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Original Article

Predictors of liver fibrosis changes assessed by paired liver biopsies in chronic hepatitis C patients treated with direct-acting antivirals

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Liver biopsy;
METAVIR score

Abstract *Background/Purpose:* There are limited studies performing paired liver biopsies in chronic hepatitis C (CHC) patients treated with direct-acting antivirals (DAA). We aimed to investigate the predictors of liver fibrosis changes assessed by paired liver biopsies in these patients.
Methods: From March 2017 to March 2020, 113 CHC patients were prospectively enrolled to receive DAA therapy at our hospital. Paired liver biopsies were performed at baseline and 12 weeks after the end of treatment.
Results: Among the entire cohort, the rate of sustained virological response (SVR) was 100%. Four baseline variables independently predicted fibrosis regression, including age <65 years [odds ratio (OR) = 2.725, p = 0.036], fibrosis stages (METAVIR scores) < 3 (OR = 4.874, p = 0.040), hemoglobin levels ≥12.5 g/dL (OR = 3.538, p = 0.029), and platelet counts ≥160 $10^3/\mu L$ (OR = 2.958, p = 0.023). Besides, five independent predictors of fibrosis progression included baseline age ≥66 years (OR = 16.351, p = 0.024), body mass index (BMI) ≥26.5 kg/m^2 (OR = 21.666, p = 0.009), sofosbuvir/ribavirin use (OR = 29.465, p = 0.031), platelet counts <119 $10^3/\mu L$ (OR = 33.739, p = 0.026), and the absence of alanine aminotransferase (ALT) levels declining from >35 U/L at baseline to ≤35 U/L at 4 weeks after baseline (OR = 284.534, p = 0.026).

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Conclusion: For DAA-treated CHC patients, those with baseline age <65 years, fibrosis stages <3, hemoglobin levels ≥12.5 g/dL, or platelet counts ≥160 $10^3/\mu L$ are more likely to attain fibrosis regression. There is a higher risk of fibrosis progression in those with baseline age ≥66 years, BMI ≥26.5 kg/m^2, sofosbuvir/ribavirin use, platelet counts <119 $10^3/\mu L$, or the absence of early ALT normalization at 4 weeks after baseline.

Introduction

Globally, 56.8 million prevalent hepatitis C virus (HCV) infections were estimated at the beginning of 2020[1] with approximately 290 thousand deaths mostly resulting from HCV-related cirrhosis and hepatocellular carcinoma (HCC) in 2019.[2] For chronic hepatitis C (CHC), direct-acting antivirals (DAA) have replaced interferon-based therapy as the standard treatment since 2016.[3—5] The purpose of DAA therapy is to eradicate HCV viremia and achieve a sustained virological response (SVR) which is considered the primary goal of anti-HCV treatment.[5] Nonetheless, post-SVR surveillance is still recommended under certain circumstances.[5,6] For patients with pretreatment F2 (METAVIR score) fibrosis and achieving SVR, follow-up of fibrosis changes is suggested to ensure that no fibrosis progression occurs after viremia eradication.[6] Besides, SVR-achieving patients with pretreatment F3 or F4 fibrosis should undergo HCC surveillance every 6 months by ultrasonography.[5,6] Given that the progression from fibrosis to cirrhosis and HCC is an extended process,[7] it is important to confirm whether DAA reverse fibrosis and reduce the risk of fibrosis-related complications in CHC patients. In addition, to further elucidate the histopathological evolution of DAA-treated CHC and prevent fibrosis progression, it is necessary to investigate the predictors of fibrosis changes in CHC patients receiving DAA.

Although noninvasive tools and biochemical markers for evaluating the severity of liver diseases have been widely performed,[8] liver biopsy remains standard for direct and tissue-specific assessment of fibrosis changes.[9,10] Several studies have reported that liver stiffness (LS)[11—25] and the levels of noninvasive fibrosis biomarkers, including aspartate aminotransferase (AST)-platelet ratio index,[12,13,17,22,23,25,26] fibrosis-4 score,[12,13,17,20,22,23] and microfibrillar-associated protein 4,[26] significantly decreased in DAA-treated CHC patients achieving SVR. Besides, some factors have been shown to correlate with the decline or a more pronounced decline in LS among DAA-treated CHC patients with or without SVR, including the absence of liver steatosis,[14] the presence of cirrhosis,[16,20] elevated levels of alanine aminotransferase (ALT) at baseline,[21] and HCV genotype 1.[21] However, all of these proposed results were based on noninvasive methodologies instead of liver biopsies. As for studies providing liver histological data,[27—29] paired liver biopsies were performed in a small group of SVR cases (Chen et al.: 21 cases[27]; Enomoto et al.: 20 cases[28]; Pan et al.: 15 cases[29]), and predictors of fibrosis changes were not investigated. Therefore, this study firstly aimed to assess liver histological changes in DAA-treated CHC patients accepting paired liver biopsies. Secondly, to improve post-SVR management of CHC patients treated with DAA, we further evaluated the predictors of fibrosis changes following treatment success.

Methods

Study cohort

From March 2017 to March 2020, a prospective cohort of 113 CHC patients, defined as those with the presence of anti-HCV antibodies in serum for at least six months and detectable serum HCV ribonucleic acid (RNA), was enrolled to receive interferon-free DAA therapy at China Medical University Hospital, Taichung, Taiwan. At each visit during pretreatment, on-treatment, and post-treatment periods, patients accepted a detailed physical examination, a complete blood count, and biochemical tests of anti-HCV antibodies, AST, ALT, bilirubin, albumin, international normalized ratio (INR), alpha-fetoprotein (AFP), and creatinine as criteria. Besides, the test of serum HCV RNA levels was performed at baseline, 4 weeks after baseline, the end of treatment (EOT), and 12 weeks after EOT. An undetectable level of serum HCV RNA at 4 weeks after baseline, EOT, or 12 weeks after EOT was defined as a rapid virological response (RVR), a virological response at EOT, or SVR12 (treatment endpoint), respectively. To assess intrahepatic histological changes, all patients received liver biopsies at baseline and 12 weeks after EOT.

Assessment of liver biopsies

Paired liver biopsies were performed by the same hepatologist (Jung-Ta Kao). Enough specimens obtained percutaneously from the right-lobe liver (mainly segment seven) were evaluated by experienced pathologists in a blinded fashion. The results of liver biopsies were assessed according to the METAVIR scoring system in which fibrosis was staged on a five-point scale (F0: no fibrosis; F1: portal fibrosis without septa; F2: portal fibrosis with rare septa; F3: numerous septa without cirrhosis; F4: cirrhosis), and activity of hepatic necroinflammation was graded according to the intensity of necroinflammatory lesions (A0: no activity; A1: mild activity; A2: moderate activity; A3: severe activity).[30]

Evaluation of histological and biochemical changes

Liver histological changes were assessed by comparing the METAVIR scores at different time points. Fibrosis changes were defined as follows: regression as decreased,

stabilization as unchanged, and progression as increased fibrosis stages. Changes in hepatic necroinflammatory activity were categorized into improved, maintaining-at-A0, unchanged, and worsened activity grades. Biochemical changes were evaluated by comparing the biochemical values at different time points (baseline vs. 4 weeks after baseline or baseline vs. EOT).

Statistical analysis

Statistical analysis was performed with SPSS 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Macintosh, Version 20.0. Armonk, NY: IBM Corp.). Nominal and ordinal data were shown as absolute frequencies with relative proportions and compared by using the Fisher's exact test. Continuous data were presented as medians with interquartile ranges (IQR) and compared by using the Mann-Whitney *U* test. The odds ratio (OR) and its 95% confidence interval (CI) were generated with binary logistic regression; variables showing a p-value <0.05 in univariate analysis were entered into multivariate analysis. All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant.

