The association between hepatocellular carcinoma and direct-acting anti-viral treatment in patients with decompensated cirrhosis

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**Summary**

**Background:** Direct-acting anti-viral therapy (DAA) has transformed hepatitis C virus (HCV) care, particularly in patients with decompensated cirrhosis. However, their impact on hepatocellular carcinoma (HCC) remains unclear.

**Aim:** To use a national registry of patients with advanced liver disease to explore the relationship between DAA therapy and HCC.

**Methods:** All patients with de novo HCC post DAA therapy were frequency matched with patients who did not develop HCC. Demographic, clinical and laboratory data were obtained. Cross-sectional imaging and multidisciplinary team reports were reviewed for dates of HCC diagnosis and HCC progression. Patients were categorised by treatment outcome and time of HCC development. Data were examined by multi-variable analysis and Kaplan-Meier estimation.

**Results:** Eighty patients with HCC were compared with 165 patients without HCC, treated between June 2014 and September 2015. Mean follow-up from start of DAA therapy was 32.4 months. Twenty-eight patients were diagnosed with early HCC (within 6 months of therapy) and 52 presented late. Baseline nonmalignant lesions (HR: 1.99), thrombocytopaenia (HR: 1.59) and diabetes (HR: 1.68) increased likelihood of HCC. Response to therapy was reduced in patients who developed liver cancer (SVR in patients with HCC = 54/80 (68%), SVR in patients without HCC = 143/165 (87%), \( P < 0.001 \), OR: 3.13, 95% CI: 1.64-5.99). We found no difference between tumour size, progression or survival between viraemic and nonviraemic patients.

**Conclusion:** There is no alteration in prognosis or cancer progression following HCC development after HCV treatment. However, baseline nonmalignant liver lesions, diabetes and thrombocytopaenia increase the risk of HCC, and HCC is associated with a decreased SVR rate.
Hepatitis C virus (HCV) infection is a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC), the second most frequent malignant cause of death worldwide.\textsuperscript{1} With the advent of direct-acting anti-viral (DAA) therapy for HCV, treatment options and curative rates have been transformed with high rates of sustained virological response (SVR).\textsuperscript{2,3} These agents have also facilitated the treatment and cure of patients with advanced liver disease who remain at risk of HCC\textsuperscript{4} and are therefore recommended to continue lifelong surveillance.\textsuperscript{5,6}

There is controversy around patients with cirrhosis who have cleared virus (ie achieved an SVR) on DAAAs and their ongoing risk of developing HCC. Conti et al reported an increased incidence of HCC following DAA treatment with 3.16% (95% CI 1.45-5.90) of 285 patients developing an HCC within 24 weeks of therapy.\textsuperscript{7} Supporting this Ravi et al found an unusually high risk (9%) of patients developing de novo HCC following DAA treatment.\textsuperscript{8} Conversely, multiple studies have shown no increase in HCC occurrence following viral clearance and a large American cohort of 62 354 patients with and without cirrhosis showed that although patients with cirrhosis who had cleared virus with DAA therapy did develop malignancy, the frequency was not increased.\textsuperscript{9} These studies have suggested that alcohol consumption, diabetes mellitus, lower platelet count and higher aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio\textsuperscript{10} are baseline characteristics that predict HCC development.

In addition to the impact of HCV clearance on HCC development, there is controversy regarding the impact of HCC on HCV treatment outcome. Prenner et al showed a greatly reduced SVR rate of 58% for patients with an HCC present on treatment initiation, with this rising to 97% in patients with a previous history of treated HCC prior to DAA commencement.\textsuperscript{11} This implies that the presence of HCC may reduce the response to treatment though this study includes patients post-liver transplantation.

The prognosis following the diagnosis of HCC in patients with HCV and cirrhosis is poor with a median survival as low as 0.7-0.9 years.\textsuperscript{12} In the SHARP trial of sorafenib in patients with advanced HCC, time to progression on imaging regardless of the initial cause was 2.8 months in the placebo group.\textsuperscript{13} It is still not known whether clearance of HCV impacts tumour progression, but anecdotal evidence has suggested that it may slow evolution.

In light of these uncertainties, we examined the NHS England early access programme (EAP), which provided access to 12 weeks of all-oral DAA therapy for patients with advanced liver disease. Patients in this programme remain on surveillance, and here, we report the incidence and factors predictive of de novo malignancy in patients developing HCC early (within 6 months) or late (after 6 months) after the onset of DAA therapy, the impact of HCC on DAA treatment response and the progression of cancers in viraemic and nonviraemic patients.

