Evolution of ferritin levels in hepatitis C patients treated with antivirals

Ming-Ling Chang1,2*, Jing-Hong Hu2,3, Ching-Hao Yen4, Kuan-Hsing Chen5, Chia-Jung Kuo1, Ming-Shyan Lin6, Cheng-Han Lee1,2, Shiang-Chi Chen7 & Rong-Nan Chien1,2*

The evolution of ferritin levels in hepatitis C virus (HCV)-infected patients with sustained virological responses (SVRs) following various therapy regimens remains elusive. An 8-year prospective cohort study of 1194 HCV-infected patients [interferon-based therapy (n = 620), direct-acting antiviral agent (DAA) therapy (n = 355)] was conducted. At baseline, sex, alanine aminotransferase (ALT), triglycerides, homeostatic model assessment of insulin resistance (HOMA-IR), estimated glomerular filtration rate (eGFR), hemoglobin, iron/total iron-binding capacity (Fe/TIBC) and IFNL3-rs12979860 genotypes were associated with ferritin levels. At 24 weeks posttherapy, ALT, triglycerides, total cholesterol, eGFR, Fe/TIBC and the therapy regimen were associated with ferritin levels in SVR patients. Among interferon-treated patients, ferritin levels increased at 24 weeks posttherapy, regardless of SVR, and 24-week posttherapy ferritin levels were higher in non-SVR patients (n = 111) than in SVR patients (n = 509); ferritin levels began decreasing at 3 years posttherapy and were lower than pretherapy levels since 4 years posttherapy in SVR patients. Among DAA-treated SVR patients (n = 350), ferritin levels decreased and remained stable since 24 weeks posttherapy. ALT, triglycerides, eGFR, and Fe/TIBC were HCV-unrelated factors associated with ferritin levels; sex, HOMA-IR, total cholesterol, hemoglobin and IFNL3-rs12979860 genotype were HCV-related factors associated with ferritin levels. In interferon-treated SVR patients, the increased trend of posttherapy ferritin levels was not reversed until 4 years posttherapy. In DAA-treated SVR patients, ferritin levels decreased since 24 weeks posttherapy.

Hepatitis C virus (HCV) is a human pathogen responsible for acute and chronic liver disease that chronically infects an estimated 71.1 million individuals worldwide1 and is classified into 8 genotypes2. Chronic HCV infection (CHC) is characterized by hepatic iron overload, hyperferraemia and hyperferritinaemia3,4, as an estimated 30–40% of CHC patients have elevated ferritin levels5. Ferritin keeps iron in a nontoxic form and reflects body iron stores6; it is also an acute phase protein and becomes elevated as inflammation occurs in chronic liver injury7. Transfusion (an important risk factor for HCV infection)8, oxidative stress9–11, steatosis12 and fibrosis12,13 are all associated with hyperferritinaemia in CHC patients. Iron overload with hyperferraemia might suppress functions of the complement system14 and accelerate the persistence of HCV infection. In addition, compared with patients who respond to interferon-based anti-HCV therapy, CHC patients who do not respond had higher baseline serum ferritin levels15,16, and a serum ferritin level that is higher during therapy than at baseline was shown to be associated with a favorable treatment response16. Serum ferritin levels thus might be affected not only by HCV-related hepatic injury but also by interferon-based anti-HCV therapy, as interferon has been associated with increases in lipid levels17 and with immune modulation in patients18. With the advent of direct-acting antiviral agents (DAAs), which target specific proteins of HCV during its life cycle19, anti-HCV therapy has resulted in a high cure rate with a short treatment duration in CHC patients, and HCV-associated ferritin level alterations may not be masked by any interferon effect. The factors that affect the ferritin levels in CHC patients and how
the anti-HCV therapeutic regimens and responses affect the serum ferritin levels remain elusive but are crucial for the prognosis of CHC patients, as ferritin is essential for iron homeostasis and is involved in a wide range of physiologic and pathologic processes, and the ferritin levels in CHC patients with sustained virological response (SVR) might serve as a prognostic marker. Comparing the pre- and posttreatment variables in SVR patients has provided an excellent opportunity to eliminate the interference caused by individual bias when reviewing the impact of HCV on alterations in ferritin levels. Accordingly, we sought to fill the aforementioned knowledge gaps by conducting an 8-year prospective cohort study analyzing the serum ferritin levels and crucial confounders of CHC patients before and after interferon-based or DAA-based anti-HCV therapy.

Materials and methods

Patients. The study group comprised subjects aged 18 years or older with CHC, defined as serum HCV-RNA detectable by polymerase chain reaction (PCR) for > 24 weeks. Subjects with human immunodeficiency virus; hepatitis B virus infection; hemochromatosis; primary biliary cholangitis; primary sclerosing cholangitis; autoimmune hepatitis; autoimmune diseases including Sjogren’s syndrome, systemic lupus erythematosus, rheumatoid arthritis and psoriasis; alcoholism; or malignancy and recipients of solid organ transplants were excluded.