Results

Patient demographics

Table 1 provides clinical profiles of the entire cohort and specific participants. In the entire cohort (n = 113), 33 cases (29.2%) had been treated with interferon-based therapy before receiving DAA; all of them were treated with pegylated interferon (PegIFN)-α/ribavirin therapy and failed to achieve SVR at 24 weeks after EOT (SVR24), thus indicated for DAA. Among these treatment-experienced patients, 21 cases (63.6%) had received a liver biopsy, in addition to paired liver biopsies performed during the course of DAA therapy, at baseline of PegIFN-α/ribavirin therapy; the median duration between liver biopsies performed at baseline of PegIFN-α/ribavirin and DAA therapy was 7.42 (IQR: 6.42—8.00) years. Compared with treatment-experienced patients, treatment-naïve patients had a significantly lower median level of body mass index (BMI) [24.20 (IQR: 22.19—26.24) vs. 26.57 (IQR: 23.76—28.55) kg/m^2, p = 0.026] or serum HCV RNA [1.25 (IQR: 0.30—3.30) vs. 3.16 (IQR: 1.42—5.23) 10^6 IU/mL, p = 0.013], a significantly lower rate of comorbid diabetes mellitus plus hypertension (13.8% vs. 33.3%, p = 0.034) or the use of daclatasvir (Daklinza®) plus asunaprevir (Sunvepra®) (0.0% vs. 9.1%, p = 0.023), and a significantly higher rate of cases without diabetes mellitus and hypertension (50.0% vs. 24.2%, p = 0.013) or with HCV genotype 2 (33.8% vs. 12.1%, p = 0.021) (Table 1). For treatment-experienced patients, the rate of males or F2 fibrosis at baseline of DAA therapy was significantly lower in those receiving liver biopsies twice than in those receiving liver biopsies thrice (males: 25.0% vs. 71.4%, p = 0.014; baseline F2 fibrosis: 0.0% vs. 38.1%, p = 0.030; Table 1).

Changes of liver fibrosis

Fig. 1 shows the profiles of liver fibrosis in the entire cohort (n = 113; Fig. 1A and B), treatment-naïve patients (n = 80; Fig. 1C and D), treatment-experienced patients (n = 33; Fig. 1E and F), and those receiving liver biopsies thrice among treatment-experienced patients (n = 21; Fig. 1G and H). Fibrosis regression, stabilization, and progression were presented in 44.2%, 46.9%, and 8.8% of the entire cohort, respectively (Fig. 1B). Most of the cases among the entire cohort, treatment-naïve, and treatment-experienced patients experienced fibrosis regression or stabilization, while cases with fibrosis progression were also observed (Fig. 1B, D, and 1F).

In terms of fibrosis changes from baseline of PegIFN-α/ribavirin therapy to baseline of DAA therapy, fibrosis regression, stabilization, and progression were observed in 33.3%, 42.9%, and 23.8% of the studied patients, respectively (Fig. 1H). More than 50% of the cases with fibrosis regression from baseline of PegIFN-α/ribavirin therapy to baseline of DAA therapy experienced further fibrosis regression at 12 weeks after the end of DAA therapy (Fig. 1H). As for cases with fibrosis progression from baseline of PegIFN-α/ribavirin therapy to baseline of DAA therapy, 80.0% showed fibrosis regression at 12 weeks after the end of DAA therapy (Fig. 1H).

Changes of hepatic necroinflammatory activity

Fig. 2 illustrates the profiles of hepatic necroinflammatory activity in the entire cohort (n = 113; Fig. 2A and B), treatment-naïve patients (n = 80; Fig. 2C and D), treatment-experienced patients (n = 33; Fig. 2E and F), and those receiving liver biopsies thrice among treatment-experienced patients (n = 21; Fig. 2G and H). Improved, maintaining-at-A0, unchanged, and worsened necroinflammatory activity were observed in 46.9%, 51.3%, 0.9%, and 0.9% of the entire cohort, respectively (Fig. 2B). Nearly all patients in the entire cohort presented improved or maintained necroinflammatory activity (Fig. 2B), while activity worsening was observed in treatment-experienced cases (Fig. 2F).

For those receiving liver biopsies thrice among treatment-experienced patients, improved, maintaining-at-A0, unchanged, and worsened activity were presented in 38.1%, 14.3%, 33.3%, and 14.3% of the studied patients, respectively (Fig. 2H). The majority (95.2%) of the studied patients showed either improved or maintained A0 activity during the course of DAA therapy regardless of the previous activity changes (Fig. 2H); of note, the other 4.8% (n = 1) experienced improved activity during the era of PegIFN-α/ribavirin therapy which later rebounded after completing DAA therapy (from A1 to A0 to A1; Fig. 2H).

Predictors of fibrosis regression

Among the entire cohort, five baseline variables and a variable of biochemical changes significantly predicted fibrosis regression in univariate analysis: baseline age <65

Table 1 Clinical profiles of the entire cohort (n = 113) and specific participants.

Variable[a]	Entire cohort (n = 113)	Entire cohort: treatment-naïve or -experienced[b]			Treatment-experienced patients: receiving liver biopsies twice or thrice[c]		
		-naïve (n = 80)	-experienced (n = 33)	p-value	Twice (n = 12)	Thrice (n = 21)	p-value
Baseline characteristics							
Sex, male/female	57 (50.4%)/ 56 (49.6%)	39 (48.8%)/ 41 (51.2%)	18 (54.5%)/ 15 (45.5%)	0.680	3 (25.0%)/ 9 (75.0%)	15 (71.4%)/ 6 (28.6%)	0.014*
Age (years)	63 (52–72)	63 (51–74)	63 (52–68)	0.585	65 (59–68)	62 (50–68)	0.464
BMI (kg/m^2)	24.49 (22.26–26.99)	24.20 (22.19–26.24)	26.57 (23.76–28.55)	0.026*	24.20 (21.93–27.02)	27.54 (23.78–29.38)	0.242
DM and/or HTN							
DM(+)/HTN(−)	4 (3.5%)	2 (2.5%)	2 (6.1%)	0.579	1 (8.3%)	1 (4.8%)	1.000
DM(−)/HTN(+)	39 (34.5%)	27 (33.8%)	12 (36.4%)	0.830	4 (33.3%)	8 (38.1%)	1.000
DM(+)/HTN(+)	22 (19.5%)	11 (13.8%)	11 (33.3%)	0.034*	5 (41.7%)	6 (28.6%)	0.471
DM(−)/HTN(−)	48 (42.5%)	40 (50.0%)	8 (24.2%)	0.013*	2 (16.7%)	6 (28.6%)	0.678
HBV coinfection	1 (0.9%)	1 (1.3%)	0 (0.0%)	1.000	0 (0.0%)	0 (0.0%)	
HCC	8 (7.1%)	6 (7.5%)	2 (6.1%)	1.000	0 (0.0%)	2 (9.5%)	0.523
HCV genotypes							
1a	4 (3.5%)	2 (2.5%)	2 (6.1%)	0.579	0 (0.0%)	2 (9.5%)	0.523
1b	66 (58.4%)	44 (55.0%)	22 (66.7%)	0.298	9 (75.0%)	13 (61.9%)	0.703
2	31 (27.4%)	27 (33.8%)	4 (12.1%)	0.021*	2 (16.7%)	2 (9.5%)	0.610
3	2 (1.8%)	2 (2.5%)	0 (0.0%)	1.000	0 (0.0%)	0 (0.0%)	
6	10 (8.8%)	5 (6.3%)	5 (15.2%)	0.153	1 (8.3%)	4 (19.0%)	0.630
DAA							
SOF + RBV	11[d] (9.7%)	9 (11.3%)	2 (6.1%)	0.504	1 (8.3%)	1 (4.8%)	1.000
DCV + ASV	3[e] (2.7%)	0 (0.0%)	3 (9.1%)	0.023*	1 (8.3%)	2 (9.5%)	1.000
LDV/SOF	34[f] (30.1%)	24 (30.0%)	10 (30.3%)	1.000	3 (25.0%)	7 (33.3%)	0.710
OBV/PTV/r + DSV	8[e] (7.1%)	7 (8.8%)	1 (3.0%)	0.434	1 (8.3%)	0 (0.0%)	0.364
OBV/PTV/r + DSV + RBV	1[g] (0.9%)	0 (0.0%)	1 (3.0%)	0.292	0 (0.0%)	1 (4.8%)	1.000
EBR/GZR	24[e] (21.2%)	15 (18.8%)	9 (27.3%)	0.322	3 (25.0%)	6 (28.6%)	1.000
GLE/PIB	20[h] (17.7%)	14 (17.5%)	6 (18.2%)	1.000	2 (16.7%)	4 (19.0%)	1.000
SOF/VEL	12[i] (10.6%)	11 (13.8%)	1 (3.0%)	0.175	1 (8.3%)	0 (0.0%)	0.364
METAVIR scores							
fibrosis stages							
F0	2 (1.8%)	1 (1.3%)	1 (3.0%)	0.501	0 (0.0%)	1 (4.8%)	1.000
F1	68 (60.2%)	48 (60.0%)	20 (60.6%)	1.000	10 (83.3%)	10 (47.6%)	0.067
F2	23 (20.4%)	15 (18.8%)	8 (24.2%)	0.608	0 (0.0%)	8 (38.1%)	0.030*
F3	3 (2.7%)	2 (2.5%)	1 (3.0%)	1.000	0 (0.0%)	1 (4.8%)	1.000
F4	17 (15.0%)	14 (17.5%)	3 (9.1%)	0.387	2 (16.7%)	1 (4.8%)	0.538
activity grades							
A0	59 (52.2%)	43 (53.8%)	16 (48.5%)	0.681	8 (66.7%)	8 (38.1%)	0.157
A1	49 (43.4%)	34 (42.5%)	15 (45.5%)	0.836	4 (33.3%)	11 (52.4%)	0.469
A2	5 (4.4%)	3 (3.8%)	2 (6.1%)	0.628	0 (0.0%)	2 (9.5%)	0.523