\section{Methods}

\subsection{Patients}

All patients enrolled in the NHS England early access programme (EAP) were encouraged to enrol in HCV Research UK (HCVRUK) with written informed consent. Details of the treatment (June 2014-September 2015) and management of the early access programme cohort have been published previously.\textsuperscript{14} In brief, patients with decompensated cirrhosis were offered 12 weeks therapy with either sofosbuvir/ledipasvir or sofosbuvir plus daclatasvir, with or without ribavirin at the clinician’s discretion. Entry to the English early access programme specified that all patients had to have either a diagnosis of hepatic decompensation in the past or have current evidence of CTP score B or C.

\subsection{Case selection}

The HCV Research UK database was interrogated for all cases of de novo HCC diagnosed from the start of the early access programme until 15 June 2017 regardless of diagnostic modality. Patients with a prior liver transplant or HCC diagnosis before the onset of DAA therapy were excluded. A control group (two controls per case) of early access programme patients with no subsequent diagnosis of HCC was then selected based on frequency matching for age, gender, Child-Turcotte-Pugh score and length of follow-up. The HCV Research UK database contained details of patient demographics and treatment used. Supplementary data relevant to this study were collected from each of the study sites using a standardised data collection form and to ensure that accuracy and data completeness sites were contacted individually to complete any missing data fields. The study was performed in accordance with the 1975 Declaration of Helsinki guidelines on ethics as reflected in a priori approval by the institution’s human research committee. HCV Research UK gained ethical approval by the National Research Ethics Service (NRES) committee East Midlands—Derby 1 (Research Ethics Committee reference 11/EM/0314). Informed consent was obtained from all patients.

\subsection{Data collection}

Baseline data included age, gender, ethnicity, alcohol usage, smoking status, diabetes mellitus, HIV status and use of proton pump inhibitors or statins. Data were also available for HCV (route of infection, genotype), date of cirrhosis diagnosis and decompensation diagnosis, previous HCV treatment and Child-Turcotte-Pugh score within the year preceding treatment. The Child-Turcotte-Pugh score was converted to a stage centrally for interpretation purposes (stage A—score 5-6, stage B—7-9, stage C—10-15). Local accredited laboratory measurements for the preceding year were collected with the highest serum HCV RNA, lowest serum sodium, lowest creatinine, highest alanine aminotransferase (ALT), aspartate transaminase (AST), highest bilirubin, lowest albumin, highest alpha-foetoprotein (AFP), highest clotting studies and lowest full blood count measurements used. The model for end-stage liver disease (MELD) score, AST to platelet ratio index (APRI) score and albumin to bilirubin (ALBI) grade were calculated centrally. Length of follow-up was defined as the date of onset of DAA treatment until the date of death, date of transplantation or date of survey, whichever occurred first.

\section{Introduction}

Hepatitis C virus (HCV) infection is a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC), the second most frequent malignant cause of death worldwide.\textsuperscript{1} With the advent of direct-acting anti-viral (DAA) therapy for HCV, treatment options and curative rates have been transformed with high rates of sustained virological response (SVR).\textsuperscript{2,3} These agents have also facilitated the treatment and cure of patients with advanced liver disease who remain at risk of HCC\textsuperscript{4} and are therefore recommended to continue lifelong surveillance.\textsuperscript{5,6}

There is controversy around patients with cirrhosis who have cleared virus (ie achieved an SVR) on DAAAs and their ongoing risk of developing HCC. Conti et al reported an increased incidence of HCC following DAA treatment with 3.16% (95% CI 1.45-5.90) of 285 patients developing an HCC within 24 weeks of therapy.\textsuperscript{7} Supporting this Ravi et al found an unusually high risk (9%) of patients developing de novo HCC following DAA treatment.\textsuperscript{8} Conversely, multiple studies have shown no increase in HCC occurrence following viral clearance and a large American cohort of 62 354 patients with and without cirrhosis showed that although patients with cirrhosis who had cleared virus with DAA therapy did develop malignancy, the frequency was not increased.\textsuperscript{9} These studies have suggested that alcohol consumption, diabetes mellitus, lower platelet count and higher aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio\textsuperscript{10} are baseline characteristics that predict HCC development.

