Incidence of HCC in chronic hepatitis C patients with advanced hepatic fibrosis who achieved SVR following DAAs: A prospective study

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Abstract
Liver cirrhosis is an important risk factor for hepatocellular carcinoma. The reported annual incidence of HCC is about 3%-8% in CHC cirrhotic patients. Based on the Cochrane systematic review, there was no clear evidence, on the long-term clinical effects of DAAs in patients achieving SVR, as regard liver cirrhosis-related HCC incidence. The aim of the study was to determine the incidence of HCC in chronic hepatitis C patients genotype IV with liver cirrhosis and advanced liver fibrosis after achieving SVR following DAA treatment in a prospective large cohort of HCV patients with long follow-up. This was a prospective observational cohort study including 2372 CHC patients with advanced liver fibrosis or cirrhosis receiving DAA therapy in outpatient clinics at the Egyptian Liver Research Institute and Hospital since January 2015. Liver fibrosis was assessed using transient elastography. Abdominal ultrasonography and AFP measurement were done at baseline and follow-up visits every 6 months, in addition to triphasic abdominal MSCT when needed. Patients were followed up after achieving SVR12 for at least 12 months. HCC developed in 109 cases during the follow-up period (mean 23.60 ± 8.25 months). Overall HCC incidence was 2.338/100 PY, 95% CI = 1.942-2.814. In patients with cirrhosis, the incidence of HCC was 2.917/100 PY, 95% CI = 2.407-3.535, while in patients with advanced liver fibrosis the incidence of HCC was 0.664/100 PY, 95% CI = 0.333-1.326. In conclusion, the incidence of HCC was reduced in chronic hepatitis C genotype 4 patients with liver cirrhosis (F4) and advanced hepatic fibrosis (F3) who achieved SVR following DAA therapy.

KEYWORDS
cirrhosis, DAAs, HCC, hepatitis C

Abbreviations: BCLC, Barcelona Clinic Liver Cancer classification; HCC, hepatocellular carcinoma; IFN, interferon; IQR, interquartile range.

Registered on www.clinicaltrial.gov under the number NCT03884062.
Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth most frequent cause of cancer-related deaths worldwide, accounting for 7% of all cancers. Liver cirrhosis is an important risk factor for HCC and the risk is high in patients with chronic viral hepatitis, with the highest incidence rates in East Asia and sub-Saharan Africa.

The reported annual incidence of HCC is about 3%-8% in chronic HCV cirrhotic patients. The 5-year cumulative risk for the development of HCC in patients with cirrhosis is 30%, depending on the cause (with the highest risk among those infected with HCV), region or ethnic group and stage of cirrhosis.

During the era of interferon (IFN) therapy, meta-analysis studies reported that achieving SVR leads to decreased risk of liver related complications, mortality, as well as decrease in the incidence of HCC compared with non-SVR patients. This was confirmed in a large prospective multicenter Japanese study where the 5-year cumulative incidence rates of HCC for SVR was 18.9%, which is significantly lower than in the nonresponder group (39.4%) (P = .03) with median follow-up of 3.5 years. Similar results were reported by El-Serag et al and Nahon et al. These findings confirm that viral clearance after IFN therapy decreased the incidence of HCC but did not eliminate it.

The availability of DAAs with excellent safety profiles and higher efficacy enables treatment of CHC patients with advanced fibrosis and cirrhosis; however, the impact of achieving SVR in patients treated with DAAs therapy on the incidence of HCC is still a matter of debate. Several studies reported unexpected high HCC occurrence or recurrence in cirrhotic patients following DAA therapy where others have not supported this observation. These studies were retrospective, performed in heterogeneous populations and had inconsistent methodologies so definitive conclusion cannot be withdrawn. This is supported by the findings of the Cochrane systematic review in 2017 which concluded that there was no clear or sufficient evidence in the long-term clinical effect of DAAs in patients achieving SVR as regard liver cirrhosis-related HCC incidence.