(*continued on next page*)

Table 1 (*continued*)

Variable[a]	Entire cohort (n = 113)	Entire cohort: treatment-naïve or -experienced[b]			Treatment-experienced patients: receiving liver biopsies twice or thrice[c]		
		-naïve (n = 80)	-experienced (n = 33)	p-value	Twice (n = 12)	Thrice (n = 21)	p-value
Anti-HCV (S/CO)	13.98 (12.67–14.96)	13.86 (12.52–14.96)	14.14 (13.25–15.01)	0.580	13.95 (13.24–14.93)	14.20 (12.72–15.16)	0.577
Serum HCV RNA (10^6 IU/mL)	1.85 (0.36–3.66)	1.25 (0.30–3.30)	3.16 (1.42–5.23)	0.013*	3.63 (2.92–5.34)	2.30 (0.66–4.32)	0.062
AST (U/L)	46 (30–72)	43 (29–74)	50 (36–69)	0.242	48 (35–67)	57 (36–72)	0.761
ALT (U/L)	55 (31–88)	55 (28–84)	56 (43–99)	0.083	49 (43–84)	61 (43–121)	0.432
Total bilirubin (mg/dL)	0.84 (0.70–1.10)	0.85 (0.61–1.10)	0.80 (0.70–1.07)	0.842	0.83 (0.63–1.10)	0.80 (0.70–1.15)	0.664
Direct bilirubin (mg/dL)	0.10 (0.10–0.20)	0.10 (0.10–0.20)	0.10 (0.10–0.20)	0.525	0.10 (0.10–0.20)	0.10 (0.10–0.20)	0.393
Albumin (g/dL)	4.3 (4.0–4.5)	4.3 (4.0–4.5)	4.4 (4.1–4.5)	0.465	4.2 (4.0–4.6)	4.4 (4.1–4.5)	0.559
INR	1.02 (0.99–1.08)	1.02 (0.99–1.08)	1.02 (0.99–1.07)	0.641	1.00 (0.97–1.04)	1.04 (1.01–1.08)	0.122
WBC counts ($10^3/\mu L$)	6.13 (4.80–7.25)	6.00 (4.65–7.00)	6.68 (5.20–7.45)	0.082	6.64 (6.17–7.38)	6.80 (4.66–8.50)	1.000
Neutrophil (%)	58.2 (51.8–63.2)	57.7 (51.2–63.2)	60.1 (53.7–64.0)	0.222	58.4 (54.7–64.5)	60.3 (53.3–64.4)	0.747
Hemoglobin (g/dL)	13.8 (12.7–14.9)	13.6 (12.3–14.7)	14.1 (13.2–15.5)	0.082	13.6 (12.5–15.1)	14.7 (13.6–15.8)	0.144
Platelet counts ($10^3/\mu L$)	172 (121–205)	171 (124–207)	176 (118–204)	0.781	180 (128–221)	160 (118–204)	0.548
AFP (ng/mL)	4.28 (2.67–7.69)	3.95 (2.67–7.43)	4.54 (2.57–7.92)	0.712	4.97 (2.71–9.91)	4.54 (2.37–7.66)	0.839
Creatinine (mg/dL)	0.91 (0.72–1.07)	0.93 (0.77–1.07)	0.85 (0.69–1.08)	0.335	0.73 (0.67–1.08)	0.91 (0.77–1.08)	0.369
GFR (mL/min/1.73m^2)	78 (60–90)	78 (58–90)	83 (69–91)	0.281	84 (56–91)	82 (69–96)	0.705
Virological response							
RVR	112 (99.1%)	80 (100.0%)	32 (97.0%)	0.292	12 (100.0%)	20 (95.2%)	1.000
At EOT	113 (100.0%)	80 (100.0%)	33 (100.0%)		12 (100.0%)	21 (100.0%)	
SVR12	113 (100.0%)	80 (100.0%)	33 (100.0%)		12 (100.0%)	21 (100.0%)	

[a] (1) When each variable was assessed, cases with missing data were excluded from analysis. (2) Categorical data were presented as absolute frequencies with relative proportions and compared by using the Fisher's exact test. Continuous data were shown as medians with interquartile ranges and compared by using the Mann-Whitney *U* test.

[b] Treatment-naïve patients were defined as those never treated with interferon-based therapy before receiving DAA. Treatment-experienced patients were defined as those ever treated with interferon-based therapy before receiving DAA; all of them had been treated with pegylated interferon-α/ribavirin therapy.

[c] In addition to receiving paired liver biopsies during the course of DAA therapy, some of the treatment-experienced patients had received a liver biopsy at baseline of pegylated interferon-α/ribavirin therapy.

[d] All with HCV genotype 2.

[e] All with HCV genotype 1b.

[f] HCV genotypes: 1a, n = 2 (5.9%); 1b, n = 17 (50.0%); 2, n = 9 (26.5%); 6, n = 6 (17.6%).

[g] With HCV genotype 1a.

[h] HCV genotypes: 1a, n = 1 (5.0%); 1b, n = 9 (45.0%); 2, n = 5 (25.0%); 3, n = 1 (5.0%); 6, n = 4 (20.0%).

[i] HCV genotypes: 1b, n = 5 (41.7%); 2, n = 6 (50.0%); 3, n = 1 (8.3%).

Abbreviations: BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; DAA, direct-acting antivirals; SOF, sofosbuvir (Sovaldi®); RBV, ribavirin; DCV, daclatasvir (Daklinza®); ASV, asunaprevir (Sunvepra®); LDV/SOF, ledipasvir/sofosbuvir (Harvoni®); OBV/PTV/r, ombitasvir/paritaprevir/ritonavir (Viekirax®); DSV, dasabuvir (Exviera®); EBR/GZR, elbasvir/grazoprevir (Zepatier®); GLE/PIB, glecaprevir/pibrentasvir (Maviret®); SOF/VEL, sofosbuvir/velpatasvir (Epclusa®); Anti-HCV, HCV antibodies; RNA, ribonucleic acid; AST, aspartate transaminase; ALT, alanine transaminase; INR, international normalized ratio; WBC, white blood cell; AFP, alpha-fetoprotein; GFR, glomerular filtration rate; RVR, rapid virological response; EOT, the end of treatment; SVR12, sustained virological response at 12 weeks after EOT.