Study design. A schematic flow chart for all enrolled patients is shown in Fig. 1. In total, 1194 CHC patients were consecutively recruited at a Taiwan tertiary referral center between January 2010 and December 2019. Of the 1194 patients, 620 had completed a course of anti-HCV therapy with weight-based pegylated interferon-α-2b and ribavirin for either 24 or 48 weeks, and 355 patients had completed a course of various combinations of DAAs (Supplementary Table 1). HCV-RNA levels, genotypes, and single-nucleotide polymorphisms of interferon-λ3 (IFNL3)-rs12979860 were assessed as previously described. Several baseline factors, including sex, age, body mass index (BMI), HCV genotype, the presence of hepatic cirrhosis, levels of HCV-RNA, serum ferritin (normal range: male: 30–400 ng/mL; female: 13–150 ng/mL), iron, iron/total iron-binding capacity (Fe/TIBC) (i.e., transferrin saturation percentage), neutrophil–lymphocyte ratio (NLR), estimated glomerular filtration rate (eGFR), homeostatic model assessment of insulin resistance (HOMA-IR) index (fasting insulin [μU/mL] × fasting glucose [mmol/L]/22.5), total cholesterol (TC), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelets and fibrosis-4 (FIB-4) index [age (years) × AST (U/L)/[PLT(10^9/L) × ALT(U/L)^1/2] were recorded in all patients. Biochemical tests were performed at the clinical pathology laboratories of the hospital using routine automated techniques in an automated clinical chemistry analyzer.
the 1194 CHC patients (Table 2). While we regarded high baseline ferritin levels as the dependent factor, the including male sex, levels of ALT and TG, HOMA-IR, Fe/TIBC and CC genotype of IFNL3-rs12979860 were independent factors were age, ALT, eGFR, TGs, Fe/TIBC and fatty liver status (Supplementary Table 2). Of 620 non-SVR patients, regardless of therapy regimen (p = 0.409 for patients who underwent interferon-based therapy; p = 0.001 for patients who underwent DAA therapy). Furthermore, pretherapy ferritin levels were not associated with SVR, regardless of therapy regimens (p = 0.911 for all patients, p = 0.417 for patients who underwent interferon-based therapy; p = 0.409 for patients who underwent DAA therapy). Therefore, ferritin levels were negatively associated with higher levels of ALT, eGFR, uric acid and hemoglobin; lower levels of NLR and FIB-4 index; and lower rates of hepatic steatosis (Table 1).

Factors associated with ferritin levels of SVR patients at 24 weeks posttherapy. At 24 weeks posttherapy, the levels of ALT, TG, and TC and the ratio of Fe/TIBC were positively associated, while the levels of eGFR and DAA therapy were negatively associated, with the ferritin levels of SVR patients (Table 3). A summary of the associations (baseline and 24 weeks posttherapy) identified between independent factors and ferritin levels is shown in Fig. 2.

Longitudinal alteration of ferritin levels in SVR patients. Among patients who received interferon-based therapy, compared with baseline, levels of ferritin increased at 24 weeks posttherapy, regardless of SVR [SVR (+) patients: 515 ± 672 vs. 365 ± 447 ng/mL, p < 0.001; SVR(−) patients: 896 ± 1281 vs. 418 ± 430 ng/mL, p = 0.001]. However, 24-week posttherapy levels of ferritin were higher in non-SVR patients than in SVR patients (896 ± 1281 vs. 515 ± 672 ng/mL, p = 0.001). Among patients who received DAA therapy and achieved an SVR (n = 350), the levels of ferritin decreased at 24 weeks posttherapy (227 ± 326 vs. 468 ± 632 ng/mL, p < 0.001), while among patients who received DAA therapy without an SVR (n = 5), the comparison between pretherapy and 24-week posttherapy levels of ferritin did not indicate a significant difference (180 ± 28.4 vs. 194 ± 129 ng/mL, p = 0.798). Among all SVR patients, lower levels of 24-week posttherapy ferritin were noted in patients who underwent DAA therapy than in those who underwent interferon-based therapy (227 ± 326 vs. 515 ± 672 ng/mL, p < 0.001) (Fig. 2). Furthermore, the percentage of patients with high ferritin levels was lower among SVR patients who underwent DAA therapy than among those who underwent interferon-based therapy (25.7% vs. 48.4%, p < 0.001).

Longitudinally, as shown in Fig. 3, for SVR patients who underwent interferon-based therapy, compared with pretherapy levels, higher posttherapy ferritin levels were noted until 2 years posttherapy (p = 0.042), while the post- and pretherapy differences disappeared at 3 years posttherapy (p = 0.585), and the posttherapy levels were lower than pretherapy levels from 4 years (p = 0.004) to 8 years posttherapy (p = 0.012) (follow-up duration:
mean ± standard deviation: 1736 ± 753 days, median: 1712 days, range 700–2920 days). For the SVR patients who underwent DAA therapy, posttherapy ferritin levels were lower than pretherapy ferritin levels from 24 weeks to 2 years posttherapy (p < 0.001) (final follow-up duration: mean ± standard deviation: 628 ± 196 days, median: 630 days, range 363–730 days). The ferritin levels in DAA-treated SVR patients remained steady beginning at 24 weeks posttherapy, as all the comparisons between posttherapy ferritin levels were nonsignificant (p values 0.471–0.646). Moreover, all the posttherapy ferritin levels of SVR patients who underwent DAA were lower than those of SVR patients who underwent interferon-based therapy with the same follow-up time (i.e., at 24 weeks and 1 and 2 years posttherapy) (Fig. 3, Supplementary Table 3).

