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Description	C 型慢性肝炎に対する DAA 治療後の肝病理組織所見の検討は、これまで十分になされていませんでした。本研究では DAA 治療によってウイルス排除に成功し、かつ血液検査で異常が見られなかった 51 症例に対し肝生検を行い、炎症、線維化、鉄沈着、脂肪化などの項目について調べました。その結果、ほとんどの症例で肝組織の改善が見られました。また、同時に約 2 割程度の症例に有意な炎症が残存していることがわかりました。 DAA によって C 型肝炎ウイルスを排除することにより、血液検査のみならず肝生検結果の改善も見られたことから、治療によって将来的には肝硬変や肝臓がんへの進展が予防できることが期待されます。一方、炎症が残存していた症例の少なくとも一部は脂肪肝炎が原因であったことから、治療後もアルコール多飲や肥満などを避けて生活習慣に注意する必要があると考えられます。 'C 型慢性肝炎　飲み薬（DAA）治療後の肝組織の改善を顕微鏡レベルで確認'. 大阪市立大学. https://www.osaka-cu.ac.jp/ja/news/2018/180723-1. (参照 2018-07-23)

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Keywords:	biopsy, DAA, HCV, histology, SVR
Abstract:	Background: Interferon-free, direct-acting antiviral (DAA) treatments can result in a sustained virologic response (SVR) in nearly 100% of patients with chronic hepatitis C virus (HCV) infection Aims: To evaluate histological improvement after achieving an SVR to DAA

	treatments in patients with chronic hepatitis C. Methods: Among 691 patients with chronic hepatitis C who achieved an SVR to DAAs, 51 underwent liver biopsy 41 ± 20 weeks after the end of treatment despite normal transaminase levels. In 20 patients, liver biopsy specimens obtained a median of 1.2 years before the start of treatment were available. Results: Among the 51 patients who underwent post-SVR biopsies, the grade of inflammation was A0 in 18 patients, A1 in 24, A2 in 8, and A3 in 1; the stage of fibrosis was F0 in 3 patients, F1 in 20, F2 in 15, F3 in 9, and F4 in 4. Among the 9 post-SVR biopsy specimens with moderate-to-severe inflammation (≥A2), 4 showed S1-to-S3 steatosis (>5% of hepatocytes affected). In the 20 paired biopsy specimens, the inflammation grade significantly regressed (P = 0.0043), but the fibrosis stage did not (P = 0.45). Histological improvement, defined as a ≥2-point decrease in the Knodell inflammatory score and no worsening of the fibrosis, was found in 11 (55%) patients. The iron accumulation had significantly regressed (P = 0.0093), but the steatosis had not (P = 0.10). Conclusions: Even if transaminases become normal after obtaining an SVR, significant histological inflammation of unknown cause was found in some patients. Additionally, improvement in liver fibrosis was not evident in the short term.

Short-term histological evaluations after achieving a sustained virologic response to direct-acting antiviral treatment for chronic hepatitis C

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Background: Interferon-free, direct-acting antiviral (DAA) treatments can result in a sustained virologic response (SVR) in nearly 100% of patients with chronic hepatitis C virus (HCV) infection.

Aims: To evaluate histological improvement after achieving an SVR to DAA treatments in patients with chronic hepatitis C.

Methods: Among 691 patients with chronic hepatitis C who achieved an SVR to DAAs, 51 underwent liver biopsy 41 ± 20 weeks after the end of treatment despite normal transaminase levels. In 20 patients, liver biopsy specimens obtained a median of 1.2 years before the start of treatment were available.

Results: Among the 51 patients who underwent post-SVR biopsies, the grade of inflammation was A0 in 18 patients, A1 in 24, A2 in 8, and A3 in 1; the stage of fibrosis was F0 in 3 patients, F1 in 20, F2 in 15, F3 in 9, and F4 in 4. Among the 9 post-SVR biopsy specimens with moderate-to-severe inflammation (≥A2), 4 showed S1-to-S3 steatosis (>5% of hepatocytes affected). In the 20 paired biopsy specimens, the inflammation grade significantly regressed ($P = 0.0043$), but the fibrosis stage did not ($P = 0.45$). Histological improvement, defined as a ≥2-point decrease in the Knodell inflammatory score and no worsening of the fibrosis, was found in 11 (55%) patients. The iron accumulation had significantly regressed ($P = 0.0093$), but the steatosis had not ($P = 0.10$).

Conclusions: Even if transaminases become normal after obtaining an SVR, significant histological inflammation of unknown cause was found in some patients. Additionally, improvement in liver fibrosis was not evident in the short term.

Keywords: biopsy, DAA, HCV, histology, SVR

Key summary

Summarize the established knowledge on this subject

- A sustained virologic response (SVR) to interferon-based therapy resulted in histological improvements in the grade of inflammation and stage of fibrosis in patients with chronic hepatitis C virus (HCV) infection.

What are the significant and/or new findings of this study?

- Histological improvement, defined as a significant decrease in inflammatory score with no worsening of fibrosis, was observed also in patients with an SVR to direct-acting antiviral (DAA) treatment.
- The residual inflammation after obtaining an SVR was attributable to steatohepatitis at least in some patients, but was of unknown cause in others.
- Although fibrous bundles/septa bridging between portal tracts became thin after DAA therapy, improvement in liver fibrosis was not evident in this short-term study.
- Hepatic iron overload commonly observed in patients with chronic HCV infection could be improved after achieving an SVR to DAAs.

Introduction

An estimated 71 million people were chronically infected with hepatitis C virus (HCV) worldwide, and approximately 399,000 people die annually from hepatitis C-related causes, including mainly cirrhosis and hepatocellular carcinoma (HCC) [1]. A sustained virologic response (SVR) is merely a surrogate marker for the virologic cure of HCV infection, and the ultimate goal of anti-HCV treatments is to prevent the development of cirrhosis and its complications. Until recently, peginterferon-ribavirin regimens had been the standard of care for patients with chronic hepatitis C. In the era of interferon therapy, a number of studies showed histologically that an SVR resulted in improvements in the grade of inflammation and stage of fibrosis in the long term [2–4].

Nowadays, interferon-free, direct-acting antiviral (DAA) treatments can result in an SVR in nearly 100% of patients with chronic HCV infection. However, long-term outcomes of patients treated with DAAs remain unknown. An increasing number of studies have shown that a DAA-induced SVR can lead to improvement in non-invasive, clinical variables [5]. However, histological improvement after achieving an SVR to DAA treatment remains to be evaluated in patients with chronic hepatitis C.

Liver biopsy is indeed invasive, but still considered the 'gold standard' for diagnosing chronic liver diseases, grading inflammatory activity, and staging liver fibrosis [6]. In chronic hepatitis C, it is also important to identify co-morbidities, such as autoimmune hepatitis, steatohepatitis, and hemochromatosis. In addition, liver biopsy provides other valuable information. For example, previous studies showed that a majority of chronically HCV-infected patients with persistently normal alanine aminotransferase (ALT) levels have some degree of histological liver damage [7]. In patients with persistently elevated ALT levels after obtaining an SVR, occult HCV infection has sometimes been described [8]. We previously reported that occult HCV

infection is extremely rare (if any) in patients who have had an SVR to DAAs [9].

The aim of this study was to evaluate the short-term histological characteristics after achieving an SVR to DAA treatment in patients with chronic hepatitis C. First, we assessed the post-SVR liver biopsy specimens obtained from 51 patients with chronic hepatitis C treated with interferon-free DAA regimens. The presence of occult HCV infection was also determined by sensitive polymerase chain reaction assays. Second, in the 20 patients for whom pre-treatment biopsy specimens were available, histological improvement was also elucidated by comparing the paired biopsy specimens.

Methods

Patients

During the period from September 2014 through June 2017, a total of 706 non-transplant patients with chronic hepatitis C started to receive interferon-free DAA treatments in our hospital. Patients were excluded if they had used immunomodulatory drugs, decompensated cirrhosis, viable HCC, hepatitis B surface antigen or had other likely causes of chronic liver disease. None of the patients showed evidence of coinfection with human immunodeficiency virus. The procedures in this study were in accordance with the Helsinki Declaration of 1964 (2013 revision) and were approved by the Ethics Committee of Osaka City University Graduate School of Medicine (No. 2905). Written informed consent was obtained from each patient.

Treatment

Patients with HCV genotype 1a or 1b infection were treated with ledipasvir 90 mg/sofosbuvir 400 mg for 12 weeks (n = 335), daclatasvir 30 mg plus asunaprevir 200 mg for 24 weeks (n = 152), ombitasvir 25 mg/paritaprevir 150 mg/ritonavir 100 mg for 12 weeks (n = 25), or elbasvir 50 mg plus grazoprevir 100 mg for 12 weeks (n = 22). Patients with HCV genotype 2a or 2b infection were treated with sofosbuvir 400 mg plus weight-based ribavirin 600–1,000 mg (n = 172). An SVR was defined as no detectable HCV RNA at the end of treatment and 24 weeks after treatment.

