Long-term surveillance of liver histological changes in chronic hepatitis C patients completing pegylated interferon-α plus ribavirin therapy: an observational cohort study

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Abstract

Background: For chronic hepatitis C (CHC) patients completing pegylated interferon (PegIFN)-α/ribavirin therapy, long-term liver histological changes remain largely unexplored.

Methods: This observational cohort study included 85 CHC patients completing PegIFN-α/ribavirin therapy with liver biopsies performed at baseline and the end of surveillance (EOS). Median years between paired biopsies were 6.75 (interquartile range: 5.63–7.54).

Results: In patients with baseline METAVIR fibrosis stages (F) <4 (able to undergo fibrosis progression; n = 77), cases achieving sustained virological response (SVR) (n = 52) had a significantly lower rate of fibrosis progression than non-SVR cases (n = 25) [3.8% versus 24.0%, p = 0.012]. Among the entire cohort (n = 85), the rate of activity response [METAVIR activity grades A] decreasing or maintaining at A0] in SVR cases (n = 59) was significantly higher than that in non-SVR cases (n = 26) [94.9% versus 65.4%, p = 0.001]. For SVR cases among the entire cohort, independent predictors of fibrosis clearance included baseline F <2 [odds ratio (OR) = 7.877, p = 0.042] and aspartate transaminase (AST) levels declining by >70% at EOS compared with baseline [OR = 9.013, p = 0.038]. For non-SVR cases among the entire cohort, baseline AST levels >80 U/l and glucose levels ≤ 105 mg/dl independently predicted significant fibrosis [F2/F3/F4] at EOS [OR = 12.558, p = 0.049] and activity response [OR = 17.741, p = 0.047], respectively.

Conclusions: Among CHC patients completing PegIFN-α/ribavirin therapy, SVR lowers the risk of liver histological progression but does not guarantee fibrosis clearance. For SVR cases, those with baseline F ≥ 2 or without significantly declined follow-up AST levels should be specifically monitored. As for non-SVR cases, those with a higher baseline AST or glucose level should preferentially receive retreatment.

Keywords: chronic hepatitis C, fibrosis, liver biopsy, necroinflammatory activity, pegylated interferon-α, ribavirin, sustained virological response

Introduction

Hepatitis C is a global health burden with above 175 million people infected with the hepatitis C virus (HCV).1 For chronic hepatitis C (CHC), therapeutic modalities including interferon-based therapy and direct-acting antivirals (DAAs) have been applied, and achieving sustained virological response (SVR) is the primary treatment goal.2,3 Among interferon-based therapeutics, the combination therapy of pegylated interferon (PegIFN)-α

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and ribavirin used to be the standard treatment for CHC, while it is no longer recommended and has been replaced by emerging DAAs to reduce side effects and improve SVR rates. However, a great number of CHC patients were treated with PegIFN-α/ribavirin therapy before the DAA era, and it remains unknown whether these patients are still at the risk of advanced liver histological events after treatment completion. 

Annually about 400 thousand people worldwide expire from HCV-related cirrhosis or hepatocellular carcinoma (HCC). The progression from HCV infection to liver fibrosis, cirrhosis, and HCC is an extended process, making the surveillance of fibrosis changes an important issue for CHC management. At present, various noninvasive tests have been utilized in the assessment of liver fibrosis. Nonetheless, a liver biopsy remains the reference standard for assessing liver fibrosis and the only method for directly estimating liver injuries. Previous studies have reported factors associated with fibrosis regression in CHC patients receiving interferon-based therapy, such as SVR, specific baseline features (advanced fibrosis, no or mild necroinflammatory activity, the absence of steatosis, younger age, and lower body mass index or serum viral load) or clinical changes (inflammation improvement and the normalization of alanine transaminase (ALT) levels), and longer follow-up. Besides, predictors of necroinflammation improvement under interferon-based therapy were proposed as well, including SVR, higher ALT levels at baseline, and the normalization of ALT levels. However, for CHC patients completing PegIFN-α/ribavirin therapy, long-term surveillance studies on liver histological changes are lacking.

Although SVR defines the success of anti-HCV treatment, it requires further confirmation as to whether SVR, an indicator of viral clearance in peripheral serum instead of the hepatic parenchyma, remains predictive for liver histological improvement over a long time frame in CHC patients completing PegIFN-α/ribavirin therapy. Besides, to develop a more comprehensive criterion for monitoring these treatment-experienced patients, it is necessary to explore the predictors of fibrosis changes in SVR and non-SVR cases among these patients. Furthermore, given that necroinflammatory activity directly reflects the severity of the underlying disease process, its changes and related predictors among these patients should also be investigated.

Methods

Study cohort
From July 2008 to June 2017 at China Medical University Hospital, Taichung, Taiwan, 97 treatment-naive CHC patients (defined as those with the presence of HCV antibody in serum for at least 6 months and detectable serum HCV RNA but not yet receiving anti-HCV treatment) starting PegIFN-α/ribavirin therapy and accepting a baseline liver biopsy for evaluating disease severity were enrolled at baseline to participate in this observational cohort study. PegIFN-α was administered subcutaneously with PegIFN-α2a prescribed at a dosage of 180 µg per week or PegIFN-α2b at a weight-based dosage of 1.5 µg/kg per week, and ribavirin was given 1000 (body weight < 75 kg) or 1200 mg (body weight ≥ 75 kg) orally per day. Dosage reduction was considered if needed. All enrolled patients were followed up extensively for as long as possible. At each visit during treatment and post-treatment follow-up, patients received detailed physical examination and biochemical evaluation. Besides, serum HCV RNA tests were performed at baseline, 4 weeks after baseline, 12 weeks after baseline, the end of treatment (EOT), and 24 weeks after EOT. Rapid virological response (RVR), early virological response (EVR), virological response at EOT, and SVR were defined as undetectable serum HCV RNA at 4 weeks after baseline, 12 weeks after baseline, EOT, and 24 weeks after EOT, respectively. To evaluate liver histological changes, all enrolled patients were invited to receive the second liver biopsy at the end of surveillance (EOS; spanning from May 2014 to December 2019 among those accepting the second liver biopsy (n = 85)), and those declining the second liver biopsy (n = 12) were excluded from the present study. From baseline to EOS, none of the included patients received retreatment with interferon-based therapy or DAAs.