Discussion
The most compelling results of the current study are as follows:

(1) At baseline, sex, ALT, TG, HOMA-IR, eGFR, hemoglobin, Fe/TIBC and IFNL3-rs12979860 genotype were associated with ferritin levels of CHC patients. (2) At 24 weeks posttherapy, ALT, TG, eGFR, Fe/TIBC, and therapy regimen were associated with ferritin levels of SVR patients. (3) Among patients who received interferon-based therapy, the levels of ferritin increased at 24 weeks posttherapy, regardless of SVR. However, 24-week post-therapy ferritin levels were higher in non-SVR patients than in SVR patients. Among SVR patients who received DAA therapy, the levels of ferritin decreased at 24 weeks posttherapy. (4) During the 8-year follow-up, the trend of increased ferritin levels was reversed beginning at 4 years posttherapy in interferon-treated SVR patients; however, a stable trend of decreased ferritin levels remained in DAA-treated SVR patients starting at 24 weeks posttherapy.

It has been proposed that HCV controls iron both by intracellular iron sequestration and intercellular iron mobilization via ferritin, as a means toward enhanced replication. However, consistent with a previous study, no significant correlations were identified between HCV viral load and any iron markers. The factors consistently associated with ferritin levels both pretherapy in CHC patients and at 24 weeks posttherapy in SVR patients, such as ALT, TG, eGFR, and Fe/TIBC, exhibited fundamental links with ferritin, regardless of HCV infection. For example, ferritin levels > 1.5 times higher than normal are usually seen in patients with a > 6 month history of elevated ALT; high TG levels are strongly associated with ferritin levels, every 100 μg/L increase in

|                           | All (n = 1194) | Interferon-based therapy (n = 620) | DAA therapy (n = 355) | p values* |
|---------------------------|---------------|-----------------------------------|----------------------|-----------|
| Male, n (%)               | 627 (52.5)    | 353 (56.9)                        | 166 (46.8)           | 0.001     |
| Age (years)               | 56.93 ± 12.75 | 53.96 ± 11.72                     | 60.33 ± 12.99        | <0.001    |
| BMI (kg/m²)               | 24.80 ± 3.85  | 24.96 ± 3.76                      | 24.80 ± 4.01         | 0.523     |
| HCV genotype              |               |                                   |                      |           |
| Genotype 1, n (%)         | 679 (56.9)    | 327 (52.7)                        | 244 (63.1)           | 0.001     |
| Genotype 2, n (%)         | 412 (34.5)    | 247 (39.8)                        | 106 (29.9)           | 0.001     |
| Genotype 3, n (%)         | 25 (2.1)      | 16 (2.6)                          | 5 (1.4)              | 0.16      |
| Log HCV RNA (logIU/mL)    | 6.00 ± 0.991  | 6.02 ± 1.05                       | 6.03 ± 0.83          | 0.809     |
| ALT (U/L)                 | 91.89 ± 96.59 | 96.92 ± 98.24                     | 81.69 ± 89.55        | 0.016     |
| eGFR (mL/min/1.73 m²)     | 95.97 ± 38.40 | 102.73 ± 36.06                    | 89.08 ± 39.86        | <0.001    |
| TG (mg/dL)                | 103.54 ± 53.45| 103.70 ± 52.77                    | 103.97 ± 53.70       | 0.939     |
| TC (mg/dL)                | 170.30 ± 33.49| 171.52 ± 31.60                    | 170.58 ± 33.78       | 0.666     |
| HOMA-IR                   | 3.23 ± 5.26   | 3.14 ± 4.45                       | 3.24 ± 5.12          | 0.77      |
| Uric acid (mg/dL)         | 5.86 ± 5.59   | 5.94 ± 1.55                       | 5.71 ± 1.62          | 0.041     |
| Ferritin (ng/mL)          | 386.0 ± 492.3 | 375.3 ± 434.1                     | 381.7 ± 532.1        | 0.858     |
| High ferritin, n (%)      | 553 (46.3)    | 296 (47.7)                        | 164 (46.2)           | 0.853     |
| Iron (μg/dL)              | 132.5 ± 56.5  | 138.9 ± 55.3                      | 136.3 ± 58.7         | 2.678     |
| Fe/TIBC (%)               | 0.404 ± 0.185 | 0.410 ± 0.181                     | 0.40 ± 0.187         | 0.736     |
| Hb (g/dL)                 | 13.92 ± 1.85  | 14.30 ± 1.65                      | 13.69 ± 1.92         | <0.001    |
| NLR                       | 1.75 ± 0.96   | 1.63 ± 0.84                       | 1.88 ± 1.03          | <0.001    |
| platelet (10⁹/µL)         | 175.0 ± 66.3  | 176.9 ± 56.56                     | 176.2 ± 72.2         | 0.884     |
| Platelet (10⁹/µL)         | 175.0 ± 66.3  | 176.9 ± 56.56                     | 176.2 ± 72.2         | 0.884     |
| Steatosis, n (%)          | 630 (52.8)    | 297 (47.9)                        | 204 (57.5)           | 0.06      |
| Liver cirrhosis, n (%)    | 423 (20.4)    | 243 (21.9)                        | 84 (23.8)            | 0.38      |
| FIB-4 index               | 3.39 ± 3.39   | 3.09 ± 3.23                       | 3.86 ± 3.64          | 0.003     |
| IFNL3-rs12979860 CC genotype, n (%) | 1018 (85.3) | 935 (85.9) | 300 (84.5) | 0.371     |