Clinical evaluations

Blood cell counts and routine biochemical tests were performed with the use of standard procedures. Hepatitis B surface antigen and core antibody were detected by chemiluminescence enzyme immunoassay. Genotypes of HCV were identified using an

HCV genotype primer kit (Institute of Immunology Co., Ltd., Tokyo, Japan). Serum HCV RNA was measured with the use of the COBAS TaqMan assay (Pleasanton, CA, USA) [10] (with a lower limit of quantification of 15 IU/mL) and Abbott RealTime assay (Des Plaines, IL, USA) [11] (with a lower limit of quantification of 12 IU/mL). Serum α-fetoprotein levels were determined by chemiluminescence enzyme immunoassay. Serum concentrations of type IV collagen 7S domain were measured by radioimmunoassay. The genetic polymorphisms rs8099917 near the interleukin 28B gene were examined in patients who consented to genetic analyses [12]. Liver stiffness and controlled attenuation parameter (CAP) values were measured by transient elastography (FibroScan®; EchoSens, Paris, France) [13,14].

Liver biopsy

The flow of patients is shown in **Figure 1**. A total of 691 (97.9%) of the 706 patients achieved an SVR (331/335 [98.8%] to ledipasvir/sofosbuvir, 145 of 152 patients [95.8%] to daclatasvir plus asunaprevir, 24 of 25 patients [96.0%] to ombitasvir/paritaprevir/ritonavir, 22 of 22 patients [100%] to elbasvir plus grazoprevir, and 169 of 172 patients [98.3%] to sofosbuvir plus ribavirin). Among the 691 patients, 57 consented to undergo liver biopsy after having SVR. After excluding 6 patients who had abnormal ALT levels (> 42 U/L for men and > 27 U/L for women), 51 patients with normal ALT levels after having an SVR were included in analyses (referred to as '*patients with post-SVR biopsy*'). In 20 patients, liver biopsy specimens taken a median (interquartile range) of 1.2 (0.4−2.4) years before the start of DAA treatment were available (referred to as '*patients with paired biopsy*').

Liver biopsy specimens were obtained from each patient with a 15-gauge Tru-Cut needle under ultrasound guidance 41 ± 20 weeks after the end of treatment. Liver

tissues were fixed in formalin immediately after biopsy and embedded in paraffin. A portion of each biopsy specimen was also immediately placed in RNAlater (Qiagen, Valencia, CA) and temporarily stored at −20°C. We attempted to amplify the HCV RNA from 25 ng of the extracted RNA from the liver needle biopsy specimen by the sensitive Abbott RealTime assay. Four-micrometer-thick sections were stained with hematoxylin-eosin, Azan-Mallory, orcein, and Prussian blue stains. All liver biopsy specimens were evaluated by an experienced histopathologist (Y.I.) blinded to the clinical data. The histological findings were assessed by grading the inflammatory activity and staging fibrosis according to the Knodell scoring system [15] and the METAVIR scoring system [16]. Iron deposition was evaluated on a scoring system proposed by Rowe et al. [17], based on Prussian blue staining as follows: grade 0 (granules absent/barely discernible at ×400 magnification), grade 1+ (granules barely discernible at ×250); grade 2+ (discrete granules resolved at ×100); grade 3+ (discrete granules resolved at ×25); and grade 4+ (masses visible at ×10 or grossly visible). Steatosis was graded in accordance with the NAFLD activity score as follows: S1 (5% to 33% of hepatocytes affected), S2 (34% to 66% affected), and S3 (>66% affected) [18]. A liver sample was considered adequate if it was longer than 15 mm and contained 6 or more portal tracts.

Statistical analysis

Statistical analysis was performed with R software (The R Foundation for Statistical Computing, Vienna, Austria). Distributions of continuous variables were analyzed by the Mann–Whitney *U*-test. Differences in proportions were tested by Fisher's exact test. The significance of changes in values between two time points was evaluated by the Wilcoxon signed-rank test. The significance of correlations was evaluated by

Spearman's rank analysis. A P value of < 0.05 was considered to be significant.

Results

Changes in clinical parameters in the study group as a whole

As expected, serum ALT, α-fetoprotein, and type IV collagen 7S levels decreased significantly and platelet count increased significantly in the study group as a whole at the end of treatment and 12 weeks after treatment (**Supplementary Fig. 1**). In the 141 patients for whom valid elastography data were available before treatment and 12 to 48 weeks after treatment, liver stiffness significantly decreased, and the CAP marginally increased (**Supplementary Fig. 2**).

Characteristics of patients with post-SVR biopsy

Although patients with post-SVR biopsy were significantly younger than patients without biopsy at the start of therapy (**Supplementary Table 1**), other baseline characteristics did not differ among the two groups. The characteristics of the patients with post-SVR biopsy at the start of therapy and at the biopsy are shown in **Table 1**. Because patients who had abnormal ALT levels after treatment were excluded, the mean ALT level was 19 ± 12 U/L at the time of biopsy.

HCV RNA detection in serum and in liver of patients with post-SVR biopsy

Rates of undetectable HCV RNA in serum during and after treatment are shown in **Figure 2**. The rates were lower with the Abbott RealTime assay than with the COBAS TaqMan assay during treatment. Of interest, even patients in whom the serum HCV-RNA remained positive on the Abbott RealTime assay at the end of treatment could achieve an SVR after treatment. HCV RNA was not detected in the post-SVR liver biopsy specimens of the 51 patients, including the 4 patients who remained positive for serum HCV-RNA at the end of treatment.

Histological evaluations of post-SVR biopsy

The results of the histological evaluations of hepatic inflammation, fibrosis, iron deposition, and steatosis in the 51 post-SVR biopsy specimens are shown in **Figure 3.** The only one biopsy specimen with severe inflammation (A3) post-SVR showed active lymphocyte and plasma cell infiltrates in the portal area, suggesting autoimmune hepatitis (**Supplementary Fig. 3**). Among the 8 post-SVR biopsy specimens with moderate inflammation (A2), 4 showed S1 to S3 steatosis (>5% of hepatocytes affected), 3 of which also showed hepatocellular ballooning, characterizing steatohepatitis (**Supplementary Fig. 4**).

Changes in histology in patients with paired biopsy

The characteristics of patients with paired biopsy at the start of therapy and at the time of biopsy are shown in **Table 2**. The changes in histological findings in the 20 patients with evaluable biopsy pairs are shown in **Figure 4.** The METAVIR grade of inflammation (improved in 13 patients /unchanged in 6/worsening in 1) had significantly regressed ($P = 0.0043$), but the stage of fibrosis (improved in 5 patients/unchanged in 11/worsening in 4) had not ($P = 0.45$). The histological improvement, defined as a ≥2-point decrease in the Knodell inflammatory score and no worsening of the fibrosis score, was found in 11 (55%) of the 20 patients. The grade of iron accumulation (improved in 11 patients/unchanged in 7/worsening in 2) had significantly regressed ($P = 0.0093$), but the steatosis score (improved in 1 patient/unchanged in 14/worsening in 5) had not ($P = 0.10$). A representative case of a 74-year-old woman is presented in **Figure 5**. The inflammatory score decreased after obtaining an SVR, as did the grade of iron accumulation. The stage of fibrosis was unchanged (F2), but fibrous bundles/septa bridging between portal tracts became thin.

Discussion

To our knowledge, this is the first report to evaluate histological outcomes in patients with chronic hepatitis C who achieved an SVR to interferon-free DAA treatments. We examined 51 liver biopsy specimens obtained with informed consent after achieving an SVR. In the first part of this study, 18% of the post-SVR liver biopsy specimens showed clinically significant inflammation (≥A2) despite normal ALT values. In the second part of this study, regression in fibrosis was not evident in the 20 paired biopsies in the short term, although histological improvement, defined as a significant decrease in inflammatory score with no worsening of fibrosis, was found in the majority of patients who achieved an SVR to DAAs.

Steatohepatitis was one of possible causes of the residual inflammation in the post-SVR liver biopsy specimens. In this study, both the histological grade of steatosis and CAP (as determined by transient elastography) increased slightly but not significantly after the treatment. Schlevogt et al. reported that DAA-induced SVR is associated with weight gain during long-term follow-up [19]. Body weight did not change significantly in our patients during the short term (**Tables 1 and 2**). In a previous study of 214 patients who received daclatasvir plus asunaprevir therapy [20], the CAP was elevated significantly after treatment. In the era of interferon-based therapies, intrahepatic steatosis was reported to be one of the independent predictors of the development of HCC after treatment [21]. Patients with steatohepatitis should be carefully followed up with periodical liver function testing and abdominal imaging even after achieving an SVR.