Liver biopsy
Biopsy specimens obtained percutaneously from the right-lobe liver were assessed by experienced pathologists blinded to patients’ data. Biopsy results were evaluated with the METAVIR scoring system; fibrosis was staged on a 5-point scale (F0:
Evaluation of liver histological changes
Liver histological changes were assessed by comparing the results of the first (baseline) and the second (EOS) liver biopsy. Fibrosis changes were defined as follows: clearance as fibrosis stages decreasing from F≥1 to F0, non-clearance regression as decreased fibrosis stages except those ending up at F0, stabilization as unchanged, and progression as increased fibrosis stages. Changes in necroinflammatory activity were categorized into activity response (defined as activity grades decreasing or maintaining at A0) and non-response [defined as unchanged (except maintaining at A0) or increased activity grades].

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.). No statistical methods were used to predetermine the sample size. Nominal and ordinal 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 for two independent samples or the Wilcoxon signed-rank test for two related samples. The odds ratio (OR) with its 95% confidence interval (CI) was calculated 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.

Ethics statement
This study was approved by the institutional review board of China Medical University Hospital (No. CMUH109-REC1-033). All procedures were performed following the ethical standards of the institutional review board and the 1964 Helsinki Declaration with its later amendments. Written informed consent was obtained from all participants.

Results

Patient characteristics
Table 1 provides the demographics of the entire cohort (85 CHC patients completing PegIFN-α/ribavirin therapy with paired liver biopsies) and its separate groups divided by whether achieving SVR or not. Among the entire cohort, 59 (69.4%) cases achieved SVR after completing PegIFN-α/ribavirin therapy, while the other 26 (30.6%) cases did not. The median duration between paired biopsies was 6.75 [interquartile range (IQR): 5.63–7.54], 6.83 (IQR: 5.58–7.67), and 6.38 (IQR: 5.71–7.02) years in the entire cohort, its SVR cases, and its non-SVR cases, respectively (SVR versus non-SVR, p=0.282) (Table 1). Compared with non-SVR cases among the entire cohort, SVR cases had a significantly higher rate of HCV genotype 2a infection (28.8% versus 7.7%, p=0.046), mild activity (METAVIR score A1) (72.9% versus 42.3%, p=0.013), RVR (64.4% versus 26.9%, p=0.002), or virological response at EOT (96.6% versus 76.9%, p=0.009) and a significantly lower rate of activity absence (METAVIR score A0) (8.5% versus 34.6%, p=0.008) or median level of HCV RNA [1.81 (IQR: 0.13–10.60) versus 6.51 (IQR: 2.74–14.12) 10⁶ copies/ml, p=0.004] at baseline (Table 1).

Liver histological and biochemical changes in SVR and non-SVR cases
Figures 1(a) and (c) show the distributions of fibrosis and activity changes in SVR and non-SVR cases among the entire cohort. In patients with baseline fibrosis stages <4 who were able to undergo fibrosis progression (n=77), SVR cases (n=52) presented a significantly lower rate of fibrosis progression than non-SVR cases (n=25) [3.8% versus 24.0%, p=0.012; Figure 1(b)]. Among the entire cohort, the rate of activity response in SVR cases was significantly higher than that in non-SVR cases [94.9% versus 65.4%, p=0.001; Figure 1(d)].

For biochemical changes compared with baseline among the entire cohort, median levels of aspartate transaminase (AST) and ALT significantly declined at 4 weeks after baseline, 12 weeks after baseline,
Table 1. Patient characteristics of the entire cohort (85 CHC patients completing PegIFN-α/ribavirin therapy with paired liver biopsies) and its separate groups divided by whether achieving SVR or not.

| Variable                        | Entire cohort (n=85) | Separate groups | Non-SVR cases (n=26) | p-value |
|---------------------------------|----------------------|-----------------|-----------------------|---------|
|                                 | SVR cases (n=59)     |                 |                       |         |
| Baseline characteristics        |                      |                 |                       |         |
| PegIFN-α, 2a/2b                 | 59 (69.4%)/26 (30.6%)| 43 (72.9%)/16 (27.1%)| 16 (61.5%)/10 (38.5%)| 0.317   |
| Sex, male/female                | 46 (54.1%)/39 (45.9%)| 29 (49.2%)/30 (50.8%)| 17 (65.4%)/9 (34.6%)  | 0.238   |
| Age [years]                     | 54 [49–61]           | 54 [50–61]      | 52 [43–61]            | 0.366   |
| HBV coinfection                 | 7 (8.2%)             | 3 (5.1%)        | 4 (15.4%)             | 0.193   |
| HCV genotype                    |                      |                 |                       |         |
| 1b                              | 42 (49.4%)           | 26 (44.1%)      | 16 (61.5%)            | 0.163   |
| 2a                              | 19 (22.4%)           | 17 (28.8%)      | 2 (7.7%)              | 0.046*  |
| Others                          | 24 (28.2%)           | 16 (27.1%)      | 8 (30.8%)             | 0.796   |
| METAVIR scores                  |                      |                 |                       |         |
| Fibrosis stages                 |                      |                 |                       |         |
| F0                              | 0 (0.0%)             | 0 (0.0%)        | 0 (0.0%)              |         |
| F1                              | 32 (37.6%)           | 21 (35.6%)      | 11 (42.3%)            | 0.630   |
| F2                              | 37 (43.5%)           | 25 (42.4%)      | 12 (46.2%)            | 0.814   |
| F3                              | 8 (9.4%)             | 6 (10.2%)       | 2 (7.7%)              | 1.000   |
| F4                              | 8 (9.4%)             | 7 (11.9%)       | 1 (3.8%)              | 0.425   |
| Activity grades                 |                      |                 |                       |         |
| A0                              | 14 (16.5%)           | 5 (8.5%)        | 9 (34.6%)             | 0.008*  |
| A1                              | 54 (63.5%)           | 43 (72.9%)      | 11 (42.3%)            | 0.013*  |
| A2                              | 16 (18.8%)           | 10 (16.9%)      | 6 (23.1%)             | 0.553   |
| A3                              | 1 (1.2%)             | 1 (1.7%)        | 0 (0.0%)              | 1.000   |
| Steatosis scores                |                      |                 |                       |         |
| S0                              | 24 (28.2%)           | 17 (28.8%)      | 7 (26.9%)             | 1.000   |
| S1                              | 57 (67.1%)           | 39 (66.1%)      | 18 (69.2%)            | 1.000   |
| S2                              | 4 (4.7%)             | 3 (5.1%)        | 1 (3.8%)              | 1.000   |
| S3                              | 0 (0.0%)             | 0 (0.0%)        | 0 (0.0%)              |         |
| Alcohol abuse                   | 10 (11.8%)           | 5 (8.5%)        | 5 (19.2%)             | 0.271   |
| Diabetes mellitus               | 24 (28.2%)           | 19 (32.2%)      | 5 (19.2%)             | 0.298   |
| Anti-HCV (S/CO)                 | 14.18 [12.76–14.96]  | 14.29 [12.84–15.36] | 13.98 [12.34–14.77]  | 0.496   |