In addition to the impact of HCV clearance on HCC development, there is controversy regarding the impact of HCC on HCV treatment outcome. Prenner et al showed a greatly reduced SVR rate of 58% for patients with an HCC present on treatment initiation, with this rising to 97% in patients with a previous history of treated HCC prior to DAA commencement.\textsuperscript{11} This implies that the presence of HCC may reduce the response to treatment though this study includes patients post-liver transplantation.

The prognosis following the diagnosis of HCC in patients with HCV and cirrhosis is poor with a median survival as low as 0.7-0.9 years.\textsuperscript{12} In the SHARP trial of sorafenib in patients with advanced HCC, time to progression on imaging regardless of the initial cause was 2.8 months in the placebo group.\textsuperscript{13} It is still not known whether clearance of HCV impacts tumour progression, but anecdotal evidence has suggested that it may slow evolution.

In light of these uncertainties, we examined the NHS England early access programme (EAP), which provided access to 12 weeks of all-oral DAA therapy for patients with advanced liver disease. Patients in this programme remain on surveillance, and here, we report the incidence and factors predictive of de novo malignancy in patients developing HCC early (within 6 months) or late (after 6 months) after the onset of DAA therapy, the impact of HCC on DAA treatment response and the progression of cancers in viraemic and nonviraemic patients.
DAA treatment type and commencement date were noted. Sustained virological response (SVR) was defined as negative for serum HCV RNA at 12 weeks (SVR12) following the completion of treatment. Patients with incomplete HCV treatment outcome data either due to death prior to SVR12 tests or those lost to follow-up were removed from the analysis.

All patients were subject to national guidelines recommending an ultrasound scan every 6 months with further cross-sectional imaging if indicated. All local imaging and multidisciplinary team (MDT) reports were collected centrally by the study team for the year prior to therapy and following therapy up until the study endpoint. Tumour size measurements were taken from radiological reports and Barcelona clinic liver cancer (BCLC) scores, Liver Reporting & Data System (L-RADS) grading, Milan criteria and response evaluation criteria in solid tumours (RECIST) criteria were applied and appropriate scores/grades generated by the study team. RECIST criteria, which take into account the size and progression of the primary lesion, secondary lesions, nodal, vascular and metastatic disease to give an overall definition for complete resolution, partial resolution, stable or progressive disease, were used to assess tumour progression with the date of cross-sectional imaging being used to define the observation period. The frequency of surveillance scans and the presence of pre-existing lesions were assessed using 6 monthly reporting windows with the date of DAA commencement being day 0. Patients with positive scans or those transplanted or died were censored at that point.

The date of HCC diagnosis was the date of the first cross-sectional imaging satisfying European Association for the Study of the Liver (EASL) HCC diagnosis guidelines, as determined following local multidisciplinary team meeting or, for cases with tissue diagnosis on explant histology, as the date of surgery. Dates and types of HCC treatment were obtained from sites as well as the date of transplant and date of death.

Given the probability that cancers diagnosed within 6 months of treatment initiation may have been present at treatment onset, we analysed data for ‘early’ cancer (within 6 months of DAA initiation) and late cancers—diagnosed after this time point. Primary endpoints were the development of HCC, sustained virological response and overall survival. Secondary endpoints were progression of nonmalignant liver lesions to HCC and the further progression of HCC.

To analyse the association of HCC development with several variables in our dataset and investigate potential confounding factors, we have used multiple logistic and Cox regression models. The binomial logistic model was built to explain the HCC status (Yes/No) with the inclusion of important predictors from an initial univariate analysis in respect to both deviance and Hosmer-Lemeshow goodness-of-fit tests while maintaining the variance inflation factor to the minimum. We also investigated potential interactions that were included as interaction terms in the model. The Cox proportional hazards regression analysis was used for a time-dependent outcome (time to develop HCC) and produced hazard rates allowing the quantification of the effect (risk) per group or unit change depending on the nature of each predictor. The effect of each variable is presented with hazard rates and 95% confidence intervals. For continuous variables, the hazard rate was calculated for a clinically meaningful increment of change.

Time-to-event analyses were performed using the nonparametric Kaplan-Meier method. The survival distributions were compared for equality for two groups at each comparison. All lost to follow-up cases were censored up to the most recent time point with available information. For each comparison, the log-rank test results are presented, but the Breslow and Tarone-Ware tests were also considered.