In 2018, Romano et al based on a prospectively recording database of all patients with hepatitis C receiving DAAs (n = 3197) in Italy with mean follow-up of 536.2 ± 197.6 days, revealed that the risk of HCC during the first year is lower than that of untreated patients. HCC incidence in the whole cohort of 2710 cirrhotic patients was 1.18 per 100 person-years (95% CI 0.92-1.49). Also, Li et al reported results of a retrospective cohort study among 2859 HCV-infected cirrhotic patients in the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES) with mean follow-up time of 396.4 days. HCC incidence rates were 2.28/100 person-years for 1160 DAA treated patients compared with 2.12 for 463 IFN treated patients and 4.53 per 100 person-years in untreated patients with cirrhosis (n = 1236) and concluded that DAAs decrease the incidence of HCC in CHC patients who achieved SVR. The same conclusion was recently reached by Carrat et al and Ide et al.

In the previous four studies, genotype IV represents a minority of patients. Furthermore, they did not clearly report the incidence of HCC in patients with advanced fibrosis (F3).

Our aim thus was to determine the incidence of HCC in chronic hepatitis C patients, genotype IV with liver cirrhosis and advanced hepatic fibrosis after achieving SVR with DAAs in a prospective large cohort of HCV patients with long follow-up period.

2 | PATIENTS AND METHODS

This is a prospective observational cohort study including 2372 consecutive chronic HCV patients with advanced liver fibrosis or cirrhosis receiving DAAs in the outpatient clinics at the Egyptian Liver Research Institute and Hospital (ELRIAH) and its satellites from January 2015 until August 2017. In the educate, test and treat programme, 310 814 patients were targeted in 73 Egyptian villages, 221 855 (71.4%) were found eligible for screening, among them 204 749 (92.3%) were screened for anti-HCV followed by HCV RNA PCR for positive patients. A total of 15 892 patients were found positive for HCV RNA and underwent full assessment. Eligible for this study were 4846 who received DAAs, and 2372 of them were included in this study (Figure 1); these were patients 18 years or older with HCV who received DAA, have advanced liver fibrosis (F3) or cirrhosis (F4), and have no history or current HCC.

Patient with either HBV or HIV co-infection, or with a history of previous IFN-treatment, liver transplantation, renal impairment, liver cell failure and other malignancies were excluded (Figure 1).

2.1 | Patients’ evaluation

All patients were evaluated before antiviral treatment. A standardized medical history and a physical examination were performed. All patients had virological, hematological and biochemical laboratory testing, abdominal ultrasound examination, FibroScan and triphasic MSCT if indicated. After start of antiviral treatment, patients were seen every 4 weeks until the end of therapy and 12 weeks after the end of therapy to assess SVR12. Patients were followed up every 6 months for at least one year from end of treatment. In each follow-up visit, hematological and biochemical parameters were determined, together with abdominal ultrasound.

2.2 | Laboratory procedures

Routine hematological and biochemical laboratory parameters were determined at ELRIAH laboratories. HCV RNA testing was performed with use of a real-time HCV RNA PCR (Cobas
Ampliprep, Cobas Taqman 48, Roche) according to the manufacturer’s instructions.

In addition to laboratory testing, abdominal ultrasonography was done using the Toshiba Apio XG ultrasound machine (Toshiba) and triphasic MSCT was done, when indicated, using Philips Brilliance CT 16 slice (Philips).

### 2.3 | Transient elastography

Transient Elastography was done by FibroScan 502 (Echosens) and three portable Echosens Mini Systems (Echosens).

A transient elastography was performed before treatment. For classification the following cut-off values were used: F0/F1/ F2 ≤ 10.2 kPa; F3 > 10.2 kPa; and F4 > 16.3 kPa. Transient elastography was considered reliable when the following criteria had been met: (a) 10 successful measurements; (b) an interquartile range (IQR) lower than 30% of the median value; and (c) a success rate of more than 60%. Liver stiffness was considered as the median of all valid measurements. For high BMI (≥30 kg/m²), examination with the XL probe, with two experienced operators, was done.