In the remaining post-SVR biopsies, the cause of the residual inflammation remains unknown. Whitcomb et al. [22] reported that among the 36 allograft liver biopsy specimens from patients who achieved an SVR after receiving a liver transplant for

chronic HCV infection, 69% had histologic features of active inflammation. Nearly all patients had been treated with interferon-based regimens. They raised the possibility that chronic HCV infection might induce a dysfunctional immune response, which remains active despite viral eradication, and suggested that the inflammatory reaction to HCV persists in the liver tissue long after serologic clearance. In the present study, the grade of residual inflammation did not correlate with the interval from the end-of-treatment to the post-SVR biopsy ($r = 0.11$, $P = 0.64$, data not shown).

We believe that the occult HCV infection could not be the cause. Elmasry et al [23] reported that positive- and negative-strand HCV RNA was detected in 4 liver tissue specimens from 9 patients in whom serum ALT levels did not normalize despite an SVR to DAAs for post-transplant recurrent HCV infection. Responding to their report, we previously demonstrated that HCV RNA was not found in any liver samples obtained from 25 non-transplant patients with sustained normal ALT levels after having an SVR [9]. In the present study, we confirmed the same results in an additional 26 patients, including some in whom serum HCV RNA remained positive on the Abbott RealTime HCV assay at the end of treatment, and then achieved an SVR [24,25].

Previous studies [5] reported that liver stiffness regressed after attaining an SVR, but it remains unclear whether such improvement in the results of non-invasive tests might really reflect the reversal of liver fibrosis or reduced inflammation (or both). Knop et al. [26] showed that the dynamics of the improvement in liver stiffness were more pronounced between baseline and end of treatment than between baseline and 24 weeks after treatment. Taken together with our paired biopsy results, the short-term improvement in non-invasive test results are mainly attributable to reduced inflammation.

In patients with chronic hepatitis B, large clinical trials of entacavir or tenofovir

disoproxil fumarate showed both short- and long-term histological improvements at 1 year and 3–7 years, respectively [27,28]. Sun et al. [29] recently proposed a classification to evaluate the dynamic changes in the quality of fibrosis, namely predominantly progressive or predominately regressive and indeterminate, which displayed an overall balance between progressive and regressive scarring. As shown in **Figure 5**, predominately regressive scarring (defined as most [> 50%] fibro-septal stroma in the liver biopsy specimens showing features of scars with thin, densely compacted stroma and largely dark staining on the connective tissue stain) was found in 4 post-SVR biopsies, but none of the pre-treatment biopsies (data not shown). Because previous studies (including ours [30,31]) have shown that progressive fibrosis in liver despite an SVR is closely associated with development of HCC, longer observation is required.

Hepatic iron overload is commonly observed in patients with chronic HCV infection, and can cause liver damage by increasing oxidative stress. The mechanisms involve mainly impaired hepcidin expression and the altered expression of other iron-metabolism-related genes. Previous studies in the interferon era [32,33] showed that successful HCV eradication restored the relative impairment of hepcidin production. In this study, we showed in liver biopsy specimens for the first time that SVR to DAAs also can improve the iron overload condition.

Prati et al. [34] proposed updated upper limits of normal for ALT (30 U/L for men and 19 U/L for women), which were more sensitive for identifying persons with HCV infection. We further divided our subjects with 'normal' ALT into two groups according to the updated upper limits of normal (≤30 U/L or 31–42 U/L for men and ≤19 U/L or 20–27 U/L for women) (**Supplementary Table 2**). The grade of inflammation was higher in patients whose ALT was normal but 31–42 U/L in men or 20–27 U/L in

women than in those whose ALT was ≤30 U/L in men or ≤19 U/L in women (P = 0.0011).

Our study had several limitations. First, because this was a retrospective study of a real-world cohort, the timing of liver biopsy was not uniform. In particular, the first paired biopsy specimens were taken a median (interquartile range) of 1.2 (0.4−2.4) years before the start of DAA treatment. However, previous studies showed that the rate of fibrosis progression was only 0.10–0.133 fibrosis unit per year in the natural history of untreated patients with chronic hepatitis C [2,35]. Therefore, the stage of liver fibrosis was probably unchanged during the lag time in the majority of patients. Second, there may be a selection bias, since the difference in age was significant between patients with post-SVR biopsy and patients without post-SVR biopsy. However, the proportions of patients with cirrhosis and other baseline characteristics did not differ between patients with post-SVR biopsy and those without biopsy. Lastly, because of the limited number of patients and short duration of observation, it was difficult to evaluate the impact of a SVR to DAAs on the regression of liver fibrosis in our study. Large, long-term studies are needed to draw final conclusions.

In summary, histological improvement, defined as a significant decrease in inflammatory score with no worsening of fibrosis, was observed in 55% of the patients with an SVR to DAA treatment. However, improvement in liver fibrosis was not evident in this short-term study. Even if ALT normalized after obtaining an SVR, clinically significant histological inflammation remained in 16% of the patients. The residual inflammation was attributable to steatohepatitis at least in some patients, but was of unknown cause in others.

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Declaration of conflict of interest

Norifumi Kawada has received lecture fees from Gilead Sciences Inc., AbbVie Inc., and MSD K.K., and research grants from Gilead and AbbVie.

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Ethics approval

The procedures in this study were in accordance with the Helsinki Declaration of 1964 (2013 revision) and approved by the Ethics Committee of Osaka City University Graduate School of Medicine on 23 July 2015 (No. 2905).

Informed consent

Written informed consent was obtained from each patient.

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Table 1. Characteristics of the 51 patients with post-SVR biopsy

	At the start of treatment	At the post-SVR biopsy
Gender (Female/male)	28/23	
Age (years)	63.6 ± 10.3	64.6 ± 10.3
Body mass index (kg/m^2)	24.3 ± 3.3	24.4 ± 3.2
Diabetes (%)	14 (27%)	
Treatment-*naïve* (%)	29 (57%)	
Clinical cirrhosis (%)	7 (14%)	
Past HCC treatment (%)	4 (8%)	
Interleukin-28B genotype TG/GG	17 (33%)	
HBcAb-*positive*	20 (39%)	
HCV genotype (1a/1b/2a/2b)	0/36/10/5	
HCV RNA (Log_{10} IU/mL)	6.1 ± 0.8	Undetectable
ALT (IU/L)	68 ± 68	19 ± 12
α-fetoprotein (ng/mL)	9.5 ± 19.7	4.9 ± 4.5
Type IV collagen 7S domain (ng/mL)	5.8 ± 2.8	5.1 ± 1.6
Platelet count (x10^3/μL)	163 ± 47	178 ± 54

NOTES: Patients with HCV genotype 1b infection were treated with ledipasvir/sofosbuvir (n = 23), daclatasvir plus asunaprevir (n = 10), elbasvir plus grazoprevir (n = 2), or ombitasvir/paritaprevir/ritonavir (n = 1). Patients with HCV genotype 2a or 2b infection were treated with sofosbuvir plus ribavirin (n = 15). Liver biopsy specimens were obtained 41 ± 20 weeks after the end of treatment.

Table 2. Characteristics of the 20 patients with paired biopsy

	At the start of treatment	At the post-SVR biopsy
Gender (female/male)	13/7	
Age (years)	62.0 ± 10.9	63.1 ± 10.7
Body mass index (kg/m^2)	23.6 ± 3.4	23.5 ± 3.3
Diabetes (%)	3 (15%)	
Treatment-*naïve* (%)	8 (40%)	
Clinical cirrhosis (%)	3 (15%)	
Past HCC treatment (%)	1 (5%)	
Interleukin-28B genotype TG/GG	9 (45%)	
HBcAb-*positive*	6 (30%)	
HCV genotype (1a/1b/2a/2b)	0/15/4/1	
HCV RNA (Log_{10} IU/mL)	6.1 ± 0.8	Undetectable
ALT (IU/L)	85 ± 91	21 ± 16
α-fetoprotein (ng/mL)	16.2 ± 31.2	5.4 ± 5.7
Type IV collagen 7S domain (ng/mL)	9.4 ± 9.8	5.3 ± 2.2
Platelet count (x10^3/μL)	154 ± 45	173 ± 55
Liver stiffness (kPa)	11.5 ± 6.4	7.7 ± 5.4
Controlled attenuation parameter (dB/m)	222 ± 48	231 ± 54
Grade of inflammation (A0/A1/A2/A3)	1/11/8/0	9/7/3/1
Stage of fibrosis (F0/F1/F2/F3/F4)	1/7/6/5/1	3/7/5/2/3
Grade of iron deposition (+0/+1/+2/+3/+4)	6/8/1/4/1	14/2/2/2/0
Grade of steatosis (S0/S1/S2/S3/S4)	13/7/0/0/0	10/9/1/0/0

NOTES: Patients with HCV genotype 1b infection were treated with ledipasvir/sofosbuvir (n = 6), daclatasvir plus asunaprevir (n = 7), or elbasvir plus grazoprevir (n = 2). Patients with HCV genotype 2a or 2b infection were treated with sofosbuvir plus ribavirin (n = 5). Liver biopsy specimens were obtained 44 ± 22 weeks after the end of treatment.

