.0 g/L and 4.0 g/L, p=0.39). Fifth, because there were so few patients in the low-albumin group with diabetes mellitus (n=14), we were unable to find any statistically significant differences in the parameters between the good-improvement group (n=5) and the poor-improvement group (n=9). However, the mean ΔAlb was lower in the patients with cirrhosis (n=9) than in those without (n=5) (3.0 g/L and 5.0 g/L, p=0.79), although not to a significant degree. Cirrhosis might be an important factor preventing improvement in the serum albumin level in the low-albumin group with diabetes mellitus.

In conclusion, this real-world multicenter study showed that all-oral, IFN-free SOF-based therapy improved the serum albumin level in patients with chronic HCV infection, especially among those in the low-albumin group. Our study also showed that the improvement in the serum albumin level during IFN-free antiviral therapy was significantly lower in the patients with diabetes mellitus than in those without.

The authors state that they have no Conflict of Interest (COI).

References

1. Niederau C, Lange S, Heintges T, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 28: 1687-1695, 1998.
2. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 358: 958-965, 2001.
3. Ogawa E, Furusyo N, Kajiwara E, et al. Evaluation of the adverse effect of premature discontinuation of pegylated interferon α-2b and ribavirin treatment for chronic hepatitis C virus infection: results from Kyushu University Liver Disease Study. J Gastroenterol Hepatol 27: 1233-1240, 2012.
4. De Clercq E. Current race in the development of DAAs (direct-acting antivirals) against HCV. Biochem Pharmacol 89: 441-452, 2014.
5. Kowdley KV, Gordon SC, Reddy KR, et al. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. N Engl J Med 370: 1879-1888, 2014.
6. Afdhal N, Zeuzem S, Kwo P, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med 370: 1889-1898, 2014.
7. Bourlière M, Bronowicki JP, de Ledinghen V, et al. Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: a randomised, double-blind, phase 2 trial (SIRIUS). Lancet Infect Dis 15: 397-404, 2015.
8. Reddy KR, Bourlière M, Sulkowski M, et al. Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: an integrated safety and efficacy analysis. Hepatology 62: 79-86, 2015.
9. European Association for Study of Liver. EASL Recommendations on Treatment of Hepatitis C 2016. J Hepatol 66: 153-194, 2017.
10. AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology 62: 932-954, 2015.
11. Omata M, Kanda T, Wei L, et al. APASL consensus statements and recommendation on treatment of hepatitis C. Hepatol Int 10: 702-726, 2016.
12. Mizokami M, Yokosuka O, Takehara T, et al. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naive and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomised, phase 3 trial. Lancet Infect Dis 15: 645-653, 2015.
13. Omata M, Nishiguchi S, Ueno Y, et al. Sofosbuvir plus ribavirin in Japanese patients with chronic genotype 2 HCV infection: an open-label, phase 3 trial. J Viral Hepat 21: 762-768, 2014.
14. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649, 1973.
15. Charlton M, Everson GT, Flamm SL, et al. Ledipasvir and sofosbuvir plus ribavirin for treatment of HCV infection in patients with advanced liver disease. Gastroenterology 149: 649-659, 2015.
16. Deterding K, Höner Zu, Siederdissen C, et al. Improvement of liver function parameters in advanced HCV-associated liver cirrhosis by IFN-free antiviral therapies. Aliment Pharmacol Ther 42: 889-901, 2015.
17. Miyaki E, Imamura M, Hiraga N, et al. Daclatasvir and asunaprevir treatment improves liver function parameters and reduces liver fibrosis markers in chronic hepatitis C patients. Hepatol Res 46: 758-764, 2016.
18. Foster GR, Irving WL, Cheung MC, et al. Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis. J Hepatol 64: 1224-1231, 2016.
19. Belli LS, Berenguer M, Cortesi PA, et al. Delisting of liver transplant candidates with chronic hepatitis C after viral eradication: a European study. J Hepatol 65: 524-531, 2016.
20. Curry MP, O'Leary JG, Bzowej N, et al. Sofosbuvir and velpatasvir for HCV in patients with decompensated cirrhosis. N Engl J Med 373: 2618-2628, 2015.
21. Bunchorntavakul C, Reddy KR. Treat chronic hepatitis C virus infection in decompensated cirrhosis - pre- or post-liver transplantation? the ironic conundrum in the era of effective and well-tolerated therapy. J Viral Hepat 23: 408-418, 2016.
22. Manns M, Samuel D, Gane EJ, et al. Ledipasvir and sofosbuvir plus ribavirin in patients with genotype 1 or 4 hepatitis C virus infection and advanced liver disease: a multicentre, open-label, randomised, phase 2 trial. Lancet Infect Dis 16: 685-697, 2016.
23. Drafting Committee for Hepatitis Management Guidelines, the Japan Society of Hepatology. JSH guidelines for the management of hepatitis C virus infection: a 2014 update for genotype 1. Hepatol Res 44 (Suppl S1): 59-70, 2014.
24. Simmonds P, Alberti A, Alter HJ, et al. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 19: 1321-1324, 1994.
25. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 43: 1317-1325, 2006.
26. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 38: 518-526, 2003.
27. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in pa-