Table 1. Baseline characteristics of the 1194 CHC patients. CHC chronic hepatitis C virus infection, DAA direct-acting antivirals, BMI body mass index, HCV hepatitis C virus, RNA ribonucleic acid, ALT alanine transaminase, eGFR estimated glomerular filtration rate, TG triglycerides, TC total cholesterol, HOMA-IR homeostatic model assessment for insulin resistance, Fe/TIBC serum Iron/total iron binding capacity, Hb hemoglobin, NLR neutrophil lymphocyte ratio, FIB-4 fibrosis-4, IFNL3 interferon-λ3. *p values between CHC patients underwent interferon-based or DAA therapy.
### Table 2. Associations of ferritin levels in CHC patients at baseline. CHC chronic hepatitis C virus infection, OR odds ratio, CI confidence interval, BMI body mass index, HCV hepatitis C virus, RNA ribonucleic acid, ALT alanine transaminase, eGFR estimated glomerular filtration rate, TG triglycerides, TC total cholesterol, HOMA-IR homeostatic model assessment for insulin resistance, Fe/TIBC serum Iron/total iron binding capacity, Hb hemoglobin, NLR neutrophil lymphocyte ratio, FIB-4 fibrosis-4, IFNL3 interferon-λ3.

| Baseline factors                              | Univariate analyses |                      | Multivariate analyses |                      |
|-----------------------------------------------|---------------------|----------------------|----------------------|----------------------|
|                                              | 95% CI of OR (OR)   | p values             | 95% CI of OR (OR)    | p values             |
| Male, yes                                     | 98.3 – 225.5 (161.9)| < 0.001              | 2.935 – 130.3 (66.6) | 0.04                 |
| Age (years)                                   | 0.667 – 5.741 (3.20)| 0.013                | 4.366 – 1.196 (1.58) | 0.264                |
| BMI (kg/m²)                                   | − 0.348 – 12.89 (4.71) | 0.26                |                       |                      |
| HCV genotype                                  | − 27.77 – 18.99 (− 4.39) | 0.712               |                       |                      |
| Log HCV RNA (logIU/mL)                        | − 60.1 – 7.4 (− 26.3)| 0.126                |                       |                      |
| ALT (U/L)                                     | 1.457 – 2.055 (1.756)| < 0.001              | 0.841 – 1.381 (1.111) | < 0.001              |
| eGFR (mL/min/1.73 m²)                         | − 1.69 – 0.1007 (− 0.849) | 0.048               | − 1.97 – 0.162 (− 1.066) | 0.021               |
| TG (mg/dL)                                    | 1.452 – 2.657 (2.055)| < 0.001              | 0.793 – 1.904 (1.349) | < 0.001              |
| TC (mg/dL)                                    | − 0.9 – 1.032 (0.066) | 0.893               |                       |                      |
| HOMA-IR                                       | 2.054 – 15.057 (8.655) | 0.01               | 0.806 – 12.073 (6.439) | 0.025               |
| Uric acid (mg/dL)                             | 33.87 – 76.18 (55.0) | < 0.001              | 14.864 – 23.74 (4.438) | 0.652               |
| Fe/TIBC (%)                                   | 1428 – 1722 (1575)   | < 0.001              | 1252 – 1565 (1409)    | < 0.001              |
| Hb (g/dL)                                     | 6.91 – 41.69 (24.3)  | 0.006                | 50.21 – 31.46 (− 31.86) | 0.001               |
| NLR                                           | − 23.8 – 43.4 (9.8)  | 0.567                |                       |                      |
| Platelet (10⁸/μL)                             | − 1.557 – 0.588 (1.07) | < 0.001             | − 3.46 – 0.875 (0.265) | 0.395               |
| Steatosis, yes                                | − 8.2 – 118.5 (55.1) | 0.188                |                       |                      |
| Liver cirrhosis, yes                          | − 107.7 – 52.4 (− 26.6) | 0.519              |                       |                      |
| Fibrosis-4 index                              | 16.4 – 34.8 (25.4)   | < 0.001              | 3.20 – 20.42 (8.606)  | 0.153               |
| IFNL3-rs12979860 CC genotype, yes             | − 227.7 – 34.7 (− 131.2) | 0.008              | 181.9 – 31.46 (106.2) | 0.005               |