(Continued)
| Variable                  | Entire cohort (n=85) | Separate groups                                                                 |
|--------------------------|----------------------|---------------------------------------------------------------------------------|
|                          | SVR cases (n=59)     | Non-SVR cases (n=26)                                                           | p-value     |
| HCV RNA (10^4 copies/ml) | 3.24 (0.28–11.07)    | 1.81 (0.13–10.60)                                                             | 6.51 (2.74–14.12) | 0.004*    |
| AST (U/l)                | 62 (40–96)           | 62 (41–94)                                                                     | 63 (39–105) | 0.964    |
| ALT (U/l)                | 85 (53–148)          | 89 (54–147)                                                                    | 77 (51–154) | 0.713    |
| Total bilirubin (mg/dl)  | 0.90 (0.71–1.12)     | 0.86 (0.70–1.09)                                                               | 1.00 (0.75–1.26) | 0.159    |
| Albumin [g/dl]           | 4.3 (4.1–4.5)        | 4.3 (4.1–4.5)                                                                  | 4.3 (4.1–4.5) | 0.792    |
| INR                      | 1.03 (0.98–1.08)     | 1.03 (0.99–1.09)                                                               | 1.02 (0.98–1.08) | 0.775    |
| Hemoglobin [g/dl]        | 14.3 (13.4–15.4)     | 14.3 (13.4–15.3)                                                               | 14.5 (13.4–15.8) | 0.478    |
| Platelet counts [10^9/µl]| 166 (133–203)        | 159 (133–202)                                                                  | 176 (132–210) | 0.369    |
| AFP [ng/ml]              | 5.17 (3.37–10.33)    | 5.54 (3.51–10.11)                                                             | 4.58 (2.52–12.39) | 0.455    |
| Glucose [mg/dl]          | 102 (95–124)         | 103 (96–136)                                                                   | 100 (93–108) | 0.122    |
| HbA1c (%)                | 5.8 (5.4–6.2)        | 5.8 (5.5–6.4)                                                                  | 5.8 (5.4–6.0) | 0.312    |
| Total cholesterol (mg/dl)| 178 (151–197)        | 179 (158–201)                                                                  | 176 (129–194) | 0.315    |
| HDL [mg/dl]              | 41.1 (36.2–51.0)     | 41.3 (38.0–51.6)                                                               | 39.5 (29.6–51.0) | 0.148    |
| LDL [mg/dl]              | 102.8 (88.3–125.4)   | 101.6 (92.9–128.7)                                                             | 103.9 (68.5–122.8) | 0.217    |
| Triglyceride [mg/dl]     | 95 (68–128)          | 96 (66–120)                                                                    | 93 (70–156) | 0.521    |
| Creatinine [mg/dl]       | 0.82 (0.66–0.96)     | 0.81 (0.65–0.96)                                                               | 0.84 (0.71–0.96) | 0.695    |
| TSH [µIU/ml]             | 1.434 (0.988–2.246)  | 1.466 (0.935–2.350)                                                            | 1.398 (1.034–2.175) | 0.911    |
| Free thyroxine [ng/dl]   | 0.85 (0.76–0.98)     | 0.84 (0.72–0.99)                                                               | 0.88 (0.81–0.95) | 0.776    |

**Virological response**

| RVR                        | 45 (52.9%)           | 38 (64.4%)                                                                   | 7 (26.9%)     | 0.002*    |
| EVR                       | 75 (88.2%)           | 54 (91.5%)                                                                   | 21 (80.8%)    | 0.271    |
| At EOT                    | 77 (90.6%)           | 57 (96.6%)                                                                   | 20 (76.9%)    | 0.009*    |

**Mean RBV dosage (mg/day)**

| 875 (800–1000) | 858 (800–1000) | 884 (800–1000) | 0.765 |

**Years between biopsies**

| 6.75 (5.63–7.54) | 6.83 (5.58–7.67) | 6.38 (5.71–7.02) | 0.282 |

(1) When each variable was assessed, cases with missing data were excluded from analysis. (2) Nominal and ordinal 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. AFP, alpha-fetoprotein; ALT, alanine transaminase; Anti-HCV, HCV antibody; AST, aspartate transaminase; EOT, end of treatment; EVR, early virological response; HbA1c, hemoglobin A1c; HBV, hepatitis B virus; HCV, hepatitis C virus; HDL, high-density lipoprotein; INR, international normalized ratio; LDL, low-density lipoprotein; PegIFN, pegylated interferon; RBV, ribavirin; RNA, ribonucleic acid; RVR, rapid virological response; SVR, sustained virological response; TSH, thyroid-stimulating hormone. *A p-value < 0.05 was considered statistically significant.
median platelet counts significantly increased in SVR cases but decreased in non-SVR cases among the entire cohort [Figure 2(c)].