### 2.4 | Diagnosis of cirrhosis

Patients were diagnosed as having liver cirrhosis (F4) when they fulfilled more than one of the following criteria.

- Definite clinical signs and laboratory parameters of liver cirrhosis (eg splenomegaly, ascites, albumin ≤ 3.5 g/dL, and platelets count ≤ 100 mm³);
- Abdominal ultrasonographic signs suggestive of cirrhosis (eg mild splenomegaly, minimal ascites, PV dilatation and collaterals);
- Transient elastography (>16.3 kPa).

Patients were diagnosed as having advanced liver fibrosis (F3) mainly by transient elastography (>10.2 and ≤16.3 kPa).

Cirrhotic patients (F4) were classified according to Child-Pugh classification scoring.

### 2.5 | Diagnosis and staging of HCC

Suspicion of HCC was raised by the detection of a hepatic focal lesion during ultrasound examination or when AFP level was elevated.
above 20 ng/mL. The diagnosis of HCC was confirmed by triphasic MSCT. Diagnosis of HCC was based on the characteristic arterial enhancement and early washout in delayed phase.

Hepatocellular carcinoma was staged according to the Barcelona Clinic Liver Cancer classification (BCLC).

### 2.6 | Antiviral treatment

All participants received a 12- or 24-week course of one of several DAA regimens in accordance with the Egyptian national treatment protocol, AASLD 2014 and 2014 WHO guidelines for treatment of genotype 4 chronic hepatitis C infection. Treatment included sofosbuvir and ribavirin (51.0% of patients), sofosbuvir and daclatasvir (27.3%), sofosbuvir, daclatasvir and ribavirin (14.1%), ombitasvir, paritaprevir, ritonavir + ribavirin (5.5%) and sofosbuvir, ledipasvir + ribavirin (2.1%) for 12 or 24 weeks of treatment.

### 2.7 | Ethics

The study protocol was approved by the Research and Ethical Committee of ELRIAH (OHRP IRB #8819). The protocol and conduct of the study complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects. All patients gave informed consent to be included in this study.

This study was registered on www.clinicaltrial.gov under the number NCT03884062.

### Table 1: Baseline characteristics of studied DAA patients according to development of HCC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-HCC Patients</th>
<th>HCC patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>2263</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.0 (50.0-62.0)</td>
<td>59.0 (55.5-65.0)</td>
<td>.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>1160 (51.3%)</td>
<td>82 (75.2%)</td>
<td>.001</td>
</tr>
<tr>
<td>Females</td>
<td>1103 (48.7%)</td>
<td>27 (24.8%)</td>
<td></td>
</tr>
<tr>
<td>HCV RNA, log_{10} IU/mL</td>
<td>5.54 (4.94-6.08)</td>
<td>5.38 (4.57-5.95)</td>
<td>.136</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>48.0 (33.0-74.0)</td>
<td>48.5 (36.5-80.5)</td>
<td>.536</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>50.0 (35.0-76.0)</td>
<td>60.0 (40.0-100.0)</td>
<td>.023</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>0.82 (0.61-1.14)</td>
<td>1.00 (0.80-1.50)</td>
<td>.001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.00 (3.60-4.30)</td>
<td>3.60 (3.14-4.00)</td>
<td>.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.78 (0.64-0.90)</td>
<td>0.80 (0.68-1.00)</td>
<td>.140</td>
</tr>
<tr>
<td>Platelets count (/mm³)</td>
<td>133.0 (90.0-184.0)</td>
<td>94.0 (62.5-141.3)</td>
<td>.001</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.5 (12.2-14.7)</td>
<td>13.5 (11.8-14.6)</td>
<td>.218</td>
</tr>
<tr>
<td>WBCs (/mm³)</td>
<td>5.60 (4.33-7.11)</td>
<td>5.11 (3.57-6.72)</td>
<td>.070</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
<td>6.90 (4.10-13.22)</td>
<td>31.00 (12.13-731.25)</td>
<td>.001</td>
</tr>
<tr>
<td>Fibrosis stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>630 (27.8%)</td>
<td>8 (7.3%)</td>
<td>.001</td>
</tr>
<tr>
<td>F4</td>
<td>1633 (72.2%)</td>
<td>101 (92.7%)</td>
<td></td>
</tr>
<tr>
<td>CTP Classification**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1228 (75.2%)</td>
<td>66 (65.6%)</td>
<td>.018</td>
</tr>
<tr>
<td>B</td>
<td>405 (24.8%)</td>
<td>35 (34.4%)</td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>450 (19.9%)</td>
<td>25 (22.9%)</td>
<td>.513</td>
</tr>
<tr>
<td>HTN</td>
<td>353 (15.6%)</td>
<td>17 (15.6%)</td>
<td>.447</td>
</tr>
<tr>
<td>Overweight²</td>
<td>1408 (62.2%)</td>
<td>60 (55.0%)</td>
<td>.160</td>
</tr>
</tbody>
</table>