Supplementary Table 1. Baseline characteristics of the patients with and without post-SVR biopsy

	Patients without post-SVR biopsy (*n* = 655)	Patients with post-SVR biopsy (*n* = 51)	*P* value
Age (years)	66.1 ± 12.3	63.6 ± 10.3	0.014
Gender (Female/male)	345/310	28/23	0.77
Treatment-*naïve*	417 (64%)	29 (57%)	0.37
Clinical cirrhosis (%)	114 (17%)	7 (14%)	0.70
Past HCC treatment (%)	77 (12%)	4 (8%)	0.50
Interleukin-28B genotype TG/GG	151 (33%)	17 (33%)	>0.99
HBcAb-*positive*	20 (39%)	234 (38%)	0.88
HCV genotype (1a/1b/2a/2b)	7/485/97/63	0/36/10/5	0.72
HCV RNA (Log_{10} IU/mL)	6.0 ± 0.8	6.1 ± 0.8	0.17
ALT (IU/L)	51 ± 44	68 ± 68	0.19
α-fetoprotein (ng/mL)	14.6 ± 42.2	9.5 ± 19.7	0.55
Type IV collagen 7S (ng/mL)	6.0 ± 2.6	5.8 ± 2.8	0.78
Platelet count ($x10^3$/μL)	162 ± 62	163 ± 47	0.99
Regimen (SOF+LDV/SOF+RBV/ DCV+ASV/GZR+EBR/OBV+PTVr)	312/157/142/23/21	23/15/10/2/1	0.91

ASV, asunaprevir; DCV, daclatasvir; EBR, elbasvir; GZR, grazoprevir; HBcAb, hepatitis B core antibody; LDV, ledipasvir; OBV, ombitasvir; PTVr, paritaprevir + ritonavir; RBV, ribavirin; SOF, sofosbuvir

Supplementary Table 2. METAVIR grade of inflammation and stage of fibrosis according to ALT levels

	Patients with normal ALT levels of ≤ 30 U/L for men and ≤ 19 U/L for women (*n* = 40)	Patients with normal ALT levels of 31–42 U/L for men and 20–27 U/L for women (*n* = 11)	Patients with abnormal ALT levels of > 42 U/L for men and > 27 U/L for women (*n* = 6)
Grade of inflammation (A0/A1/A2/A3)	16/21/2/1	2/3/6/0	2/3/1/0
Stage of fibrosis (F0/F1/F2/F3/F4)	3/19/11/5/2	0/1/4/4/2	0/1/3/1/1

Among patients with normal ALT levels, the grade of inflammation was higher in men whose ALT level was 31–42 U/L or women whose ALT level was 20–27 U/L than in men whose ALT level was ≤ 30 U/L or women whose ALT level was ≤ 19 U/L ($P = 0.0011$).

Figure Legends

Figure 1

Study flowchart. Fifty-one patients with normal ALT levels after having SVR were referred to as '*patients with post-SVR biopsy*'. Twenty patients in whom liver biopsy specimens were taken a median of 1.2 years before the start of DAA treatment were referred to as '*patients with paired biopsy*'.

Figure 2

Rates of undetectable HCV RNA in serum of the 51 patients with post-SVR biopsy during and after treatment. Serum HCV RNA was not detected at the end of treatment or at 12 weeks post-treatment in any SVR patient with use of the TaqMan assay. In contrast, with use of the RealTime assay, serum HCV RNA remained positive at the end of treatment in 4 (8%) patients, but became negative at 4–12 weeks post-treatment.

Figure 3

Results of the histological evaluations of hepatic inflammation (**A**), fibrosis (**B**), iron deposition (**C**) and steatosis (**D**) in the 51 post-SVR biopsy specimens. The grade of inflammation was A0 in 18 patients, A1 in 24, A2 in 8, and A3 in 1; the stage of fibrosis was F0 in 3 patients, F1 in 20, F2 in 15, F3 in 9, and F4 in 4; the grade of iron deposition was 0 in 29 patients, 1+ in 10, 2+ in 6, 3+ in 5, and 4+ in 1; and the grade of steatosis was S0 in 29 patients, S1 in 15, S2 in 6, and S3 in 1.

Figure 4

Changes in histological findings in the 20 patients with evaluable pairs of biopsy specimens. The grade of inflammation (**A**) had significantly regressed ($P = 0.0043$), but

the stage of fibrosis (**B**) had not ($P = 0.45$). The grade of iron accumulation (**C**) had significantly regressed ($P = 0.0093$), but the score of steatosis (**D**) had not ($P = 0.10$).

Figure 5

Liver biopsy specimens obtained from a 74-year-old woman with genotype 1b infection who relapsed after peginterferon-ribavirin plus faldaprevir treatment previously and then achieved an SVR to ledipasvir/sofosbuvir treatment (Azan-Mallory and Prussian blue stains; original magnification, all ×100): METAVIR score of A2F2 before therapy (**A**) and A1F2 after therapy (**B**). Fibrous bundles/septa bridging between portal tracts are thinner in post-SVR (arrowheads). As aminotransferases decreased (AST from 35 to 33 U/L and ALT from 31 to 26 U/L), the inflammatory score also decreased after obtaining an SVR, as did the grade of iron accumulation evaluated with Prussian blue stain.

Supplementary Figures

Supplementary Figure 1

Changes in serum ALT (**A**), α-fetoprotein (**B**), type IV collagen 7S levels (**C**), and platelet count (**D**) during and after DAA treatment in the study group as a whole. EOT, end of treatment. *, $P < 0.05$.

Supplementary Figure 2

Changes in liver stiffness (**A**) and CAP (**B**) in the 141 patients in whom valid elastography data were available before therapy and 12–48 weeks post-treatment.

Supplementary Figure 3

Liver biopsy specimens obtained from a 63-year-old woman with genotype 2a infection who relapsed after peginterferon-ribavirin treatment previously and then achieved an SVR to sofosbuvir plus ribavirin treatment (hematoxylin-eosin stain; original magnification, both ×100): METAVIR score of A1F3 before therapy (**A**) and A3F4 after therapy (**B**). Despite normalized aminotransferases (AST from 46 to 23 U/L and ALT from 70 to 19 U/L), active lymphocyte and plasma cell infiltrates were observed in the portal area, and autoimmune hepatitis was a possible cause of the severe inflammation.

Supplementary Figure 4

Liver biopsy specimens obtained from a 74-year-old woman with genotype 1b infection who did not respond to peginterferon-ribavirin treatment previously and then achieved an SVR to daclatasvir plus asunaprevir treatment [hematoxylin-eosin stain; original magnification, (**A**) ×100, (**B**) ×200]: METAVIR score of A2F4 before therapy (**A**) and A2F4 after therapy (**B**). Despite decreased aminotransferases (AST from 106 to 42 U/L

and ALT from 97 to 27 U/L), steatosis, lobular inflammation, and hepatocyte ballooning (arrowhead) were observed in the post-SVR specimen, and steatohepatitis is a possible cause of the residual inflammation.