P < 0.05 was considered statistically significant.

Data analyses were performed using IBM SPSS version 25 (Armonk, NY) and GraphPad Prism version 6.0 (San Diego, CA).

3 | RESULTS

3.1 | Baseline demographics

We identified 81 patients in the early access programme within the HCV Research UK database treated with DAA therapy between June 2014 and September 2015 who developed HCC subsequent to the onset of therapy. These were frequency matched with 178 early access programme patients who were treated with DAAs but did not develop HCC within the follow-up period. We excluded patients lost to follow up or who died before SVR outcome became known (1 HCC patient, 13 non-HCC patients). HCC was diagnosed by MRI in 45 patients, CT scan in 26, while eight patients had incidental HCC diagnosed within their explanted liver. One patient had a date of diagnosis, but no mode of diagnosis was available. The demographics of the cohort are shown in Table 1. Frequency matching provided groups with similar age, Child-Turcotte-Pugh stage and gender distributions. The cohort was predominately male (75%) and white (62%). Most patients received ribavirin-containing anti-viral therapy (95.9%) with most having previous interferon exposure (HCC = 62.5%, non-HCC = 62%). The most common treatment regimen was sofosbuvir + ledipasvir + ribavirin (65.7%). HCV genotypes 1 and 3 were the most prevalent. Staging of cirrhosis according to Child-Turcotte-Pugh, following conversion from raw scores to stages, showed most patients were Child-Turcotte-Pugh stage B (63%) followed by A (22%) and C (15%) and for model for
| Characteristic | Non-HCC (n = 165) | All HCC (n = 80) | Early HCC (<6 mo) (n = 28) | Late HCC (>6 mo) (n = 52) |
|---------------|------------------|------------------|-----------------------------|---------------------------|
| Median age (IQR), y | 57 (52.9-61.9) | 57 (51.8-60.9) | 55 (50-60.9) | 57.2 (54.2-61.4) |
| Male sex, n (%) | 123 (75) | 61 (76) | 22 (79) | 39 (75) |
| CTP grade (%) | B (62) | B (65) | B (54) | B (71) |
| Mean MELD score (IQR) | 11 (9-14) | 11 (9-14) | 10 (9-13) | 12 (9-15) |
| Median length of follow-up (IQR), mo | 33.5 (29.8-34.5) | 22.4 (13.3-32.2) | 15.3 (5.3-24.1) | 24.7 (17.2-32.9) |
| Ethnicity, n (%) | | | | |
| White-British | 100 (61) | 53 (66) | 20 (72) | 33 (63) |
| Asian | 27 (16) | 10 (13) | 4 (14) | 6 (12) |
| Other | 38 (23) | 17 (21) | 4 (14) | 13 (25) |
| Alcohol, n (%) | | | | |
| Never | 36 (22) | 15 (19) | 5 (18) | 10 (19) |
| Current | 29 (17) | 8 (10) | 3 (11) | 5 (10) |
| Past/former | 94 (57) | 57 (71) | 20 (71) | 37 (71) |
| Unavailable | 6 (4) | 0 | 0 | 0 |
| Smoking status, n (%) | | | | |
| Never | 42 (25) | 15 (19) | 2 (7) | 13 (25) |
| Currently | 62 (38) | 36 (45) | 13 (47) | 23 (44) |
| Past/former | 48 (29) | 23 (29) | 11 (39) | 12 (23) |
| Unavailable | 13 (8) | 6 (7) | 2 (7) | 4 (8) |
| Genotype, n (%) | | | | |
| Genotype 1 | 83 (50) | 34 (42) | 9 (32) | 25 (48) |
| Genotype 3 | 65 (40) | 42 (53) | 16 (57) | 26 (50) |
| Other | 17 (10) | 4 (5) | 3 (11) | 1 (2) |
| Diabetes, n (%) | | | | |
| Yes | 31 (19) | 27 (34)* | 10 (36) | 17 (33)* |
| No | 99 (60) | 41 (51) | 15 (54) | 26 (50) |
| Unavailable | 35 (21) | 12 (15) | 3 (10) | 9 (17) |
| Past history of non-HCC Ca, n | 17 | 5 | 2 | 3 |
| Previous treatment failure, n (%) | 102 (62) | 50 (63) | 19 (70) | 31 (60) |
| Treatment regimen, n (%) | | | | |
| Sof/Led | 6 (3) | 1 (1) | 1 (3) | 0 |
| Sof/Led/Riba | 115 (70) | 59 (74) | 22 (79) | 37 (71) |
| Sof/Dac | 3 (2) | 0 | 0 | 0 |
| Sof/Dac/Riba | 41 (25) | 20 (25) | 5 (18) | 15 (29) |
| SVR achieved, n (%) | 143 (87) | 54 (68) | 20 (71) | 34 (65) |
| Median albumin (IQR), g/L | 29 (26-34) | 27 (23-32)* | 28 (23-32) | 27 (22.5-31)* |
| Median alpha-foetoprotein (IQR), ng/mL | 7.0 (5-15.1) | 7.0 (4-16.5) | 9 (5.6-25) | 6.1 (3.6-12.3) |
| Median alkaline phosphatase (IQR), U/L | 148 (108-202) | 121 (101-186) | 111 (90-154) | 139 (105-189) |
| Median bilirubin (IQR), μmol/L | 34 (22-49) | 38 (23-52.75) | 32 (20-52) | 39 (25-53.5) |
| Median INR (IQR) | 1.3 (1.2-1.4) | 1.3 (1.2-1.5) | 1.3 (1.2-1.5) | 1.4 (1.2-1.5) |
| Median platelet (IQR), x10¹¹/L | 74 (53-98) | 63 (44-85.5)* | 68 (44-95) | 59 (43.5-80)* |
| Median sodium (IQR), mmol/L | 136.0 (134-138) | 136.0 (132-138) | 137.0 (133-140) | 136.0 (131.5-137) |
| Median BMI (IQR), kg/m² | 27.6 (24.6-32.3) | 27.0 (24.7-31.4) | 27.5 (24.3-33) | 27.1 (25.3-30.5) |