Note: Data are presented as frequency (%) or median (IQR).

Abbreviations: AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, diabetes mellitus; Hb, haemoglobin; HTN, hypertension; WBCs, white blood corpuscles.

²Overweight (BMI ≥ 30 kg/m²).

**For F4 patients only (1734 patients).
Statistical analyses were performed using version 24, SPSS (Statistical Package for Social Sciences) (IBM Corp.). Continuous variables were reported as median (IQR). Categorical variables were reported as frequency (%). Nonparametric tests, Mann-Whitney test for quantitative and Fisher’s exact test for qualitative comparisons, were used. Paired analysis was done by the Wilcoxon signed-rank test for quantitative data and sign test for qualitative data. Stepwise logistic regression was used for multivariable regression. We used a multivariable Cox proportional hazards model with exposure to treatment modelled as a time-varying covariate in our analysis. This analysis was adjusted for the baseline values of all predictor variables. Follow-up time was calculated as the time between the end of treatment and the last follow-up, or the date of HCC whichever occurred first.

Incidence rates and 95% CIs were estimated with an exact method based on the Poisson distribution. Incidence calculation included all HCC cases that developed from EOT till the end of follow-up. CI was calculated by Mid-P exact test using Miettinen’s modification.

### 2.8 Statistical analysis

Statistical analyses were performed using version 24, SPSS (Statistical Package for Social Sciences) (IBM Corp.). Continuous variables were reported as median (IQR). Categorical variables were reported as frequency (%). Nonparametric tests, Mann-Whitney test for quantitative and Fisher’s exact test for qualitative comparisons, were used. Paired analysis was done by the Wilcoxon signed-rank test for quantitative data and sign test for qualitative data. Stepwise logistic regression was used for multivariable regression. We used a multivariable Cox proportional hazards model with exposure to treatment modelled as a time-varying covariate in our analysis. This analysis was adjusted for the baseline values of all predictor variables.

Follow-up time was calculated as the time between the end of treatment and the last follow-up, or the date of HCC whichever occurred first.

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### 3 RESULTS

#### 3.1 Characteristics of the study population

The study included 2372 chronic HCV patients (638 patients with F3 and 1734 with F4 stage) with SVR who met the inclusion criteria in our study since January 2015 until August 2017. The follow-up period was 23.60 ± 8.25 months. We divided our patients according to development of HCC into two groups: group1 included patients who did not develop HCC in the follow-up period (2263 patients) and group 2 included patients who developed HCC for the first time (109 patients).