Figures 3(a), (c), 4(a), and (c) provide the distributions of corresponding EOS fibrosis stages or activity grades to each baseline fibrosis stage or activity grade in SVR or non-SVR cases among the entire cohort. For SVR cases among the entire cohort, patients with baseline fibrosis stages <2 ($n=21$) were more likely to achieve fibrosis clearance and avoid significant fibrosis (METAVIR score F2/F3/F4) at EOS compared with those with baseline fibrosis stages ≥2 ($n=38$) [rates: fibrosis clearance, 9.1% versus 0.0%, $p=0.423$; significant fibrosis at EOS, 45.5% versus 53.3%, $p=1.000$; Figure 3(b)]. As for non-SVR cases among the entire cohort, patients with baseline fibrosis stages <2 ($n=11$) had no significant advantage in attaining fibrosis clearance and avoiding significant fibrosis at EOS compared with those with baseline fibrosis stages ≥2 ($n=15$) [rates: fibrosis clearance, 9.1% versus 0.0%, $p=0.423$; significant fibrosis at EOS, 45.5% versus 53.3%, $p=1.000$; Figure 4(b)], whereas the rate of activity absence at EOS in patients with baseline activity grades <2 ($n=20$) was significantly higher than that in those with baseline activity grades ≥2 ($n=6$) [60.0% versus 0.0%, $p=0.017$; Figure 4(d)].

**Predictors of fibrosis clearance in SVR cases among the entire cohort**

For SVR cases among the entire cohort, six factors significantly predicted fibrosis clearance in univariate analysis [baseline characteristics: age <48
Figure 2. Levels of AST, ALT, and platelet counts at different time points in SVR (n = 59) and non-SVR cases (n = 26) among the entire cohort (n = 85). (a) AST (U/l). (b) ALT (U/l). (c) Platelet counts (10^3/μl). Data are depicted with box and whisker plots; the middle line represents the median, the upper and lower hinges indicate the first and third quartiles, and the upper and lower whiskers display the range (minimum to maximum). Dashed lines indicate the medians of baseline values. Asterisks (*) denote that the median significantly decreased or increased compared with baseline (p < 0.05; Wilcoxon signed-rank test).

1. When each variable was assessed, cases with missing data were excluded from analysis.
2. Detailed data can be found in Supplementary Table S3 online.

AST, aspartate transaminase; ALT, alanine transaminase; SVR, sustained virological response; 4 wks, 4 weeks after baseline; 12 wks, 12 weeks after baseline; EOT, end of treatment; Post-EOT 24 wks, 24 weeks after EOT; EOS, end of surveillance.
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(OR = 6.286, p = 0.024), fibrosis stages < 2 (OR = 7.179, p = 0.009), platelet counts ≥ 170 \times 10^3/µl (OR = 6.286, p = 0.014), and low-density lipoprotein (LDL) levels ≥ 135 mg/dl (OR = 5.429, p = 0.039); biochemical changes: alpha-fetoprotein (AFP) levels declining from ≥ 4 ng/ml at baseline to < 4 ng/ml at 12 weeks after baseline (OR = 6.167, p = 0.029) and AST levels declining by > 70% at EOS compared with baseline (OR = 5.104, p = 0.022); Table 2). Among these factors, two of them remained statistically significant in multivariate analysis, including baseline fibrosis stages < 2 (OR = 7.877, p = 0.042) and AST levels declining by > 70% at EOS compared with baseline (OR = 9.013, p = 0.038) (Table 2).

Predictors of activity response in non-SVR cases among the entire cohort
For non-SVR cases among the entire cohort, four factors were significantly associated with activity response in univariate analysis [baseline characteristics: glucose levels ≤ 105 mg/dl (OR = 12.375, p = 0.010), albumin levels < 4.4 g/dl (OR = 8.000, p = 0.031), and AFP levels ≤ 4 ng/ml (OR = 24.750, p = 0.008) (Table 3). Of these factors, only baseline AST levels > 80 U/l maintained statistical significance in multivariate analysis (OR = 12.588, p = 0.049; Table 3).

Figure 3. EOS METAVIR scores in SVR cases (n = 59) among the entire cohort (n = 85) with varying baseline METAVIR scores. (a) The distribution of EOS fibrosis stages. (b) The rates of fibrosis clearance and significant fibrosis (METAVIR score F2/F3/F4) at EOS. (c) The distribution of EOS activity grades. (d) The rate of activity absence (METAVIR score A0) at EOS. For (b, d), asterisks (*) denote a p-value < 0.05 (statistically significant; Fisher’s exact test).

EOS, end of surveillance; SVR, sustained virological response.
p=0.041), and AST levels declining by >30% at EOS compared with baseline (OR=11.429, p=0.037; Table 4). Among these factors, only baseline glucose levels ≤105 mg/dl significantly correlated with activity response in multivariate analysis (OR=17.741, p=0.047; Table 4).

**Discussion**

For CHC patients completing PegIFN-α/ribavirin therapy, post-treatment changes in liver fibrosis and necroinflammatory activity over a long time scale remain poorly understood. The present study evaluated long-term liver histological changes and their predictors in CHC patients completing PegIFN-α/ribavirin therapy, aiming to identify those at a higher risk of unreversed disease processes. To attain this purpose, detailed clinical factors were included in the study with liver histological status assessed by liver biopsies instead of noninvasive methodologies.

For SVR-achieving CHC patients with pretreatment advanced fibrosis (METAVIR score F3) or cirrhosis, post-SVR surveillance of HCC every 6 months with ultrasound is recommended regardless of received treatment types (interferon-based or interferon-free). This scenario reflects the concern that SVR may not definitely correspond to the clearance of hepatic injuries. To elucidate the role of SVR in long-term liver histological changes after completing PegIFN-α/ribavirin therapy, our study first evaluated whether SVR was associated with a lower risk of liver histological progression. The results showed that SVR indicated long-term advantages in preventing fibrosis progression (for patients able to undergo fibrosis progression) and attaining activity response (for the entire cohort). However, SVR did not guarantee fibrosis clearance, nonclearance regression, or activity response in the study patients. Besides, some of the non-SVR cases in our study still presented benign liver histological status at EOS.
Table 2. Predictors of fibrosis clearance in SVR cases (n = 59) among the entire cohort (n = 85).