Note: Unknown values were excluded where unknown values existed.
Abbreviations: CTP, Childs-Turcotte-Pugh; BMI, body mass index; standardised units provided where appropriate; Dac, Daclatasvir; HCC, hepatocellular carcinoma; IQR, interquartile range; mo, months; n, number; Riba, ribavirin; Sof, sofosbuvir; SVR, sustained viral response; y, years.
*Frequency matching criteria. P-values generated via a chi-squared test for categorical values and Mann-Whitney U test for continuous variables. *indicate significant of P-value <0.05.
end-stage liver disease (MELD) score, a median of 11 (7-35). In line with the inclusion criteria for the early access programme, all patients with a Child-Turcotte-Pugh score of A had a previous history of decompensation and these 37 controls and 17 HCC patients had decompensating events of ascites (22), encephalopathy (7), variceal bleeding (6) and unknown (19). Median follow-up was 32.4 months (22.5-34.2 months). Twenty-eight patients were diagnosed with an HCC within the first 6 months of treatment (19 being diagnosed during early access programme treatment). Fifty-four (67.5%) of the HCC patients (n = 80) achieved SVR12, as did 143 of 165 (86.6%) controls.

Imaging data in the year prior to early access programme onset were available for 130 of the controls and 63 of the HCC cases. 35/165 (21%) controls compared to 17/80 (21%) HCC cases did not have a surveillance ultrasound scan in this period. Similarly, there was no difference in the number of pretreatment scans between those developing cancer early (22/28, 79%) vs late (41/52, 79%, P = 0.995). However, nonmalignant lesions were seen on scans performed within 12 months of DAA onset in 23/130 (18%) of the control patients, compared to 24/63 (38%) HCC cases (P = 0.02, OR: 2.15, 95% CI:1.1-4.1). Using the nomenclature from the radiology reports, 12 of the control patients had cysts, five had nodules, three had haemangiomas and three had ‘nondescript lesions’, with seven patients having more than one of the described lesions (but always of the same type). The corresponding data for the HCC patients were six cysts, nine nodules, one haemangioma and eight ‘nondescript lesions’ with nine patients having more than one of the described lesions (but always of the same type) (Table S1). Based on the radiologist stating if a lesion had either progressed or if an HCC was diagnosed in the same anatomical region,

**FIGURE 1** Flowchart for baseline nonmalignant lesions
15 of the 24 (63%) nonmalignant lesions were considered to have progressed to HCC, with six of these patients presenting with an early HCC and the remaining nine developing a late malignancy. The breakdown for these baseline lesions is shown in Figure 1.