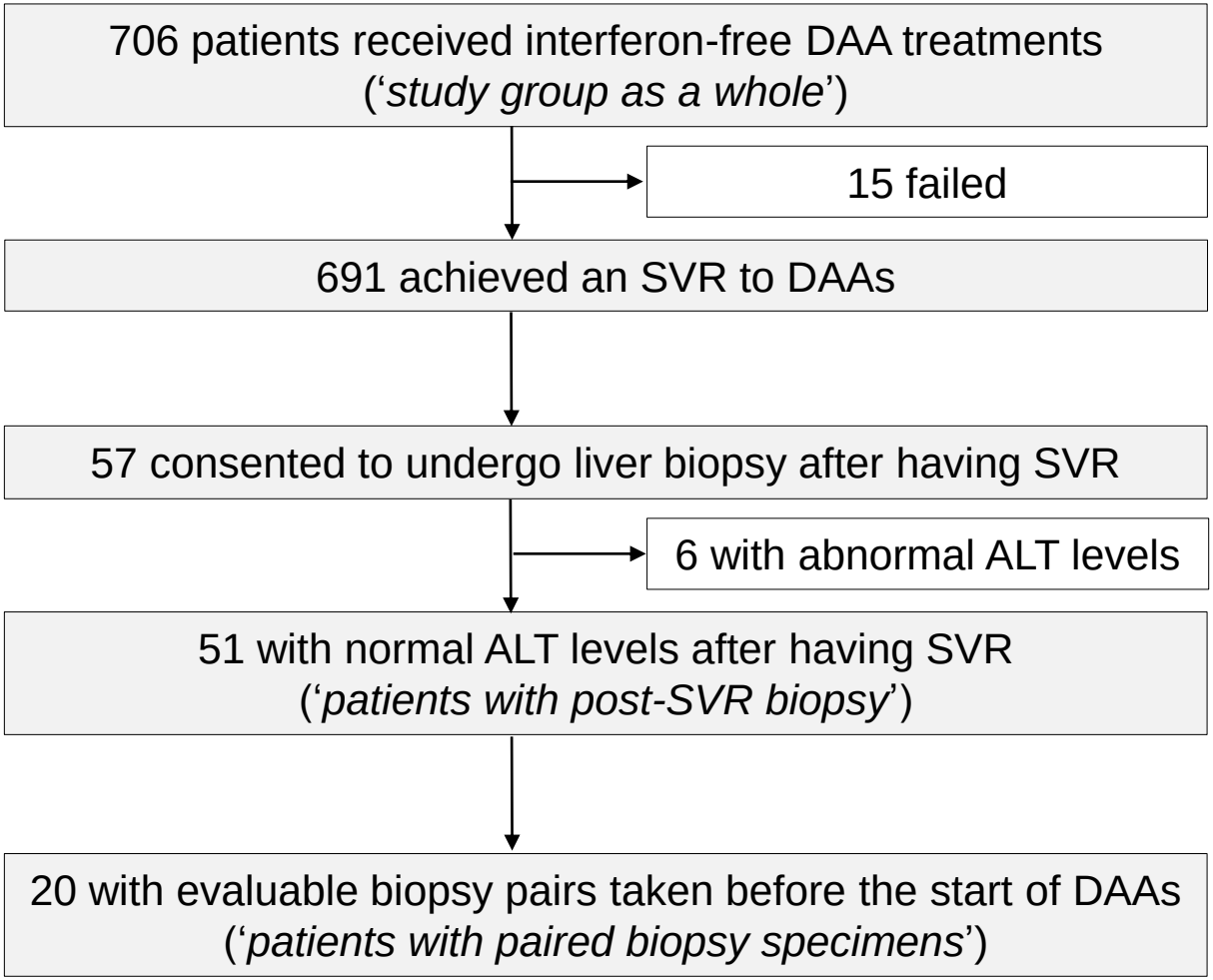

706 patients received interferon-free DAA treatments
('study group as a whole')
15 failed
691 achieved an SVR to DAAs
57 consented to undergo liver biopsy after having SVR
6 with abnormal ALT levels
51 with normal ALT levels after having SVR
('patients with post-SVR biopsy')
20 with evaluable biopsy pairs taken before the start of DAAs
('patients with paired biopsy specimens')

Figure 1

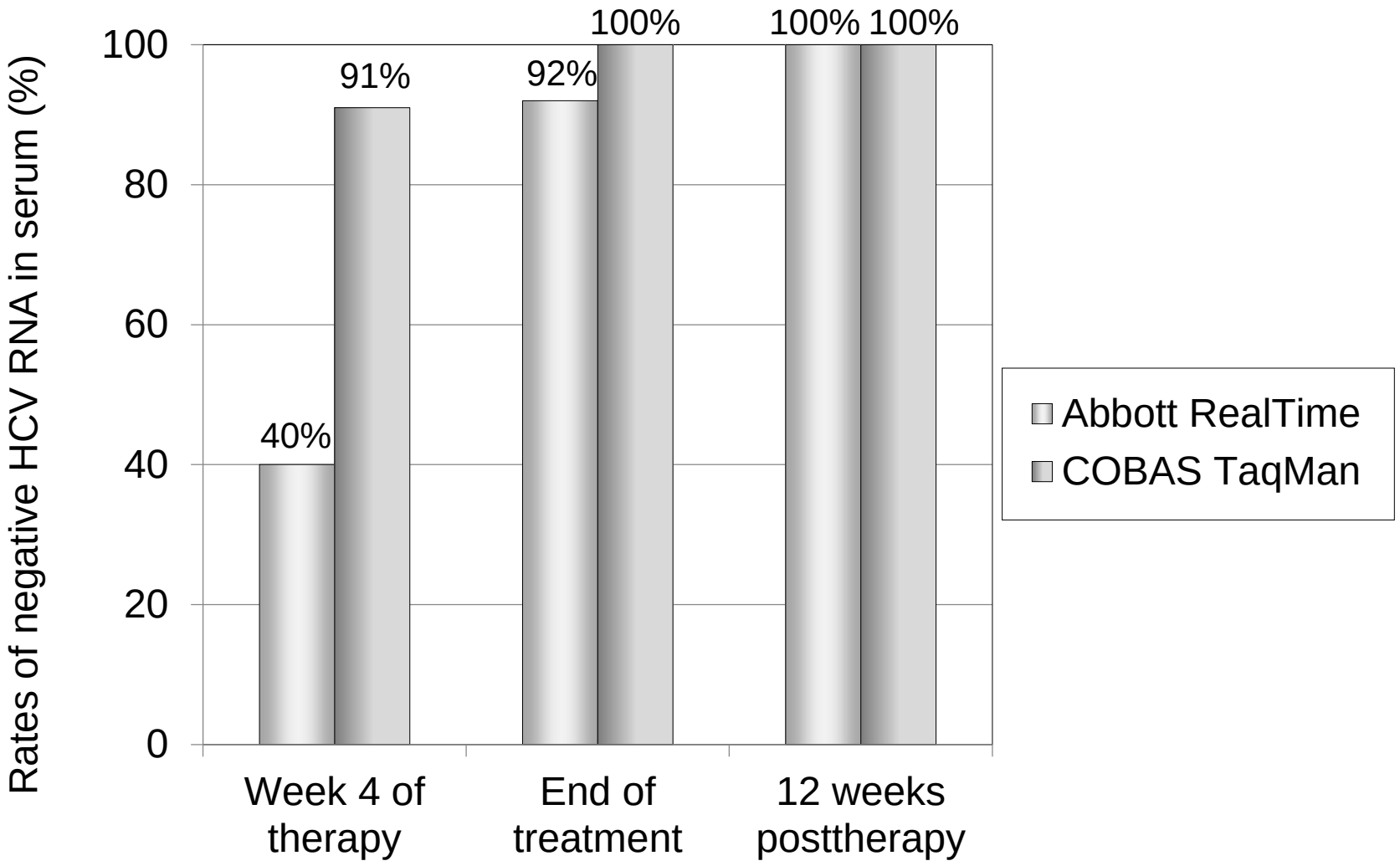

Rates of negative HCV RNA in serum (%)
100
80
60
40
20
0
40%
91%
92%
100%
100%
100%
Week 4 of therapy
End of treatment
12 weeks posttherapy
Abbott RealTime
COBAS TaqMan