Patients with HCC were significantly older (59 years vs 56 years; \( P < .001 \)) with male predominance (75.2% in HCC patients versus 51.3% in non-HCC patients; \( P < .001 \)). Compared to patients without HCC, patients with HCC had higher baseline AST, total bilirubin, AFP (\( P = .023, <.001, <.001, \) respectively) and significantly lower serum albumin and platelets (\( P < .001 \)). No significant difference was found between both groups as regard ALT and serum creatinine. Most patients who developed incident HCC had F4 stage (101 patients, 92.7%) vs 8 patients with F3 stage (7.3%). There was no significant difference between both groups in terms of comorbidities as diabetes mellitus, hypertension and overweight (\( P = .513, .447 \) and .160, respectively) (Table 1).

#### 3.2 Fibrosis changes

In cirrhotic patients (F4 before treatment), 375 patients (21.6%) showed reversal of hepatic fibrosis to F2 or less, 470 patients (27.1%) showed only one stage improvement to F3 (fibrosis regression) while 889 patients (51.3%) remained F4 with no change in fibrosis stage (stationary). For patients with advanced fibrosis (F3) before treatment, 166 (26.0%) showed reversal of fibrosis to F0 or F1, 197 patients (30.0%) showed fibrosis regression to F2, and 199 patients (31.2%) remained stationary at F3 while 76 patients (11.9%) progressed to F4.

#### 3.3 Annual incidence of HCC

Among patients who developed HCC, 101 patients (92.7%) had cirrhotic stage F4 versus 8 patients (7.3%) with F3 stage (Table 2). We found that, among all patients in our study post-DAA treatment, the annual incidence of HCC was 2.338/100 PY, 95% CI = 1.942-2.814. Moreover, in patients with cirrhosis the incidence of HCC was 2.917/100 PY, 95% CI = 2.407-3.535, while in patients with advanced liver fibrosis the incidence of HCC was 0.664/100 PY, 95% CI = 0.333-1.326 (Table 2).

Eleven out of the 109 HCC cases (10.1%) occurred during the first year of follow-up, 53 (48.6%) during the second year, and 35
(32.1%) during the third year, while 10 (9.2%) occurred after the third year of follow-up.

Among F4 patients who developed HCC (101 patients), 15 patients regressed to ≤F2 at the end of follow-up period, 16 regressed to F3, while 70 remained stationary at F4. Incidence rates were 2.052/100 PY, 95% CI = 1.192-3.308 for those who regressed to ≤F2; 1.766/100 PY, 95% CI = 1.045-2.807 for those who regressed to F3; and 3.931/100 PY, 95% CI = 3.088-4.938 for those who remained at F4 at the end of follow-up (Table 2).

Among F3 patients who developed HCC (8 patients), 3 patients regressed to ≤F2 at the end of follow-up period, 3 remained stationary at F3, while 2 progressed to F4. Incidence rates were 0.441/100 PY, 95% CI = 0.112-1.199 for those who regressed to ≤F2, 0.805/100 PY, 95% CI = 0.205-2.191 for those who remained at F3 and 1.373/100 PY, 95% CI = 0.230-4.535 for those who progressed to F4 at the end of follow-up (Table 2).

Among F2 patients who developed HCC (≤F2 at the end of follow-up period, 3 remained stationary at F4. Incidence rates were 0.805/100 PY, 95% CI = 0.205-2.191 for those who regressed to ≤F2; 1.766/100 PY, 95% CI = 1.045-2.807 for those who regressed to F3, while 70 remained stationary at F4. Incidence rates were 0.441/100 PY, 95% CI = 0.112-1.199 for those who regressed to ≤F2, 0.805/100 PY, 95% CI = 0.205-2.191 for those who remained at F3 and 1.373/100 PY, 95% CI = 0.230-4.535 for those who progressed to F4 at the end of follow-up period.