In univariate analysis comparing the 80 HCC patients with the matched population, factors associated with the development of HCC were diabetes, lower albumin, nonmalignant lesion seen on pretreatment ultrasound scan and a lower platelet count. These variables were entered into both logistic and Cox regression models for multivariate analysis, with both models returning all but albumin as statistically significant predictors. The effect size of albumin was reduced in the multivariate models due to its strong correlation with platelets (Spearman rho P-value = 0.007). In Table 2, we present the results from the Cox regression analysis in order to fully incorporate the time-dependent nature of the outcome (time from the start of treatment to HCC development).

### 3.2 | Virological response to DAA therapy in patients with and without HCC

Of 165 patients, 143 (87%) of the non-HCC patients achieved SVR12, compared with 54/80 (68%) of the HCC patients (P < 0.001; OR: 3.13, 95% CI: 1.64-5.99). Following the exclusion of those with HCC diagnosed on explant, we found 48/72 (67%) achieved SVR12 with the persistence of a significant difference (P < 0.001; OR: 3.25, 95% CI: 1.67-6.32). The difference in SVR12 rate is not accounted for by either Child-Turcotte-Pugh grade (P = 0.68) or MELD score (P = 0.95). For patients who developed an early HCC (ie within the time frame of 12 weeks therapy plus 12 weeks follow-up to determine treatment outcome), 20/28 (71%) achieved an SVR (P = 0.045, OR: 2.6, 95% CI: 1.02-6.62). In patients who developed a late HCC, the response was also lower compared to the controls, 34/52 (65%) (P < 0.001; OR: 8.26 95% CI: 4.43-15.38).

### 3.3 | Progression of liver cancers arising early after starting DAA compared to later cancers

We compared cancers that developed soon after therapy with those developing later to test the hypothesis that the elimination of the virus-associated inflammatory response leads to a more aggressive tumour. Figure 2 shows that there was no significant difference in either the progression of the tumour (Figure 2A) or overall survival (Figure 2B) between these two groups. Indeed, patients with HCC developing soon after viral elimination appeared to fare slightly better, although this was not statistically significant.

### 3.4 | Progression of liver cancer following viral clearance

To examine the hypothesis that malignancy developing in an uninfected liver (ie post-SVR) may be more aggressive than cancers that develop in an HCV-infected liver, we examined HCC prognosis by Kaplan-Meier estimation. Figure 3A,B shows that the time from cancer diagnosis to progression (P = 0.17) and death (P = 0.7), respectively, were similar in patients who did, or did not, achieve viral clearance.

The median time from onset of anti-viral treatment to HCC diagnosis for patients treated with DAAs was 8.74 months (3.43-16.8 months). Overall, main tumour size ranged from 9.5 to 120 mm with no lymph nodes, vascular involvement or metastases being found though two did not have information on size. There was no significant size difference for the primary tumour between nonviraemic (9.5-120 mm) and viraemic (14-100 mm) patients with 13 nonviraemic (28%) and 7 patients with viraemia (28%) presenting with more than one tumour. We assessed the cancer stage using the Milan criteria which determine suitability for liver transplantation in patients with cirrhosis and HCC. The proportion of patients with HCC at the point of diagnosis who fell within the Milan criteria (ie circumscribed) was 61/72, following exclusion of those diagnosed on explant. Of 47, 39 (83%) patients achieving an SVR were within Milan criteria compared to 22/25 (88%, P = 0.57) patients who did not achieve SVR. Similar assessment according to Li-RADS criteria showed one category 3 tumour, 33 category 4 and 36 category 5 cancers with two unable to be categorised. When split into nonviraemic and viraemic patients, we found one category 3, 23 category 4 and 22 category 5 cancers and 10 category 4 and 14 category 5 tumours respectively. Similar assessment according to Barcelona clinic liver cancer (BCLC) scores showed 10 grade 0, 38 grade A, 3 grade B, 9 grade C, 7 grade D cancers with 5 unable to be categorised. When split into nonviraemic and viraemic, we found 7 grade 0, 25 grade A, 2 grade B, 6 grade C, 6 grade D within the nonviraemic patients and 3