tients with chronic hepatitis C. Hepatology **20**: 15-20, 1994.

28. Lackner C, Struber G, Liegl B, et al. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. Hepatology **41**: 1376-1382, 2005.

29. Seino Y, Nanjo K, Tajima N, et al. Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. The Committee of the Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Diabetol Int **1**: 2-20, 2010.

30. Meissner EG, Wu D, Osinusi A, et al. Endogenous intrahepatic IFNs and association with IFN-free HCV treatment outcome. J Clin Invest **124**: 3352-3363, 2014.

31. Nagaishi K, Ataka K, Echizen E, Arimura Y, Fujimiya M. Mesenchymal stem cell therapy ameliorates diabetic hepatocyte damage in mice by inhibiting infiltration of bone marrow-derived cells. Hepatology **59**: 1816-1829, 2014.

32. Wan Y, Garner J, Wu N, et al. Role of stem cells during diabetic liver injury. J Cell Mol Med **20**: 195-203, 2016.

33. Marchesini G, Marzocchi R. Metabolic syndrome and NASH. Clin Liver Dis **11**: 105-117, 2007.

34. Harrison SA. Insulin resistance among patients with chronic hepatitis C: etiology and impact on treatment. Clin Gastroenterol Hepatol **6**: 864-876, 2008.

35. Takamatsu S, Noguchi N, Kudoh A, et al. Influence of risk factors for metabolic syndrome and non-alcoholic fatty liver disease on the progression and prognosis of hepatocellular carcinoma. Hepatogastroenterology **55**: 609-614, 2008.

36. Tolman KG, Fonseca V, Dalpiaz A, Tan MH. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes Care **30**: 734-743, 2007.

37. Matsunami T, Sato Y, Ariga S, et al. Regulation of oxidative stress and inflammation by hepatic adiponectin receptor 2 in an animal model of nonalcoholic steatohepatitis. Int J Clin Exp Pathol **3**: 472-481, 2010.

38. Canbay A, Friedman S, Gores GJ. Apoptosis: the nexus of liver injury and fibrosis. Hepatology **39**: 273-278, 2004.

39. Shiraishi H, Okamoto H, Yoshimura A, Yoshida H. ER stress-induced apoptosis and caspase-12 activation occurs downstream of mitochondrial apoptosis involving Apaf-1. J Cell Sci **119**: 3958-3966, 2006.

40. Cunard R, Sharma K. The endoplasmic reticulum stress response and diabetic kidney disease. Am J Physiol Renal Physiol **300**: F 1054-F1061, 2011.

41. Zhang Q, Li Y, Liang T, et al. ER stress and autophagy dysfunction contribute to fatty liver in diabetic mice. Int J Biol Sci **11**: 559-568, 2015.

42. Solomon SS, Odunusi O, Carrigan D, et al. TNF-alpha inhibits insulin action in liver and adipose tissue: a model of metabolic syndrome. Horm Metab Res **42**: 115-121, 2010.

43. Tilg H. The role of cytokines in non-alcoholic fatty liver disease. Dig Dis **28**: 179-185, 2010.