### TABLE 3

<table>
<thead>
<tr>
<th>Predictor</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.072</td>
<td>1.042-1.104</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>3.608</td>
<td>1.998-6.517</td>
<td>.011</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.174</td>
<td>0.659-2.091</td>
<td>.587</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.478</td>
<td>0.201-1.137</td>
<td>.095</td>
</tr>
<tr>
<td>Overweight (BMI ≥ 30 kg/m²)</td>
<td>0.821</td>
<td>0.489-1.378</td>
<td>.455</td>
</tr>
<tr>
<td>AFP &gt; 20 ng/mL (vs ≤20 ng/mL)</td>
<td>2.834</td>
<td>1.549-5.184</td>
<td>.001</td>
</tr>
<tr>
<td>Albumin &lt; 3.8 g/dL (vs ≥3.8 g/dL)</td>
<td>1.855</td>
<td>1.148-2.998</td>
<td>.012</td>
</tr>
<tr>
<td>Platelet count &lt; 100/mm³ (vs ≥100/mm³)</td>
<td>0.894</td>
<td>0.557-1.436</td>
<td>.644</td>
</tr>
</tbody>
</table>

### 3.4 | Predictors for the development of HCC in persons with cirrhosis or advanced liver fibrosis based on multivariate Cox proportional hazard analysis according to fibrosis stage before treatment

In this analysis, we found that, in patients with baseline F4 stage, older age (P < .001), male sex (P ≤ .011), increased AFP > 20 ng/mL vs ≤20 ng/mL (P = .001) and decreased serum albumin < 3.8 g/dL vs ≥3.8 g/dL (P = .012) were predictors for the development of HCC (Table 3). In patients with baseline F3 stage, there was no significant predictor for development of HCC.

Patients with cirrhosis who achieved SVR had higher hazard of developing HCC compared to patients with marked fibrosis (Figure 2A). Comparison of hazard of HCC development according to pre-treatment CTP stage showed that patients who were Child B had higher incidence than patients with Child A (Figure 2B).

### 3.5 | HCC tumour characteristics

One hundred nine patients developed HCC during the follow-up period. According to BCLC classification, 7.3% of cases were stage 0, 32.1% stage A, 30.3% stage B, 26.6% stage C and only 3.7% were stage D. PV invasion occurred only in 8 cases only (7.3%) (Table 4).

### 4 | DISCUSSION

Our study showed that the annual incidence of HCC is 2.9/100 PY in cirrhotic patients who achieved SVR following direct-acting antiviral treatment for hepatitis C genotype 4. This incidence is lower than that reported by Eltabakh et al where the annual incidence of HCC was 5.3/100 PY in their screening programme which included 1286 Egyptian untreated cirrhotic patients who they followed up for more than 18 months at the National Liver Institute, Menoufia University in the Nile Delta.

Our results were also similar to those reported in the ERCHIVES retrospective cohort, in which the incidence of HCC was much lower in patients with cirrhosis who achieved SVR following DAA treatment than in patients without SVR (2.12/100 py) compared with untreated cirrhotic patients (4.53/100 py). There were no HCC cases among patients with genotype 4 which was likely attributed to its small number in the study (representing < 0.5% of the cohort).17

The reduced incidence of HCC after DAAs was also confirmed by Ide et al, who prospectively investigated the incidence and risk factors of HCC after DAA treatment in a large multicenter cohort in Japan with mean follow-up period was 22.6 ± 8.3 months; most of the Japanese patients (78%) were genotype 1.19

The reduced incidence of HCC after DAAs was also confirmed by Ide et al, who prospectively investigated the incidence and risk factors of HCC after DAA treatment in a large multicenter cohort in Japan with mean follow-up period was 22.6 ± 8.3 months; most of the Japanese patients (78%) were genotype 1.19

The results of our study also showed that regression of fibrosis is associated with decreased incidence of HCC which is maximally evident in patients with liver cirrhosis (F4) who regressed to ≤F2 after achieving SVR (2.052/100 py) compared to those who remained at F4 stage (3.931/100 py). This finding highlights the beneficial effect of viral clearance on liver morbidity and also showed that resolution of hepatic fibrosis may explain, at least partially, the decreased incidence of HCC. This concept is further confirmed in patients with advanced hepatic fibrosis (F3) who progressed to F4 where higher incidence of HCC is reported (2.05/100 py) compared to those who regressed to ≤F2 (0.441/100 py). Similar results were reported by Haung et al who analysed the association of
fibrotic changes proved by paired biopsies in 265 CHC patients who achieved SVR after IFN therapy and concluded that post-treatment fibrotic modification overwhelmed pre-treatment fibrotic statuses in predicting HCC.