| Variable                              | n | Univariate analysis | Multivariate analysis |
|---------------------------------------|---|---------------------|-----------------------|
|                                       |   | OR (95% CI)         | p-value               | OR (95% CI)         | p-value               |
| **Baseline characteristics**          |   |                     |                       |                       |                       |
| PegIFN-α, 2a versus 2b                | 43 versus 16 | 0.990 (0.227–4.315) | 0.990                |                       |                       |
| Sex, male versus female               | 29 versus 30 | 0.833 (0.224–3.103) | 0.786                |                       |                       |
| Age, <48 versus ≥48                   | 8 versus 51  | **6.286 (1.270–31.102)** | **0.024***          | 4.592 (0.306–68.994) | 0.270                |
| **METAVIR scores**                    |   |                     |                       |                       |                       |
| Fibrosis stages, <2 versus ≥2         | 21 versus 38 | **7.179 (1.648–31.279)** | **0.009***          | **7.877 (1.076–57.637)** | **0.042***          |
| Activity grades, <2 versus ≥2         | 48 versus 11 | 2.632 (0.300–23.058) | 0.382                |                       |                       |
| Steatosis scores, 0 versus ≥1         | 17 versus 42 | 1.538 (0.386–6.137) | 0.542                |                       |                       |
| Diabetes mellitus, (–) versus (+)     | 40 versus 19 | 0.795 (0.202–3.136) | 0.744                |                       |                       |
| HCV RNA (10^6 copies/ml)              |   |                     |                       |                       |                       |
| ≤2 versus >2                          | 31 versus 28 | 0.705 (0.189–2.628) | 0.603                |                       |                       |
| ≤3 versus >3                          | 33 versus 26 | 0.933 (0.250–3.482) | 0.918                |                       |                       |
| AST (U/l)                             |   |                     |                       |                       |                       |
| ≤40 versus >40                        | 14 versus 44 | 1.227 (0.277–5.439) | 0.787                |                       |                       |
| ≤80 versus >80                        | 36 versus 22 | 0.680 (0.180–2.565) | 0.569                |                       |                       |
| ALT (U/l)                             |   |                     |                       |                       |                       |
| ≤55 versus >55                        | 15 versus 43 | 0.581 (0.110–3.057) | 0.522                |                       |                       |
| ≤110 versus >110                      | 37 versus 21 | 0.992 (0.253–3.883) | 0.990                |                       |                       |
| Total bilirubin (mg/dl), ≤1.2 versus >1.2 | 46 versus 12 | 0.632 (0.139–2.867) | 0.552                |                       |                       |
| Albumin (g/dl), ≥4.4 versus <4.4      | 26 versus 31 | 0.623 (0.160–2.424) | 0.495                |                       |                       |
| Platelet counts (10^9/µl), ≥170 versus <170 | 22 versus 36 | **6.286 (1.450–27.250)** | **0.014***          | 3.131 (0.436–22.478) | 0.256                |
| AFP (ng/ml), <4 versus ≥4             | 18 versus 40 | 1.347 (0.339–5.345) | 0.672                |                       |                       |
| Glucose (mg/dl), ≤105 versus >105     | 32 versus 24 | 0.556 (0.147–2.097) | 0.386                |                       |                       |
| HbA1c (%) , ≤5.7 versus ≥5.7          | 22 versus 31 | 0.541 (0.123–2.379) | 0.417                |                       |                       |
| HDL (mg/dl), ≥40 versus <40           | 34 versus 19 | 0.972 (0.244–3.869) | 0.968                |                       |                       |
| LDL (mg/dl), ≥135 versus <135         | 8 versus 45  | **5.429 (1.092–26.977)** | **0.039***          | 1.668 (0.106–26.177) | 0.716                |

(Continued)
histological changes. In sum, for CHC patients completing PegIFN-α/ribavirin therapy, long-term changes in fibrosis and necroinflammatory activity are diverse in both SVR and non-SVR cases. Therefore, we further investigated the predictors of varying liver histological changes under the separation between SVR and non-SVR cases among the entire cohort. As activity response was predominantly seen in SVR cases, only non-SVR cases were screened for the predictors of activity changes.

Among the entire cohort, younger age at baseline significantly predicted fibrosis clearance in SVR cases (univariate analysis), implying that deferred anti-HCV treatment attenuates fibrosis clearance despite treatment success. Besides, a baseline fibrosis stage <2 was an independent predictor of fibrosis clearance and meanwhile indicated a significantly lower risk of EOS significant fibrosis for SVR cases among the entire cohort. Similarly, a higher level of platelet counts (≥170 10^3/µl) was significantly predictive for fibrosis clearance in SVR cases among the entire cohort (univariate analysis). As for non-SVR cases among the entire cohort, a higher AST level of >80 U/l (multivariate analysis) and a lower albumin level of <4.4 g/dl (univariate analysis) significantly predicted significant fibrosis at EOS. These results jointly suggest that for both SVR- and non-SVR-achieving CHC patients, pretreatment severity of the disease plays a decisive role in long-term fibrosis changes following treatment completion. Future studies are required to investigate whether these results remain consistent in CHC patients treated with DAAs.

In our study, univariate analysis also showed that a baseline LDL level ≥135 mg/dl was a significant predictor of fibrosis clearance for SVR cases among the entire cohort. Previous studies have proposed that lipids play a vital role in the HCV life cycle in which viral replication and assembly require lipid raft-like domains and lipid droplets, respectively.21 Besides, the LDL receptor (LDLR) is a surface membrane glycoprotein responsible for the uptake of LDL from serum to hepatocytes.22 To facilitate the intracellular need of lipids for viral proliferation, HCV enhances LDLR expression via upregulating sterol-regulatory element (SRE) binding proteins, the proteins that activate LDLR transcription by binding to SRE-1 in the LDLR promoter, and downregulating proprotein convertase subtilisin/kexin type 9, a protein that induces LDLR degradation.23 Furthermore, CHC patients were found to present a lower level of serum LDL compared with healthy blood donors.24 These findings and our study collectively suggest that a higher level of LDL in serum indicates a less activated LDLR expression which impairs HCV propagation and therefore leads to a higher chance of fibrosis.