### TABLE 2  Results of multivariate analysis, presenting the predictors that have an effect on the development of HCC

| Variable                  | Univariate effect | Univariate P-value | Cox regression multivariate effect | Cox regression multivariate P-value |
|---------------------------|-------------------|---------------------|------------------------------------|-------------------------------------|
| Platelets                 | Mean difference: 10.0, 95% CI: 2.0-19 | 0.018               | HR: 1.59, 95% CI: 1.09-2.29 (Change of 50x10^9/L) | 0.016                               |
| Diabetes                  | OR: 2.1, 95% CI: 1.1-3.4  | 0.021               | HR: 1.68, 95% CI: 1.03-2.74        | 0.036                               |
| Nonmalignant lesions at baseline | OR: 2.6, 95% CI: 1.3-5.1 | 0.005               | HR: 1.99, 95% CI: 1.15-3.45       | 0.014                               |
| Albumin                   | Mean difference: 2.0, 95% CI: 0.4-3.6 | 0.016               | n.s                                | n.s                                |

Note: P-value significant < 0.05.
Abbreviations: CI, confidence interval; HR, hazards ratio; n.s, not significant; OR, odds ratio.
grade 0, 13 grade A, 1 grade B, 3 grade C, 1 grade D viraemics. These data are presented in Table 3 which shows no statistically significant differences between viraemic and nonviraemic patients.

4 | DISCUSSION

With the evolution of DAA treatment, the ability to treat patients successfully, particularly those previously considered difficult to cure, has changed practice. Recent studies showing a raised incidence of HCC following treatment have raised concerns about prescribing DAA therapy for patients with advanced cirrhosis. Here, we show data from the NHS England early access programme cohort, a nationwide unselected cohort of decompensated cirrhotic patients, in order to address the issues: (a) are there any baseline features predictive of HCC development, (b) are patients who are diagnosed with HCC during treatment less likely to achieve SVR, (c) are HCCs diagnosed during DAA treatment more aggressive than those developing later. We studied all liver cancers with known treatment outcomes and found that the presence of a ‘lesion’ on previous scans, diabetes and thrombocytopenia was associated with subsequent development of malignancy. These findings are consistent with previous

FIGURE 2  A, Time from HCC diagnosis to the first progression split by early vs late HCC. Kaplan-Meier estimation depicted. Mantel-Cox comparison test P = 0.25. B, Time from HCC diagnosis to death split by early vs late HCC. Kaplan-Meier estimation depicted. Mantel-Cox comparison test P = 0.12
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At present, we would recommend more intensive HCC surveillance in patients with these characteristics to allow early identification of lesions at a stage where they may be amenable to therapy.

The significance of pretreatment nonmalignant lesions presents a challenge for hepatologists. The LI-RADS criteria were developed to try and overcome this, but diagnostic uncertainty remains. We have shown that patients with apparently nonmalignant lesions on scans taken within 12 months of the onset of DAA therapy are more likely to go on to develop HCC. This is in keeping with the notion that many HCCs diagnosed after the onset of DAA therapy were already present beforehand, a phenomenon previously noted by others. Nahon et al found that 5/15 patients had a nonmalignant nodule observed within 6 months prior to starting DAA treatment and subsequently developing HCC with this shown as a statistically significant risk factor for HCC development. Alternate to this, Toyoda et al recently found no effect of previously identified nonhypervascular hypointense nodules (NHHNs) on HCC incidence; however, these were all compensated cirrhotic patients with all nodules found on contrast-enhanced MRI scans as opposed to the less sensitive ultrasound scanning, which most of our patients received. Our study is in agreement with a recently published Spanish study with both studies suggesting an increased rate of de novo HCC in those with noncharacterised nodules or other lesions; however, as our