### Table 3. Associations of ferritin levels in SVR patients at 24 weeks posttherapy. OR odds ratio, BMI body mass index, ALT alanine transaminase, eGFR estimated glomerular filtration rate, TG triglycerides, TC total cholesterol, HOMA-IR homeostatic model assessment for insulin resistance, Fe/TIBC serum Iron/total iron binding capacity, Hb hemoglobin, NLR neutrophil lymphocyte ratio, FIB-4 fibrosis-4, IFNL3 interferon-λ3, DAA direct-acting antivirals.

| 24-week post-therapy factors                  | Univariate analyses |                      | Multivariate analyses |                      |
|-----------------------------------------------|---------------------|----------------------|----------------------|----------------------|
|                                              | 95% CI of OR (OR)   | p values             | 95% CI of OR (OR)    | p values             |
| Male, yes                                     | − 47.83 – 132.4 (42.29) | 0.357              |                       |                      |
| Age, (years)                                  | − 1.49 – 5.71 (2.1)  | 0.251                |                       |                      |
| BMI (kg/m²)                                   | − 16.7 – 7.286 (− 4.7) | 0.44                |                       |                      |
| ALT (U/L)                                     | 6.19 – 11.91 (9.052) | < 0.001              | 3.007 – 8.107 (5.557) | < 0.001              |
| eGFR (mL/min/1.73 m²)                         | − 4.526 – 1.821 (− 3.17) | < 0.001             | − 3.9 – 1.61 (− 2.76) | < 0.001              |
| TG (mg/dL)                                    | 0.351 – 1.228 (0.779) | 0.001               | 0.146 – 0.909 (0.527) | 0.017               |
| TC (mg/dL)                                    | 0.956 – 3.423 (2.189) | 0.001               | 0.211 – 2.346 (1.278) | 0.019               |
| HOMA-IR                                       | − 0.689 – 29.63 (14.47) | 0.061              | 5.03 – 20.97 (7.53)  | 0.239               |
| Uric acid (mg/dL)                             | 33.59 – 91.36 (62.474) | < 0.001             | 7.11 – 40.52 (16.70) | 0.169               |
| Fe/TIBC (%)                                   | 1811 – 2338 (2075)   | < 0.001              | 1521 – 2030 (1776)   | < 0.001              |
| Hb (g/dL)                                     | − 34.76 – 18.93 (− 7.91) | 0.563              |                       |                      |
| NLR                                           | − 34.1 – 66.98 (16.45) | 0.523               |                       |                      |
| Platelet (10⁸/μL)                             | − 2.06 – 0.591 (1.29) | 0.001               | − 1.12 – 0.661 (− 0.234) | 0.608               |
| Steatosis, yes                                | − 177.8 – 86.3 (45)  | 0.495                |                       |                      |
| Liver cirrhosis, yes                          | − 77.0 – 256.8 (89.92) | 0.29                |                       |                      |
| Fibrosis-4 index                              | − 2.56 – 42.1 (19.755) | 0.083              | − 4.36 – 47.8 (21.72) | 0.102               |
| IFNL3-rs12979860 CC genotype, yes             | − 189.3 – 80.9 (− 54.2) | 0.431              |                       |                      |
| Therapy (interferon = 1, DAA = 2)             | − 352 – 170 (− 261)  | < 0.001              | − 331 – 161 (− 246)  | < 0.001              |

Table 2. Associations of ferritin levels in CHC patients at baseline. CHC chronic hepatitis C virus infection, OR odds ratio, CI confidence interval, BMI body mass index, HCV hepatitis C virus, RNA ribonucleic acid, ALT alanine transaminase, eGFR estimated glomerular filtration rate, TG triglycerides, TC total cholesterol, HOMA-IR homeostatic model assessment for insulin resistance, Fe/TIBC serum Iron/total iron binding capacity, Hb hemoglobin, NLR neutrophil lymphocyte ratio, FIB-4 fibrosis-4, IFNL3 interferon-λ3. 