Table 2. (Continued)

| Variable | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|
|          | n                   | OR (95% CI)           | p-value   | OR (95% CI) | p-value |
|          | OR (95% CI) | p-value | OR (95% CI) | p-value |
| Biochemical changes | | | | |
| Baseline versus 12 weeks after baseline | | | | |
| AFP levels declining from ≥4 to <4 ng/ml, (+) versus (–) | 8 versus 43 | 6.167 (1.205–31.550) | 0.029* | 3.562 (0.348–36.474) | 0.285 |
| Baseline versus EOS | | | | |
| AST levels declining by >70%, (+) versus (–) | 19 versus 39 | 5.104 (1.268–20.544) | 0.022* | 9.013 (1.130–71.896) | 0.038* |

[1] To calculate the OR, each variable was categorized into a binary form with the latter designated as the reference factor. [2] For each variable, cases with missing data were excluded from both univariate and multivariate analysis. [3] Variables entered into multivariate analysis were those showing a p-value <0.05 in univariate analysis. [4] Additional data can be found in Supplementary Tables S4 and S5 online. AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; EOS, end of surveillance; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; PegIFN, pegylated interferon; RNA, ribonucleic acid.

*p-Value <0.05 was considered statistically significant. Bold values represent statistical significance in univariate or multivariate analysis.
Table 3. Predictors of significant fibrosis (METAVIR score F2/F3/F4) at EOS in non-SVR cases (n=26) among the entire cohort (n=85).

| Variable                          | n     | Univariate analysis | Multivariate analysis |
|-----------------------------------|-------|---------------------|-----------------------|
|                                   |       | OR (95% CI)         | p-value               |
| **Baseline characteristics**      |       |                     |                       |
| PegIFN-α, 2a versus 2b           | 16 versus 10 | 1.000 (0.206–4.856) | 1.000                 |
| Sex, male versus female           | 17 versus 9  | 0.350 (0.065–1.895)  | 0.223                 |
| Age, ≥48 versus <48              | 17 versus 9  | 6.417 (0.999–41.212) | 0.050                 |
| HBV coinfection, (+) versus (-)  | 4 versus 22 | 0.278 (0.025–3.104)  | 0.298                 |
| **METAVIR scores**               |       |                     |                       |
| Fibrosis stages, ≥2 versus <2    | 15 versus 11 | 1.371 (0.288–6.535)  | 0.692                 |
| Activity grades, ≥2 versus <2    | 6 versus 20 | 7.500 (0.733–76.773) | 0.090                 |
| Steatosis scores, ≥1 versus 0    | 19 versus 7  | 1.481 (0.258–8.499)  | 0.659                 |
| Alcohol abuse, (+) versus (-)    | 5 versus 21 | 5.333 (0.506–56.236) | 0.164                 |
| Diabetes mellitus, (+) versus (-)| 5 versus 21 | 5.333 (0.506–56.236) | 0.164                 |
| HCV RNA (10^6 copies/ml)         |       |                     |                       |
| >2 versus ≤2                     | 21 versus 5 | 1.650 (0.227–11.993) | 0.621                 |
| >3 versus ≤3                     | 17 versus 9 | 1.406 (0.277–7.131)  | 0.681                 |
| **AST (U/l)**                     |       |                     |                       |
| >40 versus ≤40                   | 18 versus 8 | 4.714 (0.734–30.278) | 0.102                 |
| >80 versus ≤80                   | 11 versus 15 | 12.375 (1.828–83.767) | 0.010*                |
| **ALT (U/l)**                     |       |                     |                       |
| >55 versus ≤55                   | 18 versus 8 | 4.714 (0.734–30.278) | 0.102                 |
| >110 versus ≤110                 | 7 versus 19 | 3.437 (0.527–22.432) | 0.197                 |
| Total bilirubin (mg/dl), >1.2 versus ≤1.2 | 7 versus 19 | 0.291 (0.045–1.898)  | 0.197                 |
| Albumin (g/dl), <4.4 versus ≥4.4 | 15 versus 10 | 8.000 (1.215–52.693) | 0.031*                |
| **Platelet counts (10^3/µl), <170 versus ≥170** | 13 versus 13 | 2.560 (0.527–12.431) | 0.244                 |
| **AFP (ng/ml), ≥4 versus <4**    | 15 versus 10 | 24.750 (2.333–262.586) | 0.008*                |
| Glucose (mg/dl), >105 versus ≤105 | 10 versus 16 | 3.889 (0.718–21.061)  | 0.115                 |
| HbA1c (%), ≥5.7 versus <5.7      | 15 versus 10 | 3.500 (0.638–19.195)  | 0.149                 |
| HDL (mg/dl), <40 versus ≥40      | 12 versus 11 | 1.750 (0.329–9.298)  | 0.511                 |

(Continued)
Table 3. (Continued)

| Variable | n | Univariate analysis | Multivariate analysis |
|----------|---|---------------------|-----------------------|
|          |   | OR (95% CI)         | p-value               | OR (95% CI)         | p-value               |
| LDL (mg/dl), <135 versus ≥135 | 20 versus 4 | 3.000 (0.265–33.974) | 0.375 |          |                        |

Biochemical changes

Baseline versus 12 weeks after baseline

| Variable | n | Univariate analysis | Multivariate analysis |
|----------|---|---------------------|-----------------------|
|          |   | OR (95% CI)         | p-value               | OR (95% CI)         | p-value               |
| AFP levels declining from ≥4 to <4 ng/ml, [–] versus [+] | 18 versus 4 | 1.000 (0.115–8.730) | 1.000 |          |                        |

Baseline versus EOS

| Variable | n | Univariate analysis | Multivariate analysis |
|----------|---|---------------------|-----------------------|
|          |   | OR (95% CI)         | p-value               | OR (95% CI)         | p-value               |
| AST levels declining by ≥70%, [–] versus [+] | 24 versus 2 | 1.000 (0.056–17.903) | 1.000 |          |                        |

[1] To calculate the OR, each variable was categorized into a binary form with the latter designated as the reference factor. [2] For each variable, cases with missing data were excluded from both univariate and multivariate analysis. [3] Variables entered into multivariate analysis were those showing a p-value < 0.05 in univariate analysis. [4] Additional data can be found in Supplementary Tables S6 and S7 online. AFP, alpha-fetoprotein; ALT, alanine transaminase; CI, confidence interval; EOS, end of surveillance; HBV, hepatitis B virus; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; PegIFN, pegylated interferon; RNA, ribonucleic acid.

