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

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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 (Δ Alb).

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 Δ Alb (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 Δ Alb >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 Δ Alb 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

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associated 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).

IFN-free regimens of sofosbuvir (SOF, an NS5B polymerase inhibitor)-based therapies are recommended worldwide for the treatment of HCV infection (9). 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). Improvements in the serum albumin level lead to an improvement in the Child-Pugh score (15, 16, 18). 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.

Patients 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 level / upper limit of normal / PLT count [10^9/L] × ALT [IU/l] (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 META VIR score of F4 (27) or EOT were assessed (14). Liver cirrhosis was defined by a liver biopsy showing a META VIR score of F4 (27) or HCC (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 (Δ Alb).

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, Inc., 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, Inc., Tokyo, Japan) orally once-daily and RBV (Rebetol; MSD, Tokyo, Japan or Copegus; Chugai...
Patients with chronic hepatitis and compensated liver cirrhosis chronically infected with genotype 1 or 2 HCV who started 12-week IFN-free SOF-based therapy from May 2015 until January 2017 (n=486)

- Genotype 1 HCV-infected patients who received SOF/LDV (n=285)
  - Discontinued: Cholangiocarcinoma, 1
  - Severe adverse events: Anemia, 1
  - Accidental events during treatment: Nausea, 1
  - HCC development during or at the end of the treatment: (n=5) (n=4)

- Genotype 2 HCV-infected patients who received SOF/RBV (n=201)
  - Discontinued: Renal dysfunction, 1
  - Severe adverse events: (n=1)
  - Accidental events during treatment: Pneumothorax, 1
  - HCC development during or at the end of the treatment: (n=2) (n=0)

Genotype 1 HCV-infected patients entered into the analysis (n=276)

Genotype 2 HCV-infected patients entered into the analysis (n=195)

Patients who completed 12-week therapy without severe adverse events for the analysis (n=471)

Figure 1. Patient acquisition flow diagram. HCV: hepatitis C virus, IFN: interferon, SOF: sofosbuvir, LDV: ledipasvir, RBV: ribavirin, HCC: hepatocellular carcinoma

Pharmaceutical Co., Ltd., Tokyo, Japan) 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 Inc., Chicago, IL, 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’s U test and Student’s t-test. To compare continuous variables before and after treatment, a paired t-test or Wilcoxon’s 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. In-
formed consent was not obtained because this study utilized 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 cholesterol, 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 patients with serum albumin ≥35 g/L (low-albumin group, n=65) showed a higher age, lower total protein, lower total cholesterol, 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.

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**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/m²), 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 m²), 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) | 151 (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 (mg/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 (×10⁹/L), mean (SD) | 5.060 (1.615) | 5.130 (1.652) | 4.634 (1.306) | 0.025 |
| Neutrophil count (×10⁹/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 (×10⁹/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 |

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

Continuous data are expressed as mean values with the standard deviation. Categorical variables are reported as frequencies and percentages.
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).

Figure 3. A comparison of the mean Δ Alb (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 Δ Alb 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). Δ Alb, 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).

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

| Variables                               | Univariate OR (95% CI) | p    | Multivariate OR (95% CI) | p    |
|-----------------------------------------|------------------------|------|--------------------------|------|
| Age (years)                             | 0.99 (0.95-1.04)       | 0.74 | 1.01 (0.99-1.03)         | 0.38 |
| 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 (×10⁹/L)         | 0.99 (0.99-1.00)       | 0.40 |                          |      |
| Neutrophil count (×10⁹/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
the low-albumin group (serum albumin at baseline ≤ 35 g/L) showed a significantly higher mean Δ Alb (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 Δ Alb ≥ 5 g/L as the good-improvement group (n=41) and those with Δ Alb < 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 systemic 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 133×10^9/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 Δ Alb 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).

**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/m²), mean (SD)      | 22.8 (3.5)                   | 22.6 (3.5)                    | 0.83 |
| Total bilirubin (mg/dL), mean (SD)      | 9.5 (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 m²), 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 (×10^9/L), mean (SD) | 4,469 (1,384)              | 4,681 (1,294)                  | 0.60 |
| Neutrophil count (×10^9/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)     | 164 (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 |

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

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

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 intracellular IFN-stimulated genes (30). We noted that ALT quickly
Diabetes mellitus (-)

Liver cirrhosis (Child-Pugh score) can 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 developed 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 the degree of liver steatosis, inflammation or fibrosis directly. However, using the FIB4-index and APRI, we were normalized during IFN-free antiviral therapy (Fig. 4B), indicating 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 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), oxidative stress (36, 37), endoplasmic reticulum stress (38) 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 the degree of liver steatosis, inflammation or fibrosis directly. However, using the FIB4-index and APRI, we were

**Figure 5.** A comparison of the mean Δ Alb (g/L) in the low-albumin group by the presence of diabetes mellitus. In the low-albumin group, the mean Δ Alb 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). Δ Alb, 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 Δ Alb <5 g/L) than in the good-improvement group (median Δ Alb ≥5 g/L) (38% (9/24), 12% (5/41), P=0.016). Δ Alb, changes in the serum albumin level from baseline to the end of treatment.
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 Δ albumin. 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 Δ Alb 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 Δ Alb 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).

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