Figure 2

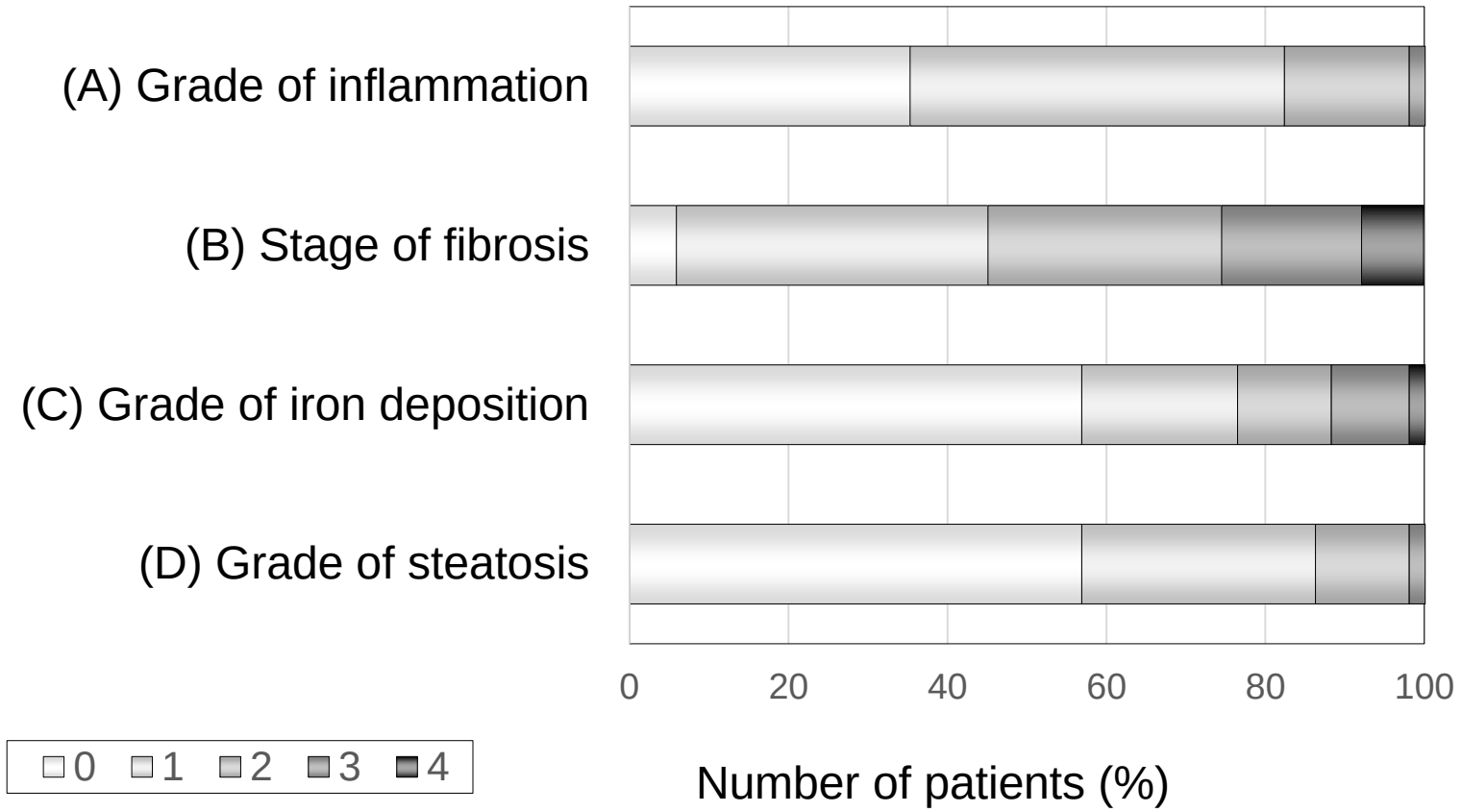

(A) Grade of inflammation
(B) Stage of fibrosis
(C) Grade of iron deposition
(D) Grade of steatosis
0
20
40
60
80
100
0
1
2
3
4
Number of patients (%)

Figure 3

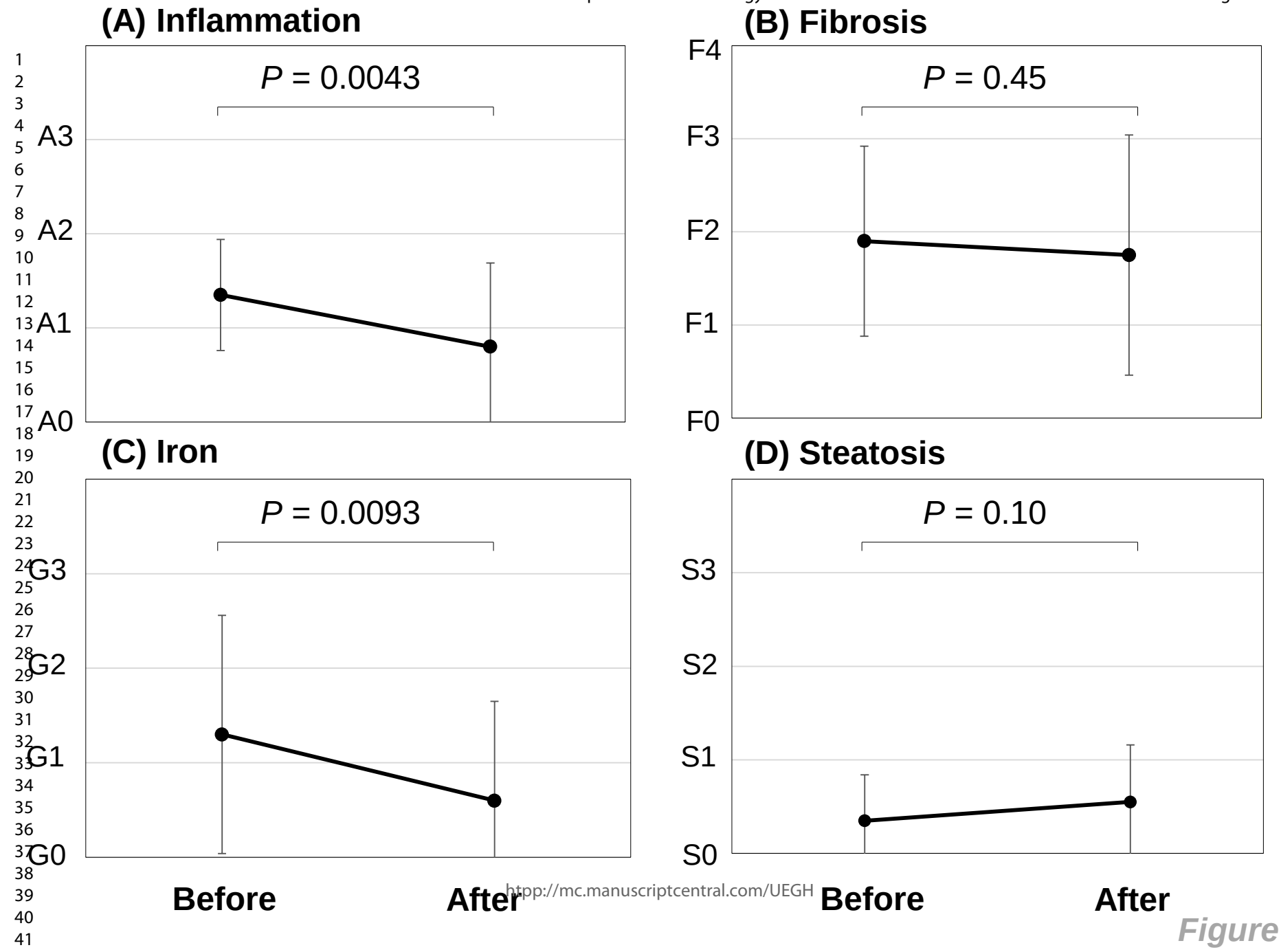

(A) Inflammation
P = 0.0043
A3
A2
A1
A0
(B) Fibrosis
P = 0.45
F4
F3
F2
F1
F0
(C) Iron
P = 0.0093
G3
G2
G1
G0
Before
After
(D) Steatosis
P = 0.10
S3
S2
S1
S0
Before
After