[5] Binary logistic regression: A p-value < 0.05 was considered statistically significant.

Bold values represent statistical significance in univariate or multivariate analysis.

Clearance for SVR-achieving CHC patients treated with PegIFN-α/ribavirin therapy.

In terms of factors predicting activity changes in our study, baseline glucose levels ≤ 105 mg/dl were independently predictive for activity response of non-SVR cases among the entire cohort. Previous studies have reported that HCV infection is linked with altered glucose metabolism. By decreasing insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1, HCV infection induces insulin resistance which leads to the suppression of phosphoinositide 3-kinase and protein kinase B, eventually inhibiting glucose uptake and promoting gluconeogenesis in hepatocytes. Besides, HCV replication downregulates cell surface expression of glucose transporters, which also suppresses glucose uptake in hepatocytes. Furthermore, through reactive oxygen species-dependent JNK activation, HCV infection promotes nuclear accumulations of forkhead box O1, a transcription factor that upregulates gene expression of the enzymes for hepatic gluconeogenesis including phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, thus enhancing glucose production by gluconeogenesis in hepatocytes. Taken together, HCV infection suppresses glucose uptake and induces gluconeogenesis in hepatocytes, causing hyperglycemia and high glucose levels in HCV-infected hepatocytes. To explain how HCV infection benefits from glucose metabolic disorders, previous studies have proposed that abundant glucose promotes HCV replication or assembly mainly through two mechanisms. First, fatty acid synthesis (FAS) and its downstream production of lipid droplets are required for HCV replication and assembly, and glucose meets the need for FAS through glycolysis and the tricarboxylic acid cycle. Second, glucose shortage leads to the activation of adenosine monophosphate-activated protein kinase (AMPK) which inhibits FAS and hepatic lipid accumulations, and high glucose levels prevent AMPK from being activated, thereby enhancing HCV replication.

In addition to baseline factors mentioned above, several variables of biochemical changes also served as the predictors of liver histological changes in the present study. For SVR cases among the entire cohort, an early decline in AFP levels (from ≥4 ng/ml at baseline to <4 ng/ml at 12 weeks after baseline) significantly predicted fibrosis clearance in univariate analysis. Previous studies have found that elevated serum AFP levels indicate more advanced fibrosis in treatment-naïve CHC patients. To explain this phenomenon, Kuhlmann et al. proposed that when the liver is injured, serum AFP levels rise due to AFP synthesis by regenerating adult hepatocytes with AFP gene activation or...
Table 4. Predictors of activity response (activity grades decreasing or maintaining at A0) in non-SVR cases (n = 26) among the entire cohort (n = 85).

| Variable                  | n              | Univariate analysis | Multivariate analysis |
|---------------------------|-----------------|---------------------|-----------------------|
|                           |                | OR (95% CI)         | p-value               | OR (95% CI)         | p-value               |
| **Baseline characteristics** |                 |                     |                       |                      |                       |
| PegIFN-α, 2a versus 2b    | 16 versus 10   | 1.467 (0.282–7.627) | 0.649                 |                     |                       |
| Sex, male versus female   | 17 versus 9    | 1.920 (0.358–10.286)| 0.446                 |                     |                       |
| Age, <48 versus ≥48       | 9 versus 17    | 7.111 (0.723–69.985)| 0.093                 |                     |                       |
| **METAIVR scores**        |                 |                     |                       |                      |                       |
| Fibrosis stages, <2 versus ≥2 | 11 versus 15 | 0.208 (0.037–1.181) | 0.076                 |                     |                       |
| Activity grades, <2 versus ≥2 | 20 versus 6    | 0.300 (0.029–3.071) | 0.310                 |                     |                       |
| Steatosis scores, 0 versus ≥1 | 7 versus 19  | 0.615 (0.104–3.658) | 0.593                 |                     |                       |
| Alcohol abuse, (–) versus (+) | 21 versus 5  | 1.333 (0.179–9.912) | 0.779                 |                     |                       |
| Diabetes mellitus, (–) versus (+) | 21 versus 5 | 1.333 (0.179–9.912) | 0.779                 |                     |                       |
| HCV RNA [10^6 copies/ml]  |                 |                     |                       |                      |                       |
| ≤2 versus >2              | 5 versus 21    | 0.267 (0.035–2.019) | 0.201                 |                     |                       |
| ≤3 versus >3              | 9 versus 17    | 0.521 (0.097–2.790) | 0.446                 |                     |                       |
| **AST (U/l)**             |                 |                     |                       |                      |                       |
| ≤40 versus >40            | 8 versus 18    | 0.833 (0.147–4.723) | 0.837                 |                     |                       |
| ≤80 versus >80            | 15 versus 11   | 0.563 (0.105–3.023) | 0.502                 |                     |                       |
| **ALT (U/l)**             |                 |                     |                       |                      |                       |
| ≤55 versus >55            | 8 versus 18    | 0.385 (0.068–2.164) | 0.278                 |                     |                       |
| ≤110 versus >110          | 19 versus 7    | 0.686 (0.104–4.522) | 0.695                 |                     |                       |
| Total bilirubin (mg/dl), ≤1.2 versus >1.2 | 19 versus 7 | 0.686 (0.104–4.522) | 0.695                 |                     |                       |
| **Albumin (g/dl), ≥4.4 versus <4.4** | 10 versus 15 | 1.167 (0.208–6.559) | 0.861                 |                     |                       |
| **Platelet counts (10^3/µl), ≥200 versus <200** | 7 versus 19 | 0.268 (0.044–1.640) | 0.154                 |                     |                       |
| **AFP (ng/ml), <4 versus ≥4** | 10 versus 15 | 1.167 (0.208–6.559) | 0.861                 |                     |                       |
| **Glucose (mg/dl), ≤105 versus >105** | 16 versus 10 | 16.333 (2.197–121.425) | 0.006*               | 17.741 (1.033–304.675) | 0.047*               |
| **HbA1c (%), <5.7 versus ≥5.7** | 10 versus 15 | 2.667 (0.414–17.169) | 0.302                 |                     |                       |
| **HDL (mg/dl), ≥40 versus <40** | 11 versus 12 | 0.583 (0.097–3.506) | 0.556                 |                     |                       |
| **LDL (mg/dl), ≥135 versus <135** | 4 versus 20 | 1.615 (0.140–18.581) | 0.700                 |                     |                       |