Table 3. Associations of ferritin levels in SVR patients at 24 weeks posttherapy. OR odds ratio, BMI body mass index, ALT alanine transaminase, eGFR estimated glomerular filtration rate, TG triglycerides, TC total cholesterol, HOMA-IR homeostatic model assessment for insulin resistance, Fe/TIBC serum Iron/total iron binding capacity, Hb hemoglobin, NLR neutrophil lymphocyte ratio, FIB-4 fibrosis-4, IFNL3 interferon-λ3, DAA direct-acting antivirals.
ferritin levels was correlated with 0.26 mL/min per 1.73 m² decrease in eGFR, and the positive association between ferritin levels and transferrin saturation (Fe/TIBC) reflects the correlation between iron store and iron availability. In contrast, the pretherapy-only factors (sex, HOMA-IR, hemoglobulin and IFNL3-rs12979860 genotype) and posttherapy-only factors (TC and anti-HCV therapy) suggested potential links, direct or indirect, between HCV infection and ferritin levels. Consistently, higher hepatic iron concentrations were observed in male CHC patients; the connection between HOMA-IR and ferritin levels in CHC patients has been reported and may correlate with the grade of hepatic iron deposition; the negative association between hemoglobin and ferritin levels among CHC patients seemed to reflect the links between fibrosis (portal hypertension subsequent to hepatic fibrosis might lead to variceal bleeding with anemia and then low hemoglobin levels) and ferritin levels, as elevated serum ferritin levels are independently associated with advanced liver fibrosis; the association might occur through advanced hepatic fibrosis, hepatic steatosis or high necroinflammatory activity. However, the baseline ferritin levels were not associated with SVR among interferon-treated patients in the current study, and a prevalent IFNL3-rs12979860 CC genotype in Taiwan might blunt the impact of baseline ferritin levels on SVR. Interestingly, HCV nonstructural proteins upregulate the ferritin heavy chain, which in turn inhibits apoB-100 secretion, and serum ferritin and apoB-100 concentrations are inversely correlated in HCV-infected patients. The positive association between ferritin and TC among SVR patients might reflect the reversal of HCV-associated inhibition of the secretion of apoB-100, the main apolipoprotein of TC, after HCV clearance. Given that sex, HOMA-IR, TC, hemoglobin and IFNL3-rs12979860 genotype were HCV-related factors associated with serum ferritin levels, special caution is demanded in male CHC patients with high HOMA-IR and TC but low Hb levels and an IFNL3-rs12979860 non-CC genotype due to the potential poor prognosis linked with high ferritin levels.
Therapy regimens also affect ferritin levels in CHC patients. Serum ferritin levels were significantly decreased at the end of therapy, at 12 weeks, 24 weeks, or up to 1 year from baseline in SVR patients following DAA therapy. Since ferritin might work as an acute phase protein and reflect hepatic injury, it is conceivable that ferritin levels decreased after SVR in CHC patients following DAA therapy. Compared with baseline levels, the lower ferritin levels persisted up to 2 years posttherapy among DAA-treated SVR patients in the current study, suggesting a long-term improvement in iron homeostasis. On the other hand, although iron overload improved after SVR in CHC patients who underwent interferon-based therapy, how ferritin levels evolved in these patients remains inconclusive, as ferritin levels had been reported to increase, decrease or remain steady in studies with case numbers ranging from 73 to 191. Based on a cohort of 620 CHC patients who underwent interferon-based therapy, our study demonstrated that the levels of ferritin increased at 24 weeks posttherapy, rather than decreased, regardless of SVR, although the 24-week posttherapy ferritin levels were lower in the SVR patients than in the non-SVR patients. The fact that ferritin levels increased at 24 weeks posttherapy in CHC patients who underwent interferon monotherapy regardless of viral clearance suggests that interferon per se increases ferritin levels. On the other hand, ribavirin-induced hemolysis was shown to significantly increase serum ferritin levels, intrahepatic iron deposition and liver fibrosis in renal transplant patients receiving ribavirin monotherapy. Additionally, ribavirin-induced hemolysis floods hepatocytes and Kupffer cells with heme, which is metabolized and detoxified by heme oxygenase-1 to carbon monoxide, biliverdin and free iron, which induces ferritin. Together, pegylated interferon and ribavirin of interferon-based therapy might synergistically increase serum ferritin levels in CHC patients. This idea explained why both SVR and non-SVR patients treated with interferon-based therapy had increased 24-week posttherapy ferritin levels. Surprisingly, it took at least 4 years to eliminate interferon- and ribavirin-related biases, as the trend of increased ferritin levels was not reversed until 4 years posttherapy. Thus, compared with interferon-based therapy, DAA therapy is not only more effective, safer and more tolerable but also leads to a less vulnerable iron homeostasis status, evidenced by earlier reversal of high ferritin levels after SVR.

Given that most non-SVR patients following interferon-based therapy had received further therapeutic courses of DAA, their long-term post-interferon-based therapy ferritin levels cannot be acquired. Thus, the major limitation of the current study is that the long-term posttherapy ferritin levels between the SVR and non-SVR patients following interferon-based therapy cannot be compared. However, as mentioned, the comparisons between the pre- and posttherapy ferritin levels in the same individuals might be a better alternative to compare the levels between SVR and non-SVR patients in terms of eliminating the individual biases.

In summary, ALT, TG, eGFR, and Fe/TIBC were HCV-unrelated factors associated with ferritin levels, while sex, HOMA-IR, TC, hemoglobin and IFNL3-rs12979860 genotype were HCV-related factors associated with serum ferritin levels. During a follow-up of 8 years, in interferon-treated SVR patients, the trend of increased posttherapy ferritin levels was not reversed until 4 years posttherapy. In SVR patients treated with DAA therapy, ferritin levels decreased at 24 weeks posttherapy and remained stable afterward. These specific evolutions and associations of ferritin levels indicated that a tailored follow-up protocol for hyperferritinaemia and associated complications in CHC patients with SVR needs to be conducted according to antiviral regimens.

Data availability
The data that support the findings of this study are available on request from the corresponding author (MLC).