*A p-value <0.05 was considered statistically significant.

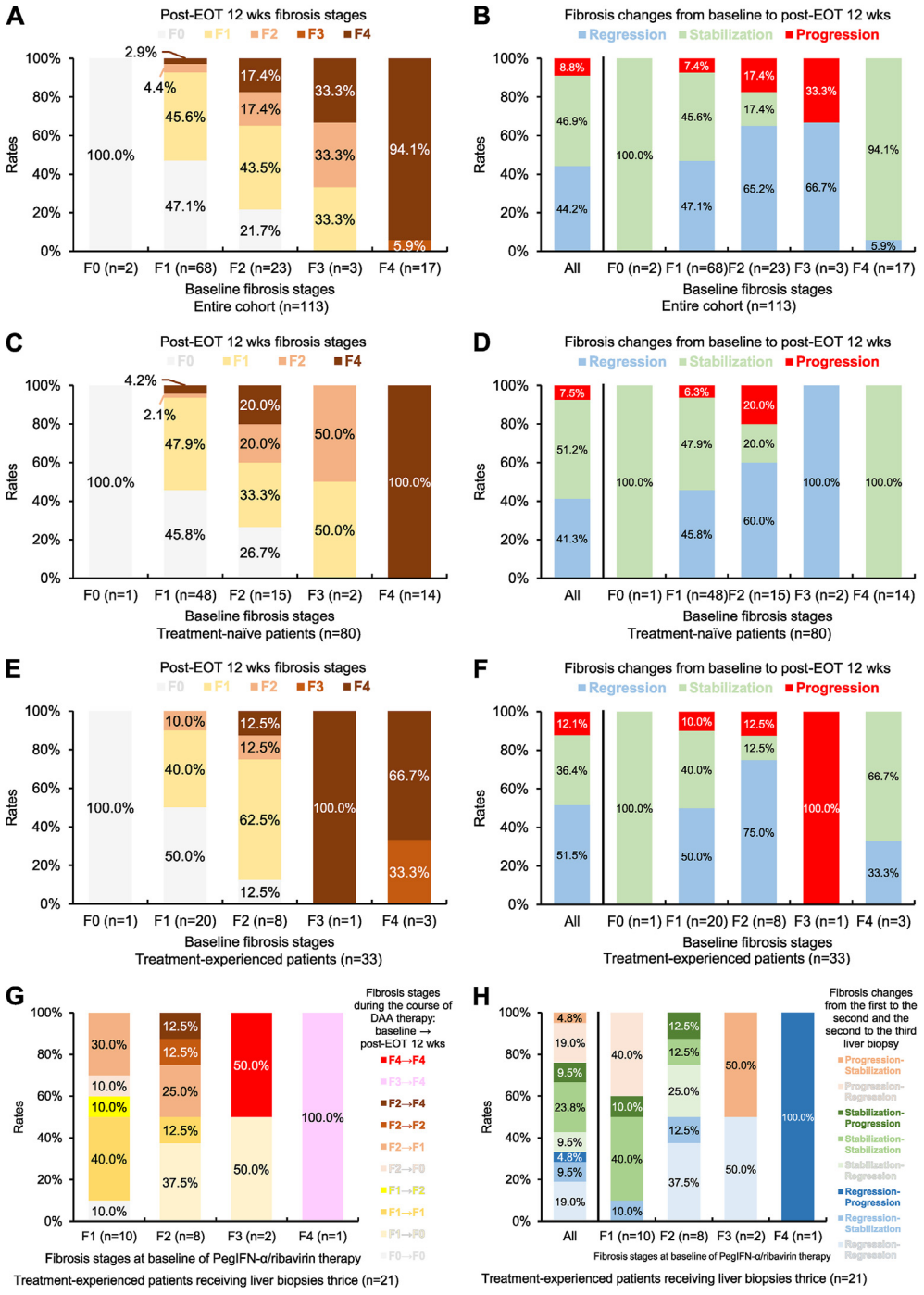

Figure 1. The profiles of liver fibrosis in the entire cohort (n = 113) and specific participants. **(A, C, and E)** The distribution of fibrosis stages at 12 weeks after the end of DAA therapy (post-EOT 12 wks) in **(A)** the entire cohort, **(C)** treatment-naïve patients (defined as those never treated with interferon-based therapy before receiving DAA; n = 80), or **(E)** treatment-experienced patients (defined as those ever treated with interferon-based therapy before receiving DAA; all treated with PegIFN-α/ribavirin therapy; n = 33) with varying fibrosis stages at baseline of DAA therapy. **(B, D, and F)** The distribution of fibrosis changes (regression, stabilization, or progression) from baseline to post-EOT 12 wks during the course of DAA therapy in **(B)** the entire cohort, **(D)** treatment-naïve patients, or **(F)** treatment-experienced patients with varying fibrosis stages at baseline of DAA therapy. **(G)** The distribution of fibrosis stages at baseline and post-EOT 12 wks during the course of DAA therapy in treatment-experienced patients receiving liver biopsies thrice (at baseline of PegIFN-α/ribavirin therapy and during the course of DAA therapy) (n = 21) with varying fibrosis stages at baseline of PegIFN-α/ribavirin therapy. **(H)** The distribution of fibrosis changes from baseline of PegIFN-α/ribavirin therapy to baseline of DAA therapy and baseline to post-EOT 12 wks during the course of DAA therapy in treatment-experienced patients receiving liver biopsies thrice with varying fibrosis stages at baseline of PegIFN-α/ribavirin therapy. Abbreviations: DAA, direct-acting antivirals; post-EOT 12 wks, 12 weeks after the end of treatment (DAA therapy); PegIFN-α, pegylated interferon-α.