Figure 4

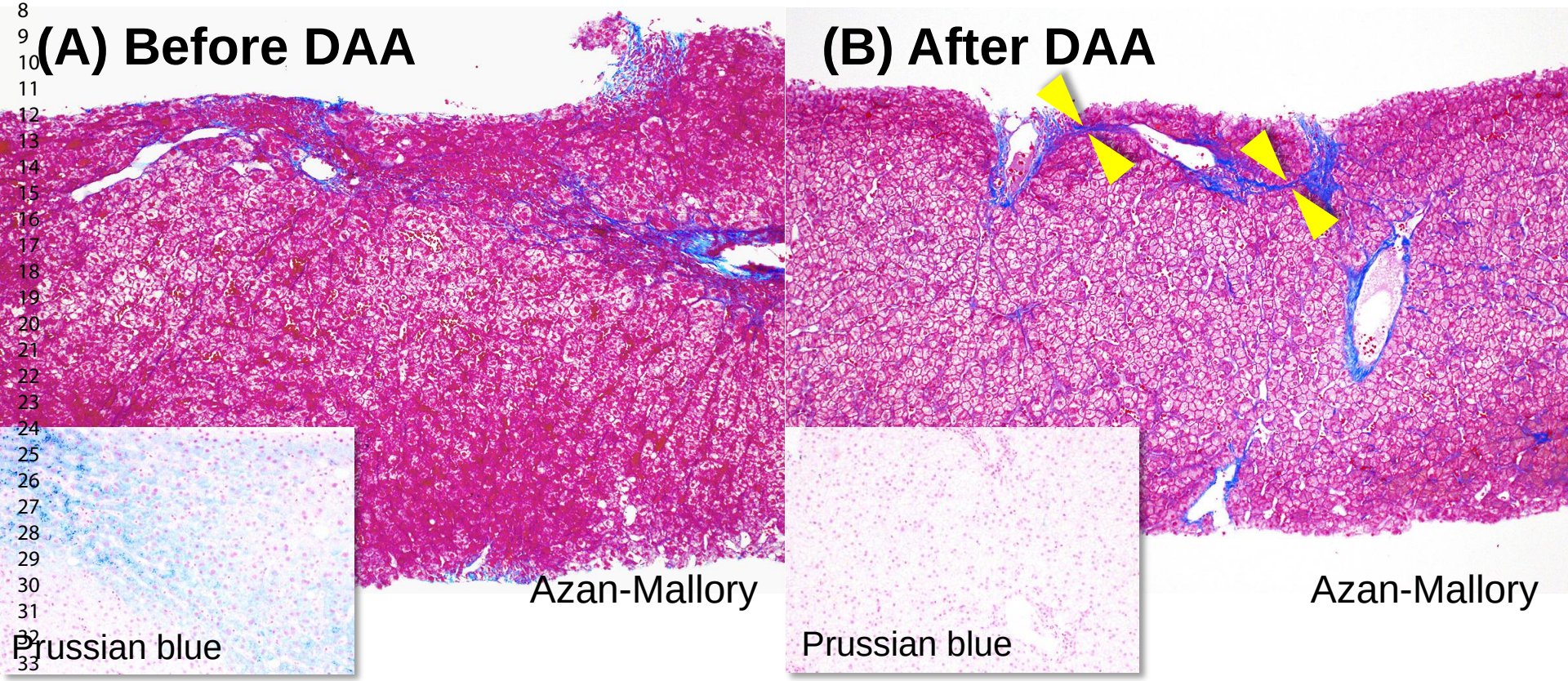
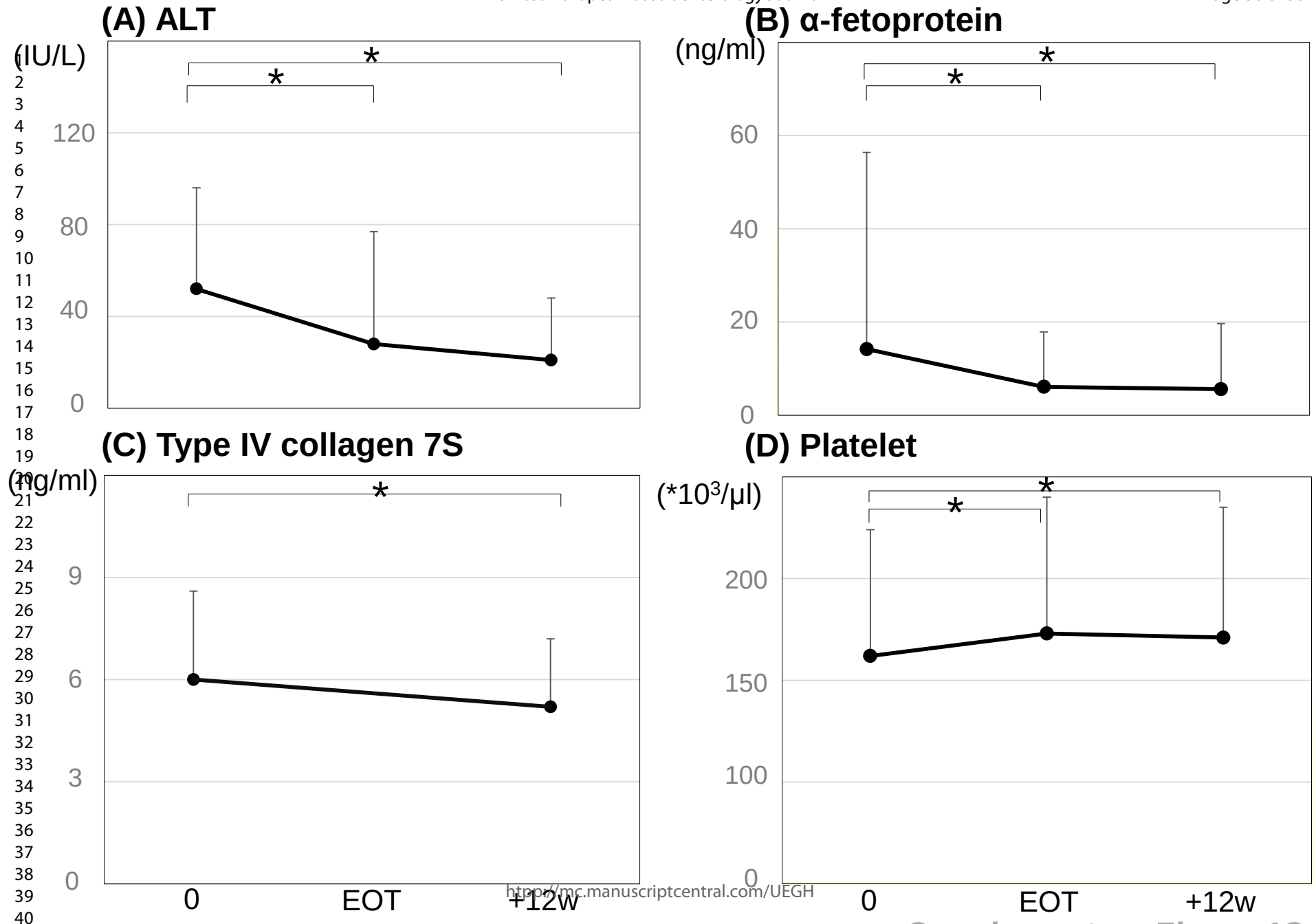
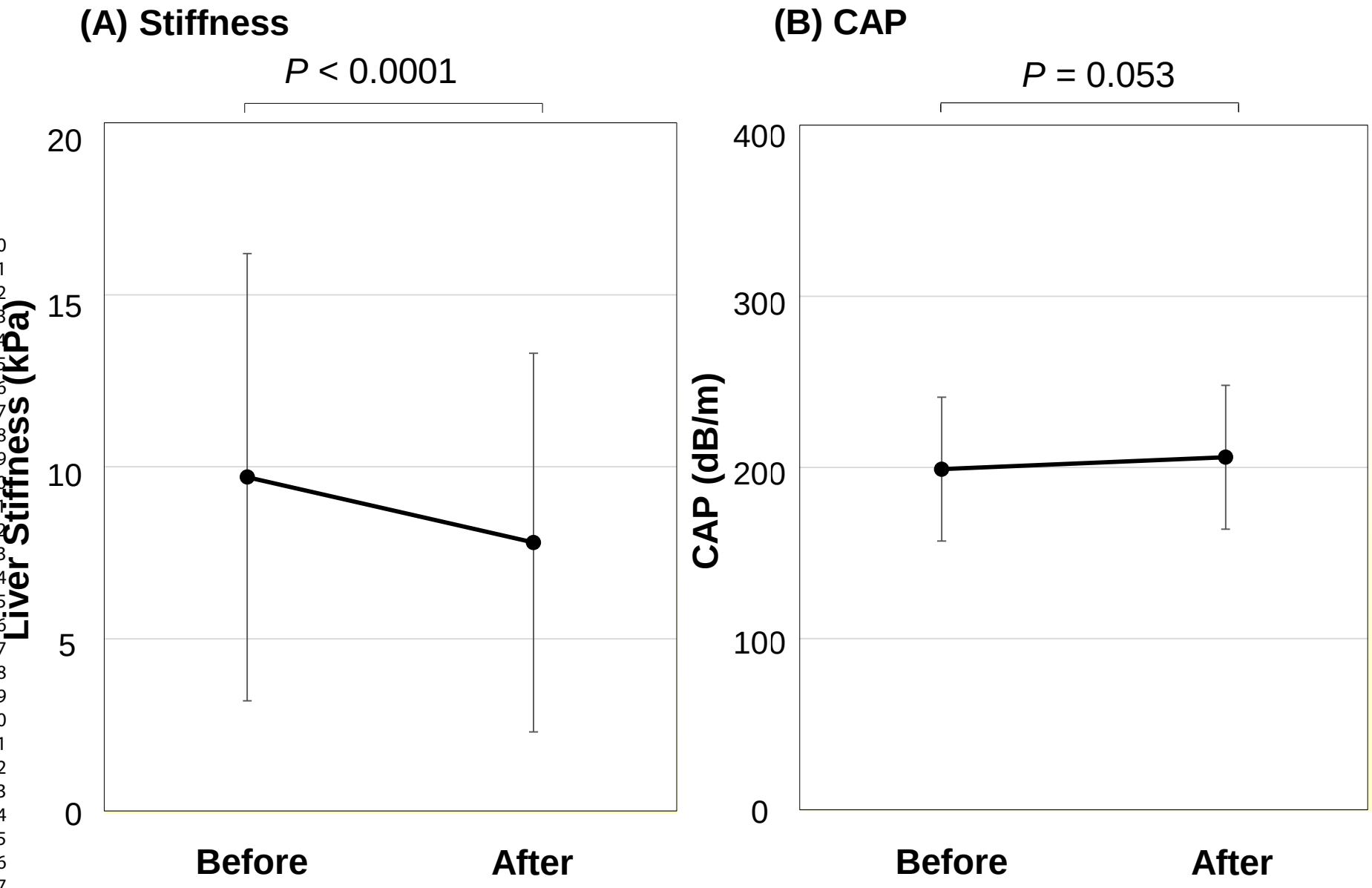

(A) Before DAA
Azan-Mallory
Prussian blue
(B) After DAA
Azan-Mallory
Prussian blue