Received: 20 August 2020; Accepted: 2 November 2020
Published online: 12 November 2020

References
1. Spearman, C. W., Dusheiko, G. M., Hellard, M. & Sonderup, M. Hepatitis C. Lancet 394, 1451–1466 (2019).
2. Borgia, S. M. et al. Identification of a novel hepatitis C virus genotype from Punjab, India: Expanding classification of hepatitis C virus into 8 genotypes. J. Infect. Dis. 218, 1722–1729 (2018).
3. Foka, P. et al. Alterations in the iron homeostasis network: A driving force for macrophage-mediated hepatitis C virus persistency. Virulence 7, 679–690 (2016).
4. Shan, Y. et al. Association of hepatitis C virus infection with serum iron status: Analysis of data from the third National Health and Nutrition Examination Survey. Clin. Infect. Dis. 40, 834–841 (2005).
5. Georgopoulou, U. et al. Hepcidin and the iron enigma in HCV infection. Virulence 5, 465–476 (2014).
6. Fiorelli, G. Serum ferritin and erythrocyte indices in iron overload. Blood Transfus. 5, 187–188 (2007).
7. Ruddell, R. G. et al. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepaticstellate cells. Hepatology 49, 887–900 (2009).
8. Richard, S. & Billet, H. H. Liver function tests in sickle cell disease. Clin. Lab. Haematol. 24, 21–27 (2002).
9. Nishina, S. et al. Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. Gastroenterology 134, 226–238 (2008).
10. Fujita, N. et al. Comparison of hepatic oxidative DNA damage in patients with chronic hepatitis B and C. J. Viral. Hepat. 15, 498–507 (2008).
11. Fujita, N. et al. Hepatic oxidative DNA damage correlates with iron overload in chronic hepatitis C patients. Free Radic. Biol. Med. 42, 353–362 (2007).
12. Lange, C. M. et al. Serum ferritin levels are associated with a distinct phenotype of chronic hepatitis C poorly responding to pegylated interferon-alpha and ribavirin therapy. Hepatology 55, 1038–1047 (2012).
13. Vaghe, C. et al. Serum iron markers in patients with chronic hepatitis C. Infect. Hepat. 13, e13136 (2013).
14. Walker, E. M. Jr & Walker, S. M. Effects of iron overload on the immune system. Ann. Clin. Lab. Sci. 30, 354–365 (2000).
15. Distante, S. et al. Raised serum ferritin predicts non-response to interferon and ribavirin treatment in patients with chronic hepatitis C infection. Liver 22, 269–275 (2002).
16. Barut, S. et al. Serum ferritin levels in chronic hepatitis C patients during antiviral therapy and prediction of treatment response. Scand. J. Infect. Dis. 44, 761–765 (2012).
Author contributions
M.L.C. and R.N.C.: study design and implementation, manuscript drafting, and critical revision of the manuscript for important intellectual content. J.H.H., C.H.Y., K.H.C., C.J.K., M.S.L. and S.C.C.: data collection and interpretation.

Acknowledgements
The authors thank Ms. Shu-Chun Chen, Ms. Chia-Hui Tsai, Mr. Chun-Kai Liang and Mr. Shuen-Shian Shiau from the Liver Research Center, Chang Gung Memorial Hospital, Taiwan, for their assistance with data mining. The authors thank Ms. Shu-Chun Chen, Ms. Chia-Hui Tsai, Mr. Chun-Kai Liang and Mr. Shuen-Shian Shiau for their assistance with data mining.

Funding
This study was supported by grants from the Chang Gung Medical Research Program (CMRPG3I0412 and CMRPG3K0721) and the National Science Council (MOST 108-2314-B-182-051, 109-2314-B-182-024 and 109-2314-B-182-034).