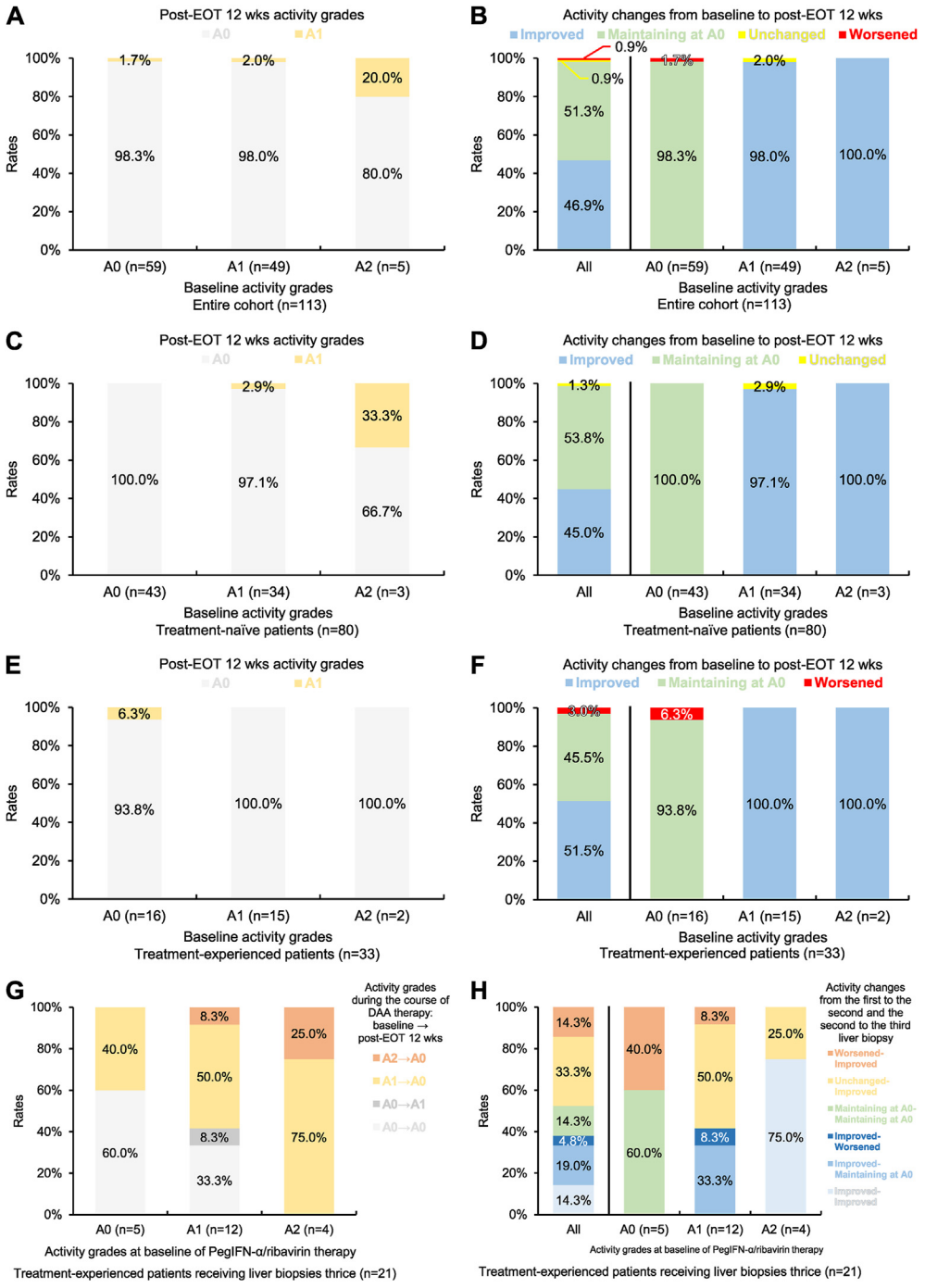

Figure 2. The profiles of hepatic necroinflammatory activity in the entire cohort (n = 113) and specific participants. **(A, C, and E)** The distribution of activity grades at 12 weeks after the end of DAA therapy (post-EOT 12 wks) in **(A)** the entire cohort, **(C)** treatment-naïve patients (defined as those never treated with interferon-based therapy before receiving DAA; n = 80), or **(E)** treatment-experienced patients (defined as those ever treated with interferon-based therapy before receiving DAA; all treated with PegIFN-α/ribavirin therapy; n = 33) with varying activity grades at baseline of DAA therapy. **(B, D, and F)** The distribution of activity changes (improved, maintaining at A0, unchanged, or worsened) from baseline to post-EOT 12 wks during the course of DAA therapy in **(B)** the entire cohort, **(D)** treatment-naïve patients, or **(F)** treatment-experienced patients with varying activity grades at baseline of DAA therapy. **(G)** The distribution of activity grades at baseline and post-EOT 12 wks during the course of DAA therapy in treatment-experienced patients receiving liver biopsies thrice (at baseline of PegIFN-α/ribavirin therapy and during the course of DAA therapy) (n = 21) with varying activity grades at baseline of PegIFN-α/ribavirin therapy. **(H)** The distribution of activity changes from baseline of PegIFN-α/ribavirin therapy to baseline of DAA therapy and baseline to post-EOT 12 wks during the course of DAA therapy in treatment-experienced patients receiving liver biopsies thrice with varying activity grades at baseline of PegIFN-α/ribavirin therapy. Abbreviations: DAA, direct-acting antivirals; post-EOT 12 wks, 12 weeks after the end of treatment (DAA therapy); PegIFN-α, pegylated interferon-α.

years (OR = 2.951, p = 0.006), fibrosis stages <3 (OR = 5.790, p = 0.008), albumin levels ≥4.2 g/dL (OR = 2.533, p = 0.026), hemoglobin levels ≥12.5 g/dL (OR = 2.857, p = 0.032), and platelet counts ≥160 $10^3/\mu L$ (OR = 4.157, p = 0.001); AST levels declining from >31 U/L at baseline to ≤31 U/L at 4 weeks after baseline (OR = 2.135, p = 0.049) (Table 2). Among these variables, four of them which were all baseline variables remained statistically significant in multivariate analysis, including age <65 years (OR = 2.725, p = 0.036), fibrosis stages <3 (OR = 4.874, p = 0.040), hemoglobin levels ≥12.5 g/dL (OR = 3.538, p = 0.029), and platelet counts ≥160 $10^3/\mu L$ (OR = 2.958, p = 0.023) (Table 2).

Predictors of fibrosis progression

Univariate analysis showed that four baseline variables and five variables of biochemical changes significantly predicted fibrosis progression among the entire cohort: baseline age ≥66 years (OR = 6.049, p = 0.027), BMI ≥26.5 kg/m^2 (OR = 4.385, p = 0.031), sofosbuvir/ribavirin use (OR = 9.143, p = 0.003), and platelet counts <119 $10^3/\mu L$ (OR = 3.905, p = 0.045); the absence of AST levels declining from >31 U/L at baseline to ≤31 U/L at 4 weeks after baseline (OR = 10.312, p = 0.030), the absence of ALT levels declining from >35 U/L at baseline to ≤35 U/L at 4 weeks after baseline (OR = 10.723, p = 0.027), the absence of ALT levels declining by >10% at 4 weeks after baseline compared with baseline levels (OR = 10.444, p = 0.001), the absence of AST levels declining from >31 U/L at baseline to ≤31 U/L at EOT (OR = 8.491, p = 0.046), and the absence of ALT levels declining by >10% at EOT compared with baseline levels (OR = 6.963, p = 0.008) (Table 3). Among these variables, four baseline variables and a variable of biochemical changes maintained statistical significance in multivariate analysis, including age ≥66 years (OR = 16.351, p = 0.024), BMI ≥26.5 kg/m^2 (OR = 21.666, p = 0.009), sofosbuvir/ribavirin use (OR = 29.465, p = 0.031), and platelet counts <119 $10^3/\mu L$ (OR = 33.739, p = 0.026) at baseline and the absence of ALT levels declining from >35 U/L at baseline to ≤35 U/L at 4 weeks after baseline (OR = 284.534, p = 0.026) (Table 3).

Discussion

In the era of DAA dominating CHC treatment, failure of viremia eradication is no longer a major concern due to the prominent effectiveness of DAA in achieving SVR. Therefore, a further purpose of improving liver fibrosis and necroinflammation should be considered. Recent studies have proposed that LS and fibrosis biomarkers significantly decreased in DAA-treated CHC patients,[11–26] but they are all based on noninvasive methodologies. To eliminate the uncertainty, it requires direct and tissue-specific studies via liver biopsy which are now lacking. Besides, to reverse liver fibrosis and necroinflammation more effectively, it is necessary to elucidate the unknown predictors of liver histological changes in DAA-treated CHC patients. In this study, we performed paired liver biopsies to assess changes of fibrosis and necroinflammatory activity in CHC patients receiving DAA and investigated the predictors of fibrosis changes, aiming to establish the surveillance standard of fibrosis evolution for DAA-treated CHC patients.

Our study identified that fibrosis progression was possibly presented in CHC patients achieving SVR with DAA. Liver fibrogenesis is mainly attributed to activated myofibroblasts which are transdifferentiated from hepatic stellate cells (HSCs).[31] For fibrosis regression, mechanisms of HSC responses strengthened by DAA include reversion to a quiescent phenotype, apoptosis/autophagy, and cellular senescence.[32] In addition, fibrolytic macrophages (Ly-$6C^{lo}$ macrophages in mice; $CD14^+$ macrophages in humans) are recruited to enhance degradation of extracellular matrix during fibrosis regression.[33] Nevertheless, significant fibrosis degradation is less likely once there is severe architectural distortion, vascular collapse, and portal hypertension,[32] explaining SVR with DAA did not guarantee fibrosis regression in our study.