(Continued)
Variable | n | Univariate analysis | p-value | Multivariate analysis | p-value
--- | --- | --- | --- | --- | ---
**Biochemical changes**

**Baseline versus 24 weeks after EOT**

ALT levels remaining $\leq 42$ or declining from $>42$ to $42$ U/l, (+) versus (-) 11 versus 12

| OR (95% CI) | p-value |
| --- | --- |
| 0.114 (0.016–0.806) | 0.029* |

Platelet counts remaining $\geq 200$ $10^3/\mu l$, (+) versus (-) 5 versus 17

| OR (95% CI) | p-value |
| --- | --- |
| 0.077 (0.007–0.901) | 0.041* |

**Baseline versus EOS**

AST levels declining by $>30$%, (+) versus (-) 11 versus 15

| OR (95% CI) | p-value |
| --- | --- |
| 11.429 (1.155–113.115) | 0.037* |

(1) To calculate the OR, each variable was categorized into a binary form with the latter designated as the reference factor. (2) For each variable, cases with missing data were excluded from both univariate and multivariate analysis. (3) Variables entered into multivariate analysis were those showing a p-value < 0.05 in univariate analysis. (4) Additional data can be found in Supplementary Tables S8 and S9 online. AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; CI, confidence interval; EOS, end of surveillance; EOT, end of treatment; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; PegIFN, pegylated interferon; RNA, ribonucleic acid.

*Binary logistic regression: A p-value < 0.05 was considered statistically significant.

Bold values represent statistical significance in univariate or multivariate analysis.

differentiating biliary epithelial cells with fetal gene reactivation. Therefore, the level of AFP in serum may be considered as an indicator for the severity of liver injuries. Given so, we inferred that diminished liver injuries at an early stage benefit post-SVR clearance of fibrosis. Besides, considering that a higher baseline AFP level ($\geq 4$ ng/ml) significantly predicted EOS significant fibrosis of non-SVR cases in our study cohort (univariate analysis), a more severe pretreatment liver injury may lead to a more advanced final fibrotic outcome if anti-HCV treatment fails. Future studies are needed to validate these inferences in DAA-treated CHC patients. As for other predictive biochemical changes in our study, fibrosis clearance of SVR cases among the entire cohort was featured by a much more significant decline in AST levels (by $>70$%; multivariate analysis) in the same time frame (from baseline to EOS). A significant decline in AST levels (by $>30$%) from baseline to EOS also indicated concurrent changes in necroinflammatory activity (activity response) for non-SVR cases among the entire cohort (univariate analysis). However, the normalization of ALT levels (remaining $\leq 42$ or declining from $>42$ to $42$ U/l) or the maintenance of higher platelet counts (remaining $\geq 200$ $10^3/\mu l$) from baseline to 24 weeks after EOT was linked with activity nonresponse in non-SVR cases among the entire cohort (univariate analysis), implying that the liver with improved or maintained functions allows for a more progressive disease following the failure of anti-HCV treatment.

The major limitation of this study is that we were unable to enroll and include a large number of participants as most of the CHC patients are unwilling to receive a liver biopsy despite its accuracy. Besides, sample size calculation was not performed. However, the distribution of HCV genotypes in our study cohort (Supplementary Table S10 online) was similar to that in the general CHC population in Taiwan (genotypes 1b and 2a being the dominant types with genotypes 1 and 2 accounting for around 53% and 40% of the CHC population, respectively), suggesting that patients included in our study were somewhat representative and generalizable. Another limitation of this study is that liver biopsies were not performed at time points other than baseline and EOS (such as 4 or 12 weeks after baseline, EOT, and 24 weeks after EOT), making it unfeasible to compare liver histological changes over short and long time frames in CHC patients completing PegIFN-α/ribavirin therapy. Nonetheless, biochemical values measured at different time points reflecting the variation of liver functions helped elucidate the pattern of liver histological
changes in our study. However, different biochemical parameters may be inconsistent in indicating liver histological changes. To determine which of them shares the most similar changing pattern with liver histological status over short and long time scales in CHC patients receiving anti-HCV treatment, future studies combining liver biopsies and biochemical tests performed at multiple time points are needed. At last, although our study was conducted in CHC patients completing PegIFN-α/ribavirin therapy, the results providing a preliminary insight could also benefit the DAA era as it remains unknown whether HCV eradication with interferon-based therapy is the same as with DAAs.35

**Conclusion**

For CHC patients completing PegIFN-α/ribavirin therapy, SVR indicates a lower risk of liver histological progression but does not guarantee benign fibrosis or activity changes under long-term follow-up. Based on the independent predictors of liver histological changes found in our study, we suggested the following surveillance criterion for CHC patients completing PegIFN-α/ribavirin therapy: (1) for SVR cases, those with baseline fibrosis stages ≥2 or the absence of significantly declined follow-up AST levels (by >70% compared with baseline) should be specifically monitored for fibrosis changes as fibrosis is less likely to be cleared in these patients; (2) for non-SVR cases, retreatment with DAAs should be preferentially performed in those with baseline AST levels >80 U/l or glucose levels >105 mg/dl as they are more likely to present advanced liver histological outcomes after the failure of PegIFN-α/ribavirin therapy.

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**Author contributions**

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**Tzu-Yu Kao**: Data curation; Formal analysis; Investigation; Software; Writing – review & editing.

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**Chun-Chi Kao**: Data curation; Formal analysis; Investigation; Resources; Writing – review & editing.

**Cheng-Yuan Peng**: Investigation; Resources; Writing – review & editing.

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**Data accessibility statement**

All data generated or analyzed during this study are included in this published article and its supplementary material files.

**Supplemental material**

Supplemental material for this article is available online.

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