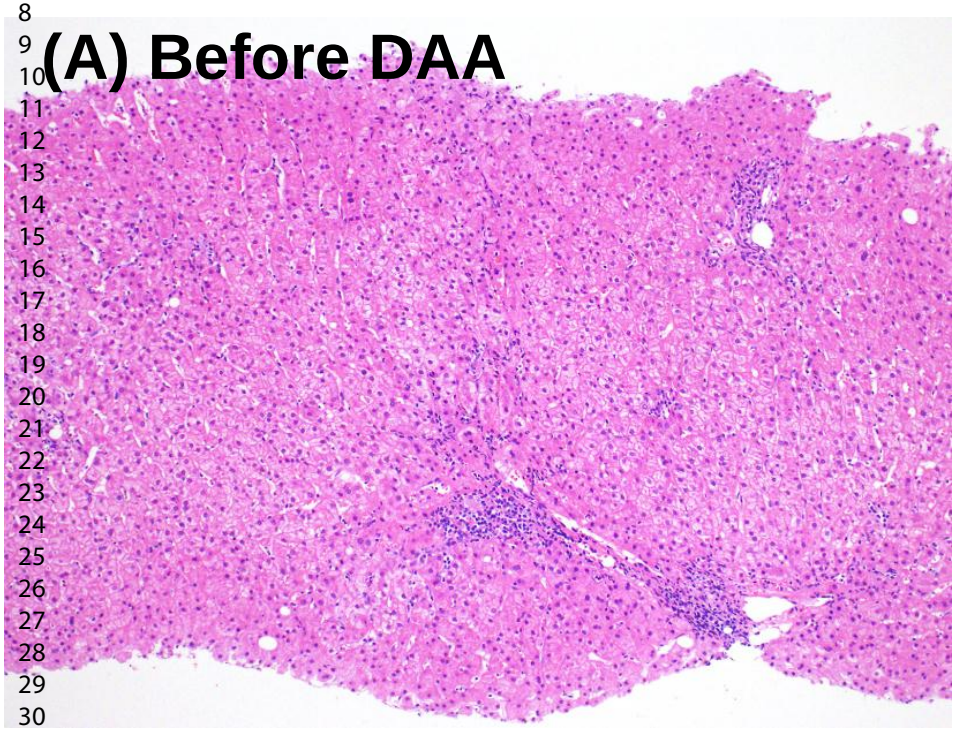
Figure 5

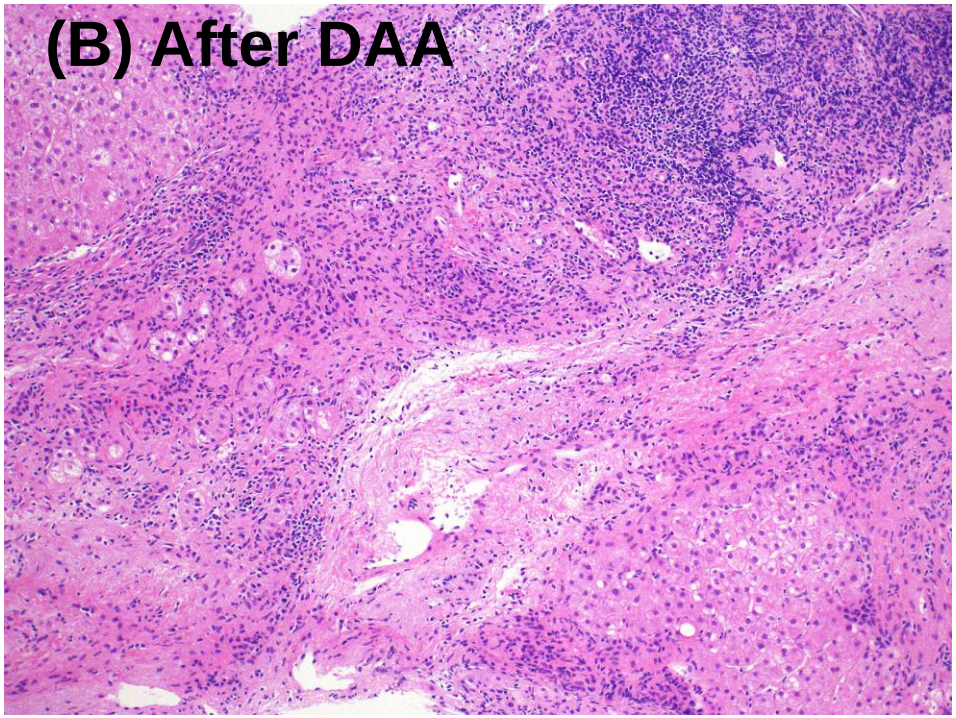


Supplementary Figure 1S

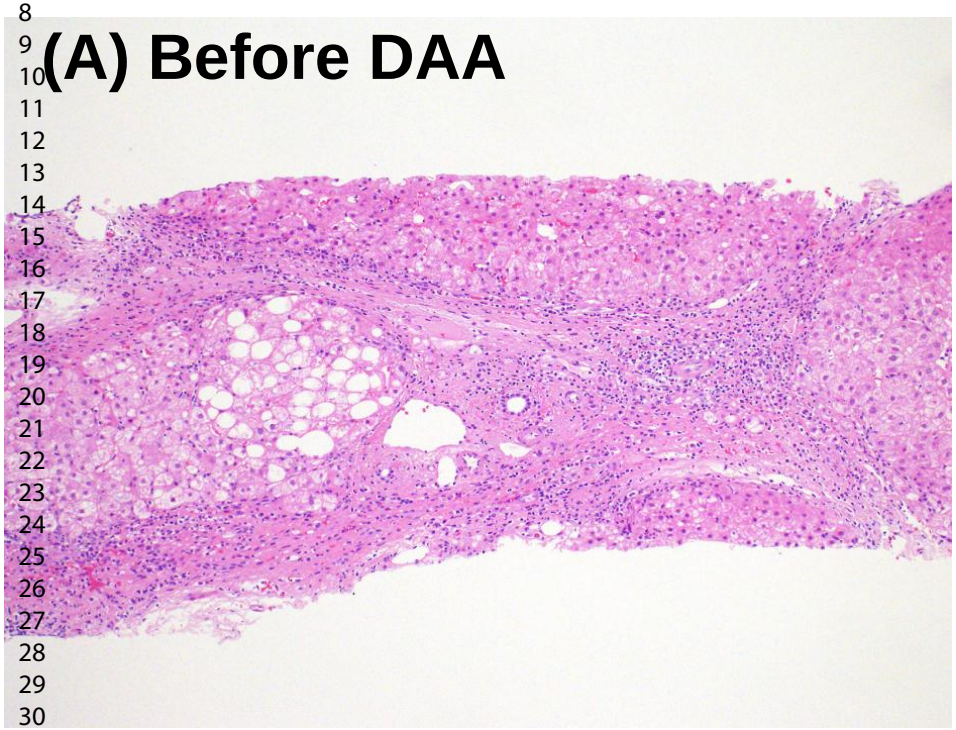


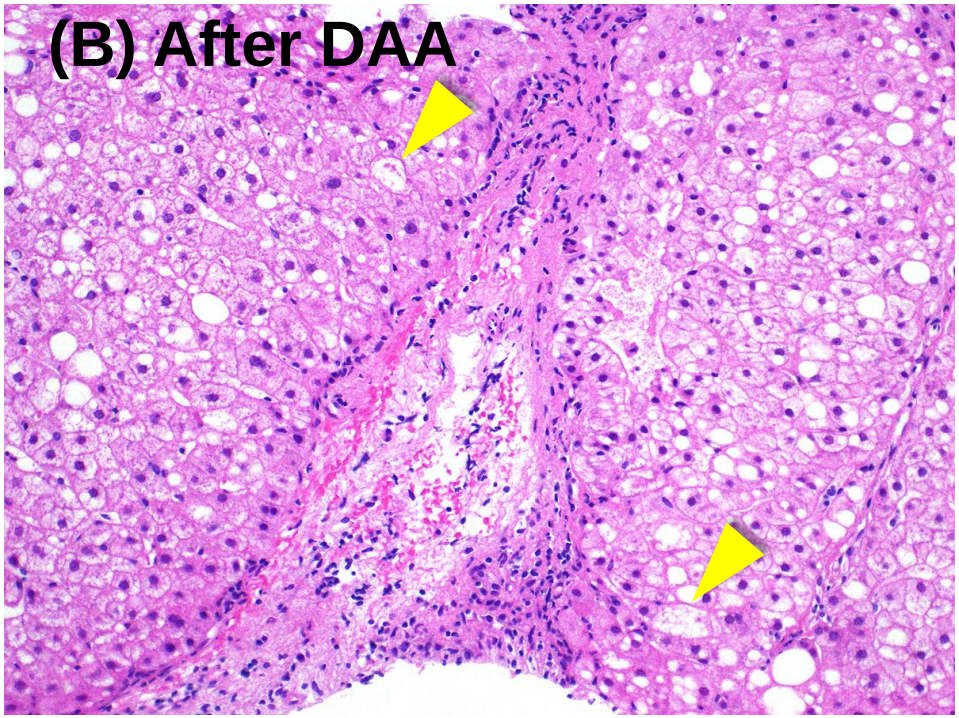
Supplementary Figure 2S





Supplementary Figure 3S





Supplementary Figure 4S