So far it remains a critical issue whether HCV eradication with an interferon-based therapy is the same as with a DAA-based therapy.[32] In our study, whether being previously treated with PegIFN-α/ribavirin therapy did not significantly affect the chance of achieving fibrosis regression (OR = 1.513, p = 0.319; Table 2) or the risk of gaining fibrosis progression (OR = 1.701, p = 0.436; Table 3) after completing DAA therapy. These results suggest that antifibrotic effects of PegIFN-α/ribavirin therapy and DAA may be independent in CHC patients. Furthermore, CHC patients may present contrary responses to PegIFN-α/ribavirin therapy and DAA in terms of fibrosis changes as shown in our study (regression-progression, 4.8%; progression-regression, 19.0%; Fig. 1H). Our study also found that among CHC patients attaining fibrosis regression but failing in HCV eradication with PegIFN-α/ribavirin therapy, the risk of fibrosis progression does exist after retreatment with DAA (Fig. 1H). However, those with fibrosis progression during the era of PegIFN-α/ribavirin therapy benefit from DAA as our study showed that most of them (80.0%) had fibrosis regression following DAA retreatment (Fig. 1H). The above-mentioned findings may be explained by different mechanisms of viral clearance between the two anti-HCV therapies, while basic studies exploring the underlying mechanisms are warranted.

In this study, baseline age <65 years independently predicted fibrosis regression, and baseline age ≥66 years was an independent predictor of fibrosis progression. This result indicates a younger age benefits fibrosis improvement under DAA therapy. Besides, the baseline fibrosis stage is an ideal indicator of fibrosis changes in DAA-treated CHC patients. Our study showed baseline fibrosis stages <3 was an independent predictor of fibrosis regression, suggesting patients with advanced pretreatment fibrosis as fibrosis stages ≥3 were at a higher risk of unimproved fibrosis though receiving DAA and achieving SVR12. Our previous study during the era of interferon-based therapy identified a lower fibrosis stage at baseline of PegIFN-α/ribavirin therapy as fibrosis stages <2 independently predicted fibrosis clearance during long-term follow-up in SVR24 cases.[34] These results collectively imply that baseline fibrosis status determines fibrosis changes following viremia eradication regardless of the types of anti-HCV treatment. The present study also uncovered higher baseline hemoglobin levels as ≥12.5 g/dL and platelet counts as

Table 2 Predictors of fibrosis regression in the entire cohort (n = 113).

Variable[a]	n	Univariate analysis[b]		Multivariate analysis[b,c]	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment-experienced vs. -naïve	33 vs. 80	1.513 (0.670–3.419)	0.319		
Baseline characteristics					
Sex, male vs. female	57 vs. 56	1.726 (0.815–3.654)	0.154		
Age (years), <65 vs. ≥65	58 vs. 55	**2.951 (1.362–6.390)**	**0.006***	**2.725 (1.069–6.945)**	**0.036***
BMI (kg/m^2), <26.5 vs. ≥26.5	80 vs. 32	0.588 (0.258–1.344)	0.208		
DM, (−) vs. (+)	87 vs. 26	1.684 (0.677–4.187)	0.262		
HTN, (−) vs. (+)	52 vs. 61	1.542 (0.730–3.257)	0.257		
HCV genotypes					
1b, (+) vs. (−)	66 vs. 47	0.722 (0.340–1.535)	0.398		
2, (+) vs. (−)	31 vs. 82	1.259 (0.549–2.885)	0.586		
others (1a, 3, or 6), (+) vs. (−)	16 vs. 97	1.310 (0.454–3.776)	0.618		
DAA					
SOF + RBV, (+) vs. (−)	11 vs. 102	0.439 (0.110–1.749)	0.243		
LDV/SOF, (+) vs. (−)	34 vs. 79	1.176 (0.525–2.638)	0.693		
OBV/PTV/r + DSV ± RBV, (+) vs. (−)	9 vs. 104	0.333 (0.066–1.681)	0.183		
EBR/GZR, (+) vs. (−)	24 vs. 89	1.661 (0.671–4.113)	0.273		
GLE/PIB, (+) vs. (−)	20 vs. 93	0.626 (0.229–1.711)	0.361		
SOF/VEL, (+) vs. (−)	12 vs. 101	2.810 (0.794–9.942)	0.109		
METAVIR scores					
fibrosis stages, <3 vs. ≥3	93 vs. 20	**5.790 (1.589–21.095)**	**0.008***	**4.874 (1.076–22.070)**	**0.040***
activity grades, 0 vs. >0	59 vs. 54	2.039 (0.957–4.348)	0.065		
Serum HCV RNA (10^6 IU/mL)					
≤0.4 vs. >0.4	29 vs. 84	1.032 (0.441–2.413)	0.942		
≤0.5 vs. >0.5	30 vs. 83	1.142 (0.494–2.642)	0.756		
≤0.8 vs. >0.8	36 vs. 77	1.012 (0.456–2.244)	0.977		
≤1.0 vs. >1.0	42 vs. 71	0.783 (0.362–1.696)	0.535		
≤1.2 vs. >1.2	47 vs. 66	0.766 (0.359–1.633)	0.490		
≤1.5 vs. >1.5	51 vs. 62	0.797 (0.377–1.683)	0.551		
≤1.6 vs. >1.6	53 vs. 60	0.702 (0.332–1.482)	0.353		
≤2.0 vs. >2.0	58 vs. 55	0.787 (0.374–1.656)	0.529		
≤2.4 vs. >2.4	63 vs. 50	0.658 (0.311–1.392)	0.274		
≤2.5 vs. >2.5	65 vs. 48	0.575 (0.270–1.224)	0.151		
≤2.8 vs. >2.8	70 vs. 43	0.636 (0.296–1.368)	0.247		
≤3.0 vs. >3.0	72 vs. 41	0.642 (0.297–1.391)	0.261		
≤3.5 vs. >3.5	84 vs. 29	0.553 (0.236–1.295)	0.172		
AST (U/L)					
≤31 vs. >31	29 vs. 84	1.244 (0.534–2.903)	0.613		
≤62 vs. >62	70 vs. 43	2.193 (0.994–4.840)	0.052		
≤93 vs. >93	100 vs. 13	2.956 (0.767–11.387)	0.115		
ALT (U/L)					
≤35 vs. >35	33 vs. 80	1.071 (0.474–2.421)	0.868		
≤70 vs. >70	72 vs. 41	1.195 (0.550–2.593)	0.653		
≤105 vs. >105	92 vs. 21	1.755 (0.649–4.749)	0.268		
Total bilirubin (mg/dL), ≤1.2 vs. >1.2	94 vs. 19	2.571 (0.857–7.713)	0.092		
Direct bilirubin (mg/dL), ≤0.4 vs. >0.4	106 vs. 7	5.158 (0.600–44.331)	0.135		
Albumin (g/dL), ≥4.2 vs. <4.2	73 vs. 40	**2.533 (1.119–5.737)**	**0.026***	0.797 (0.271–2.345)	0.681
INR, ≤1.1 vs. >1.1	94 vs. 18	3.214 (0.985–10.488)	0.053		
Hemoglobin (g/dL), ≥12.5 vs. <12.5	86 vs. 27	**2.857 (1.095–7.454)**	**0.032***	**3.538 (1.135–11.021)**	**0.029***
Platelet counts (10^3/μL), ≥160 vs. <160	68 vs. 45	**4.157 (1.808–9.557)**	**0.001***	**2.958 (1.160–7.541)**	**0.023***
AFP (ng/mL), <4 vs. ≥4	52 vs. 61	1.154 (0.548–2.430)	0.706		
Biochemical changes					
Baseline vs. 4 weeks after baseline					
AST levels declining from >31 to ≤31 U/L, (+) vs. (−)	56 vs. 57	**2.135 (1.002–4.547)**	**0.049***	1.540 (0.647–3.669)	0.329
ALT levels					

Table 2 (*continued*)