References
1. Harnamoto, S. et al. Changes in serum lipid concentrations in patients with chronic hepatitis C virus positive hepatitis responsive or non-responsive to interferon therapy. J. Gastroenterol. Hepatol. 20, 204–208 (2005).
2. Teijaro, J. R. Pleiotropic roles of type 1 interferons in antiviral immune responses. Adv. Immunol. 132, 135–158 (2016).
3. Halfon, P. & Locarnini, S. Hepatitis C virus resistance to protease inhibitors. J. Hepatol. 55, 192–206 (2011).
4. Knovich, M. A. et al. Ferritin for the clinician. Blood Rev. 23, 95–104 (2009).
5. Chang, M. L. et al. Distinct patterns of the lipid alterations between genotype 1 and 2 chronic hepatitis C patients after viral clearance. PLoS ONE 9, e104783 (2014).
6. Cheng, Y. T. et al. Rheumatoid factor and immunoglobulin M mark hepatitis C-associated mixed cryoglobulinaemia: An 8-year prospective study. Clin. Microbiol. Infect. 26, 366–372 (2020).
7. Chang, M. L. et al. Interactive effects of type 1 interferons on viral replication and mixed cryoglobulinemia on complement levels. Dig. Dis. Sci. 1, 1. https://doi.org/10.1007/s10620-020-06507-9 (2020).
8. Chang, M. L., Jeng, W. J. & Liao, Y. F. Clinical events after cessation of lamivudine therapy in patients recovered from hepatitis B flare with hepatic decompensation. Clin. Gastroenterol. Hepatol. 13, 979–986 (2015).
9. Sanal, M. G. Biomarkers in nonalcoholic fatty liver disease—the emperor has no clothes?. World J. Gastroenterol. 21, 3232–3231 (2015).
10. Suarez-Ortegon, M. F. et al. Ferritin, metabolic syndrome and its components: A systematic review and meta-analysis. Atherosclerosis 275, 97–106 (2018).
11. Zhu, Y. et al. Association between iron status and risk of chronic kidney disease in Chinese adults. Front. Med. (Lausanne) 6, 303 (2020).
12. Kotze, M. J. et al. Pathogenic mechanisms underlying iron deficiency and iron overload: New insights for clinical application. EJIFCC 20, 108–123 (2009).
13. Silva, I. S. et al. Iron overload in patients with chronic hepatitis C virus infection: clinical and histological study. J. Gastroenterol. Hepatol. 20, 243–248 (2005).
14. Himoto, T. et al. Insulin resistance derived from zinc deficiency in non-diabetic patients with chronic hepatitis C. Exp. Ther. Med. 1, 707–711 (2010).
15. Sumida, Y. et al. Hepatic iron accumulation may be associated with insulin resistance in patients with chronic hepatitis C. Hepatol. Res. 37, 932–940 (2007).
16. Furutani, M. et al. Y/Linsulin resistance/beta-cell function and serum ferritin level in non-diabetic patients with hepatitis C virus infection. Liver Int. 23, 294–299 (2003).
17. Hwang, E. W. et al. Implications of rapid virological response in hepatitis C therapy in the US veteran population. Aliment. Pharmacol. Ther. 35, 105–115 (2012).
18. Jorgoouma, F. et al. Impairment of metabolic function in chronic hepatitis C is related to factors associated with resistance to therapy. Am. J. Gastroenterol. 96, 2456–2461 (2001).
19. Petta, S. et al. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. Am. J. Gastroenterol. 103, 1136–1144 (2008).
20. Sumida, Y. et al. Impact of amino acid substitutions in hepatitis C virus genotype 1b core region on liver steatosis and glucose tolerance in non-cirrhotic patients without overt diabetes. J. Gastroenterol. Hepatol. 26, 836–842 (2011).
21. Sebastiani, G. et al. Hepatic iron, liver steatosis and viral genotypes in patients with chronic hepatitis C. J. Viral. Hepat. 13, 199–205 (2006).
22. Mancone, C. et al. Ferritin heavy chain is the host factor responsible for HCV-induced inhibition of apoB-100 production and is required for efficient viral replication. J. Proteome. Res. 11, 2786–2797 (2012).
23. Mehta, R. et al. Safety and efficacy of sofosbuvir and daclatasvir for hepatitis C virus infection in patients. J. Clin. Exp. Hepatol. 8, 3–6 (2018).
24. Origa, R. et al. Treatment of hepatitis C virus infection with direct-acting antiviral drugs is safe and effective in patients with hemoglobinopathies. Am. J. Hematol. 92, 1349–1355 (2017).
25. Ilinomata, S. et al. Changes in the serum hepcidin–to-ferritin ratio with erythroferrone after hepatitis C virus eradication using direct-acting antiviral agents. Intern. Med. 58, 2915–2922 (2019).
26. Carvalho, J. R. et al. Lipids, glucose and iron metabolic alterations in chronic hepatitis C after viral eradication—comparison of the new direct-acting antiviral agents with the old regimens. Scand. J. Gastroenterol. 53, 857–863 (2018).
27. Fujita, N. et al. Patients with chronic hepatitis C achieving a sustained virological response to peginterferon and ribavirin therapy recover from impaired hepcidin secretion. J. Hepatol. 49, 702–710 (2008).
28. Mancia, A. et al. Randomised clinical trial: Sofosbuvir and ledipasvir in patients with transfusion-dependent thalassaemia and HCV genotype 1 or 4 infection. Aliment. Pharmacol. Ther. 40, 424–431 (2017).
29. Amanzada, A. et al. Vitamin D status and serum ferritin concentration in chronic hepatitis C virus type 1 infection. J. Med. Virol. 85, 1534–1541 (2013).
30. Ladero, J. M. et al. Oscillations in serum ferritin associated with antiviral therapy in chronic hepatitis C. Rev. Esp. Enferm. Dig. 101, 31–40 (2009).
31. Nagashima, M. et al. Elevated serum ALT levels during pegylated interferon monotherapy may be caused by hepatic iron overload. Intern. Med. 1, 76–85 (2008).
32. Kamar, N. et al. Factors accelerating liver fibrosis progression in renal transplant patients receiving ribavirin monotherapy for chronic hepatitis C. J. Med. Virol. 76, 61–68 (2005).
33. Soosta, K. & Maliakall, B. Ribavirin induced hemolysis: A novel mechanism of action against chronic hepatitis C virus infection. World J. Gastroenterol. 20, 16184–16190 (2014).
34. Saho, S. et al. Toward the elimination of hepatitis C in the United States. Hepatology 67, 2449–2459 (2018).

Scientific Reports | (2020) 10:39744 | https://doi.org/10.1038/s41598-020-76871-z

nature research
109-2629-B-182-002) to MLC. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. All authors have read the journal’s authorship agreement and policy on disclosure of potential conflicts of interest.

**Competing interests**
The authors declare no competing interests.

**Additional information**
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-76871-z.

Correspondence and requests for materials should be addressed to M.-L.C. or R.-N.C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020