In our study, we identified four main pre-treatment risk factors which were older age, male gender, lower albumin < 3.8 gm/dL and AFP > 20 ng/mL. Similar risk factors were also reported by several studies. Interestingly diabetes mellitus is not reported as a risk factor for development of HCC in our study or the other studies in patients who achieved SVR after DAAs, but it was reported as a risk factor in CHC patients treated with IFN.

In our study, we included 638 CHC F3 patients with annual HCC incidence of 0.66 /100py which is much lower than that reported in CHC patients with liver cirrhosis (2.9/100 py). Data about HCC incidence in F3 chronic hepatitis C patients following DAAs in the literature are limited; Romano et al reported an incidence of HCC in 959 F3 patients of 0.46/100 py who were followed up for about 18 months from start of treatment, and in which genotype 4 represented only 7.5% of the cases. Similar results were reported by Sanchez-Azofra et al in their multicenter observational study including 474 CHC patients with baseline F3 who achieved SVR following DAA treatment with median follow-up of 23.6 months and reported an HCC incidence of 0.35/100 py.

The strength of our work is that it is a prospective study that included a large number of chronic hepatitis C genotype 4 patients not only with liver cirrhosis (F4) but also advanced hepatic fibrosis (F3), who achieved SVR following DAA treatment with a median 2 year follow-up.

Our study has several limitations; first, lack of a control group of untreated patients. However, leaving patients without treatment would have been unethical in our screening outreach programme. A second limitation was the absence of patients who did not achieve SVR, simply, because all relapers in the outreach programme were retreated with second or even a third course of treatment. Third, lack of liver biopsy for assessment of liver fibrosis stages. However according to society guidelines including AASLD-IDSA, AGA, ALEH and EASL noninvasive methods had replaced the standard liver biopsy for assessment of liver fibrosis stages.

In conclusion, the incidence of HCC is reduced in chronic hepatitis C genotype 4 patients with liver cirrhosis (F4) and advanced hepatic fibrosis (F3) who achieved SVR following DAA treatment.

TABLE 4 Tumour characteristics of HCC developed during study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour laterality</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>51 (46.7%)</td>
</tr>
<tr>
<td>Left</td>
<td>23 (21.1%)</td>
</tr>
<tr>
<td>Both lobes</td>
<td>35 (32.1%)</td>
</tr>
<tr>
<td>Tumour number</td>
<td></td>
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<tr>
<td>Single</td>
<td>51 (46.8%)</td>
</tr>
<tr>
<td>Multiple</td>
<td>58 (53.2%)</td>
</tr>
<tr>
<td>Tumour size cm ≤3</td>
<td>53 (48.6%)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>56 (51.4%)</td>
</tr>
<tr>
<td>PV invasion</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>101 (92.7%)</td>
</tr>
<tr>
<td>Yes</td>
<td>8 (7.3%)</td>
</tr>
<tr>
<td>BCLLC</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8 (7.3%)</td>
</tr>
<tr>
<td>A</td>
<td>35 (32.1%)</td>
</tr>
<tr>
<td>B</td>
<td>33 (30.3%)</td>
</tr>
<tr>
<td>C</td>
<td>29 (26.6%)</td>
</tr>
<tr>
<td>D</td>
<td>4 (3.7%)</td>
</tr>
</tbody>
</table>

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
GS conceived and designed the study. GS, NM, ME and RS supervised clinical work in this programme. NNHM performed the statistical analyses and drafted material and methods. GS, NM and NNHM interpreted the data. GS drafted the paper. GS, RS, ME, NM and NNHM revised the paper. MK revised the final version. All authors provided input into the manuscript and approved the final version.

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