Variable[a]	n	Univariate analysis[b]		Multivariate analysis[b,c]	
		OR (95% CI)	p-value	OR (95% CI)	p-value
declining from >35 to ≤35 U/L, (+) vs. (−)	57 vs. 56	1.291 (0.614–2.718)	0.501		
declining by >10%, (+) vs. (−)	99 vs. 14	3.314 (0.871–12.608)	0.079		
Baseline vs. EOT					
AST levels declining from >31 to ≤31 U/L, (+) vs. (−)	51 vs. 62	1.908 (0.898–4.053)	0.093		
ALT levels					
declining from >35 to ≤35 U/L, (+) vs. (−)	60 vs. 53	1.233 (0.585–2.598)	0.582		
declining by >10%, (+) vs. (−)	100 vs. 13	2.956 (0.767–11.387)	0.115		

[a] To calculate the OR, each variable was categorized into a binary form with the latter designated as the reference factor.
[b] Cases with missing data were excluded from both univariate and multivariate analysis.
[c] Variables entered into multivariate analysis were those showing a p-value <0.05 in univariate analysis.
Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; HCV, hepatitis C virus; DAA, direct-acting antivirals; SOF, sofosbuvir (Sovaldi®); RBV, ribavirin; LDV/SOF, ledipasvir/sofosbuvir (Harvoni®); OBV/PTV/r, ombitasvir/paritaprevir/ritonavir (Viekirax®); DSV, dasabuvir (Exviera®); EBR/GZR, elbasvir/grazoprevir (Zepatier®); GLE/PIB, glecaprevir/pibrentasvir (Maviret®); SOF/VEL, sofosbuvir/velpatasvir (Epclusa®); RNA, ribonucleic acid; AST, aspartate transaminase; ALT, alanine transaminase; INR, international normalized ratio; AFP, alpha-fetoprotein; EOT, the end of treatment.
*Binary logistic regression: A p-value <0.05 was considered statistically significant.
[bold] represents data showing statistical significance in univariate or multivariate analysis. This is for a better visual effect.

≥160 $10^3/\mu L$ were independent predictors of fibrosis regression, and lower platelet counts as <119 $10^3/\mu L$ independently predicted fibrosis progression. As anemia and thrombocytopenia reflect the severity of chronic liver disease,[35,36] we infer pretreatment disease severity indicated by hemoglobin levels and platelet counts, instead of other biochemical values, affects fibrosis changes in DAA-treated CHC patients achieving SVR. Future studies are needed to assess whether correcting hemoglobin levels and platelet counts at baseline of DAA therapy benefits fibrosis improvement in CHC patients.

Baseline BMI plays an independent role in predicting fibrosis changes: a higher baseline BMI as ≥26.5 kg/m^2 independently predicted fibrosis progression in our study. Previous studies have identified BMI as a critical factor for fibrosis changes in CHC patients.[37–40] Firstly, Ratziu et al. reported a higher BMI as ≥28 kg/m^2 is independently predictive for septal fibrosis (METAVIR F2–F4) among untreated CHC patients with abnormal liver function.[37] Secondly, Hourigan et al. proposed that in treatment-naïve CHC patients, BMI significantly correlates with the severity of steatosis which is highly associated with fibrosis stages.[38] Thirdly, Ortiz et al. showed obesity defined as BMI ≥25 kg/m^2 accelerates fibrosis progression under the natural history of CHC.[39] At last, Poynard et al. demonstrated a lower BMI as <27 kg/m^2 predicts the absence of post-treatment septal fibrosis in CHC patients receiving interferon-based therapy.[40] These studies support that obesity as increasing BMI promotes steatogenesis which leads to fibrogenesis in the natural course of CHC.[41] Besides, obesity, an inflammatory condition, impairs the therapeutic response of interferon-based therapy by diminishing the biological response to interferon or reducing the bioavailability of PegIFN-α.[41] As shown in our study, the pro-fibrosis role of higher BMI remains significant even under DAA therapy. Hence, for CHC patients with obesity, weight reduction should be considered before receiving DAA.

Our study also identified the use of sofosbuvir (Sovaldi®) plus ribavirin increased the risk of fibrosis progression. In our study, although all patients receiving sofosbuvir/ribavirin (n = 11) were of HCV genotype 2, among HCV genotype 2 cases (n = 31) the rate of fibrosis progression was significantly higher in patients treated with sofosbuvir/ribavirin than in those treated with other regimens (36.4% vs. 0.0%, p = 0.010). Besides, the variable of HCV genotype 2 did not significantly predict fibrosis progression (OR = 1.877, p = 0.357; Table 3). Therefore, the role of sofosbuvir/ribavirin use in fibrosis progression may be considered independent of HCV genotypes. Future studies are warranted to confirm whether the use of sofosbuvir/ribavirin should be avoided in CHC patients.

In addition to baseline characteristics, we assessed whether changes in biochemical values were associated with fibrosis evolution. Although baseline ALT levels at various cut-off points were insignificant in predicting fibrosis regression (Table 2) and progression (Table 3), the absence of ALT levels declining from >35 U/L at baseline to ≤35 U/L at 4 weeks after baseline independently predicted fibrosis progression. These results indicate early ALT normalization reflects improved or stabilized liver fibrosis under DAA therapy.

In clinical practice, liver biopsy is the only method for directly estimating liver injuries which helps define disease severity more accurately.[9,10] However, only a small number of patients are willing to receive this procedure, making studies collecting liver biopsy samples rare and valuable. To the best of our knowledge, this is the first study that enrolled adequate DAA-treated CHC patients and performed paired liver biopsies for evaluating liver histological changes, providing tissue-specific evidence. Nonetheless, there were limitations of this study. First, our study was unable to assess

Table 3 Predictors of fibrosis progression in the entire cohort (n = 113).

Variable[a]	n	Univariate analysis[b]		Multivariate analysis[b,c]	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Treatment-experienced vs. -naïve	33 vs. 80	1.701 (0.447–6.471)	0.436		
Baseline characteristics					
Sex, male vs. female	57 vs. 56	0.629 (0.168–2.361)	0.492		
Age (years), ≥66 vs. <66	49 vs. 64	**6.049 (1.223–29.928)**	**0.027***	**16.351 (1.455–183.779)**	**0.024***
BMI (kg/m^2), ≥26.5 vs. <26.5	32 vs. 80	**4.385 (1.147–16.766)**	**0.031***	**21.666 (2.132–220.139)**	**0.009***
DM, (+) vs. (−)	26 vs. 87	2.455 (0.636–9.469)	0.192		
HTN, (+) vs. (−)	61 vs. 52	3.774 (0.764–18.634)	0.103		
HCV genotypes					
1b, (+) vs. (−)	66 vs. 47	0.441 (0.117–1.659)	0.226		
2, (+) vs. (−)	31 vs. 82	1.877 (0.492–7.161)	0.357		
others (1a, 3, or 6), (+) vs. (−)	16 vs. 97	1.589 (0.306–8.265)	0.582		
DAA					
SOF + RBV, (+) vs. (−)	11 vs. 102	**9.143 (2.082–40.151)**	**0.003***	**29.465 (1.368–634.588)**	**0.031***
LDV/SOF, (+) vs. (−)	34 vs. 79	0.555 (0.111–2.760)	0.472		
OBV/PTV/r + DSV ± RBV, (+) vs. (−)	9 vs. 104	3.429 (0.608–19.322)	0.163		
EBR/GZR, (+) vs. (−)	24 vs. 89	0.920 (0.182–4.649)	0.920		
METAVIR scores					
fibrosis stages, ≥3 vs. <3	20 vs. 93	0.491 (0.059–4.114)	0.512		
activity grades, >0 vs. 0	54 vs. 59	2.780 (0.681–11.353)	0.154		
Serum HCV RNA (10^6 IU/mL)					
>0.4 vs. ≤0.4	84 vs. 29	1.421 (0.284–7.113)	0.669		
>0.5 vs. ≤0.5	83 vs. 30	1.493 (0.299–7.464)	0.625		
>0.8 vs. ≤0.8	77 vs. 36	0.431 (0.116–1.594)	0.207		
>1.0 vs. ≤1.0	71 vs. 42	0.358 (0.095–1.352)	0.130		
>1.2 vs. ≤1.2	66 vs. 47	0.441 (0.117–1.659)	0.226		
>1.5 vs. ≤1.5	62 vs. 51	0.517 (0.138–1.943)	0.329		
>1.6 vs. ≤1.6	60 vs. 53	0.560 (0.149–2.101)	0.390		
>2.0 vs. ≤2.0	55 vs. 58	0.680 (0.181–2.552)	0.567		
>2.4 vs. ≤2.4	50 vs. 63	0.511 (0.125–2.085)	0.349		
>2.5 vs. ≤2.5	48 vs. 65	0.552 (0.135–2.257)	0.409		
>2.8 vs. ≤2.8	43 vs. 70	0.378 (0.076–1.870)	0.233		
>3.0 vs. ≤3.0	41 vs. 72	0.410 (0.083–2.032)	0.275		
>3.5 vs. ≤3.5	29 vs. 84	0.704 (0.141–3.522)	0.669		
AST (U/L)					
>31 vs. ≤31	84 vs. 29	1.421 (0.284–7.113)	0.669		
>62 vs. ≤62	43 vs. 70	1.094 (0.290–4.121)	0.894		
>93 vs. ≤93	13 vs. 100	2.091 (0.393–11.117)	0.387		
ALT (U/L)					
>35 vs. ≤35	80 vs. 33	0.588 (0.155–2.236)	0.436		
>70 vs. ≤70	41 vs. 72	1.189 (0.315–4.486)	0.798		
>105 vs. ≤105	21 vs. 92	1.105 (0.217–5.627)	0.904		
Total bilirubin (mg/dL), >1.2 vs. ≤1.2	19 vs. 94	1.265 (0.247–6.484)	0.778		
Direct bilirubin (mg/dL), >0.4 vs. ≤0.4	7 vs. 106	1.796 (0.194–16.613)	0.606		
Albumin (g/dL), <4.2 vs. ≥4.2	40 vs. 73	1.943 (0.527–7.164)	0.318		
INR, >1.1 vs. ≤1.1	18 vs. 94	1.344 (0.261–6.919)	0.724		
Hemoglobin (g/dL), <12.5 vs. ≥12.5	27 vs. 86	1.411 (0.338–5.881)	0.637		
Platelet counts (10^3/μL), <119 vs. ≥119	26 vs. 87	**3.905 (1.034–14.750)**	**0.045***	**33.739 (1.537–740.532)**	**0.026***
AFP (ng/mL), ≥4 vs. <4	61 vs. 52	3.774 (0.764–18.634)	0.103		
Biochemical changes					
Baseline vs. 4 weeks after baseline					
AST levels declining from >31 to ≤31 U/L, (−) vs. (+)	57 vs. 56	**10.312 (1.260–84.377)**	**0.030***	1.178 (0.002–723.459)	0.960
ALT levels					
declining from >35 to ≤35 U/L, (−) vs. (+)	56 vs. 57	**10.723 (1.310–87.749)**	**0.027***	**284.534 (1.960–41314.002)**	**0.026***
declining by >10%, (−) vs. (+)	14 vs. 99	**10.444 (2.535–43.026)**	**0.001***	0.320 (0.008–12.389)	0.541

Table 3 (*continued*)

Variable[a]	n	Univariate analysis[b]		Multivariate analysis[b,c]	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Baseline vs. EOT					
AST levels declining from >31 to ≤31 U/L, (−) vs. (+)	62 vs. 51	**8.491 (1.038–69.461)**	**0.046***	0.646 (0.001–584.253)	0.900
ALT levels					
declining from >35 to ≤35 U/L, (−) vs. (+)	53 vs. 60	2.891 (0.708–11.809)	0.139		
declining by >10%, (−) vs. (+)	13 vs. 100	**6.963 (1.653–29.335)**	**0.008***	3.913 (0.101–151.254)	0.464

[a] To calculate the OR, each variable was categorized into a binary form with the latter designated as the reference factor.
[b] Cases with missing data were excluded from both univariate and multivariate analysis.
[c] Variables entered into multivariate analysis were those showing a p-value <0.05 in univariate analysis.
Abbreviations: OR, odds ratio; CI, confidence interval; BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; HCV, hepatitis C virus; DAA, direct-acting antivirals; SOF, sofosbuvir (Sovaldi®); RBV, ribavirin; LDV/SOF, ledipasvir/sofosbuvir (Harvoni®); OBV/PTV/r, ombitasvir/paritaprevir/ritonavir (Viekirax®); DSV, dasabuvir (Exviera®); EBR/GZR, elbasvir/grazoprevir (Zepatier®); RNA, ribonucleic acid; AST, aspartate transaminase; ALT, alanine transaminase; INR, international normalized ratio; AFP, alpha-fetoprotein; EOT, the end of treatment.
*Binary logistic regression: A p-value <0.05 was considered statistically significant.
[bold] represents data showing statistical significance in univariate or multivariate analysis. This is for a better visual effect.

the predictive roles of virological responses in fibrosis changes due to the prominent antiviral effect of DAA. In this study, 99.1% of the entire cohort attained RVR, and all patients achieved EOT virological response as well as SVR12, making it infeasible to categorize the patients based on the presence/absence of virological responses. In terms of fibrosis changes under DAA therapy, previous studies utilizing noninvasive methodologies mainly focused on SVR-achieving patients[11–19,22,24–26] or only included a small number of non-SVR cases.[21] By contrast, Bachofner et al. proposed SVR determined whether LS significantly declined in DAA-treated CHC patients with HCV genotypes 1–4.[23] However, in a more specific study focusing on patients with HCV genotype 1b and treated with daclatasvir/asunaprevir, a significant LS decrease was reported in both SVR and non-SVR cases.[20] To elucidate the predictive role of SVR in fibrosis changes under DAA therapy, future studies are recommended to enroll more non-SVR cases and perform paired liver biopsies if available. As for the second limitation of this study, the follow-up of fibrosis outcomes was limited by the time point of the second liver biopsy (12 weeks after EOT). To assess long-term liver histological changes and confirm whether they are linear over time or vary, future studies are suggested to perform at least three times of liver biopsies (baseline, 12 weeks after EOT, and long-term follow-up) in DAA-treated CHC patients, which is also the agenda of this study.

In conclusion, viremia eradication with DAA indicated by SVR does not guarantee concurrent fibrosis regression in CHC patients, and fibrosis progression is observed in some DAA-treated CHC patients even with SVR. To attain fibrosis regression and prevent progression, CHC patients should receive DAA as early as possible (when age <65 years or fibrosis stages <3) and avoid the use of sofosbuvir/ribavirin. Besides, correcting baseline hemoglobin levels as ≥12.5 g/dL, platelet counts as ≥160 $10^3/\mu L$, and BMI as <26.5 kg/m^2 may be considered. For CHC patients already treated with DAA and achieving SVR, specific surveillance for fibrosis progression is advised in those with baseline age ≥66 years, BMI ≥26.5 kg/m^2, sofosbuvir/ribavirin use, platelet counts <119 $10^3/\mu L$, or the absence of ALT levels declining from >35 U/L at baseline to ≤35 U/L at 4 weeks after baseline.

Ethics approval statement

This study was approved by the institutional review board of China Medical University Hospital (No. CMUH109-REC1-033). All procedures performed in the study were in accordance with the ethical standards of the institutional review board, the 2013 Declaration of Helsinki, and the 2018 Declaration of Istanbul.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Patient consent statement

Written informed consent was obtained from all participants.

CRediT authorship contribution statement

Ming-Han Hsieh: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Tzu-Yu Kao:** Investigation. **Ting-Hui Hsieh:** Investigation. **Chun-Chi Kao:** Investigation. **Cheng-Yuan Peng:**

Resources, Validation. **Hsueh-Chou Lai:** Resources, Validation. **Hsing-Hung Cheng:** Resources, Validation. **Mao-Wang Ho:** Resources, Validation. **Chih-Yu Chi:** Resources, Validation. **Jung-Ta Kao:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing — review & editing.

Conflict of interest disclosure

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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