FIGURE 3 A, Time from HCC diagnosis to the first progression split by ongoing viraemia vs viral clearance. Kaplan-Meier estimation depicted. Mantel-Cox comparison test $P = 0.17$. B, Time from HCC diagnosis to death split by ongoing viraemia vs viral clearance inclusive of only EAP patients. Kaplan-Meier estimation depicted. Mantel-Cox comparison test $P = 0.7$.
follow-up period is a year longer, this suggests that the progression of these nodules occurs early following DAA initiation. Vigilance is clearly indicated in patients with pre-existing liver lesions. We found that patients diagnosed with HCC within 6 months of the onset of DAA therapy are less likely to achieve SVR12. Prenner et al reported that in a cohort of 137 patients with pre-existing HCC treated with regimens incorporating sofosbuvir, ledipasvir, simeprevir, ombitasvir/paritaprevir/ritonavir and ribavirin, 21% failed to achieve SVR, significantly more than those patients without HCC at baseline (P = 0.009). These data may be interpreted as indicating a difficulty for DAAs to penetrate a small pre-existing liver cancer effectively. Alternatively, a strain of HCV which has a higher oncogenic effect may be present which renders DAAs less effective when coupled with the above. However, in our study, we also detected a lower SVR12 rate (65%) in patients who were diagnosed with HCC more than 6 months after the onset of therapy. This suggests that either virus-infected premalignant/malignant cells that are treatment resistant are present for a very long time before presenting as overt malignancy or viral or host factors that predispose to malignancy are also involved in treatment failure. Whatever the mechanism of tumour development, physicians should be aware that patients who fail DAA therapy may be at increased risk of HCC development to allow early detection of malignancy. We have previously shown that, in the English early access programme, there is no difference in the frequency of liver cancer in treated or untreated patients, and here, we address the question of whether cancers in a ‘virus free’ environment are more aggressive than those in patients with persisting virus. Given the uncertainty about the delay from cancer initiation to presentation (it is unknown whether small, invisible, lesions are present for months or weeks prior to detection), we studied all cancers that developed in patients who did, or did not, respond to therapy as well as examining HCC developing 6 months after therapy. We chose 6 months as an arbitrary, convenient time period that was likely to exclude cancers present before treatment was initiated although we accept that other periods could have been selected. We found no difference in outcomes in either of the groups between HCC in infected or noninfected livers leading us to conclude that viral clearance does not alter cancer behaviour. We accept that the ideal study would have involved untreated patients with comparable degrees of cirrhosis, but we do not believe such a study to be ethical.

Our study is a nationwide prospectively collected real-world study of decompensated cirrhotic patients. The standard of data collection was high throughout the study and carried out to a clinical trial standard, although not formally audited. In our opinion, the results of this study are readily translatable to everyday patient care.

Although our study is one of the larger studies examining HCC in the post-DAA era, we nevertheless had only 80 HCC patients treated with DAAs. This may limit our ability to detect small yet significant differences in populations and is compounded by the relatively short period of follow-up. Another limitation of our study is the selection of controls which although frequency matched to remove bias for age, gender, stage of disease and length of follow-up, were not otherwise

| All (n = 72) | Nonviraemic (n = 47) | Viraemic (n = 25) |
|-------------|---------------------|-----------------|
| Size of primary lesion, mm | 9.5-120 | 9.5-120 | 14-100 |
| More than 1 lesion, n (%) | 20 (28) | 13 (28) | 7 (28) |
| Fits within Milan criteria (%), n (%) | 61 (85) | 39 (83) | 22 (88) |
| Li-RADS criteria, n (%) | | | |
| LR-3 | 1 (1) | 1 (2) | 0 |
| LR-4 | 33 (46) | 23 (49) | 10 (40) |
| LR-5 | 36 (50) | 22 (47) | 14 (56) |
| Unavailable | 2 (3) | 1 (2) | 1 (4) |
| Barcelona Grade, n (%) | | | |
| 0 | 10 (14) | 7 (15) | 3 (12) |
| A | 38 (53) | 25 (53) | 13 (52) |
| B | 3 (4) | 2 (4) | 1 (4) |
| C | 9 (13) | 6 (13) | 3 (12) |
| D | 7 (9) | 6 (13) | 1 (4) |
| Unavailable | 5 (7) | 1 (2) | 4 (16) |

Abbreviations: Li-RADS, liver imaging reporting and data system; n, number, standardised units provided where appropriate.
matched. However, as liver function has the greatest impact on the development of hepatocellular carcinoma, we felt that these measures would be most sensitive for this. We removed all patients without data for SVR and this may have led to missing of ultra-aggressive cancers in the very early stages of follow-up. We chose to use the worst value for the blood tests in the year prior to treatment to provide an assessment of ‘baseline, most severe’ liver function. We accept that other approaches are possible but as liver function values are often modified by specific treatments (e.g. albumin infusions), we believe that it is most appropriate to use the worst value within a reasonable time period to avoid potentially artificially adjusted values. As this is a real-world observational study, some data were unavailable due to patient engagement or ability to gain this from the records; nevertheless the clear outcomes from the majority patients where data were available provide us with confidence that the conclusions are robust. Finally, the question of whether the presence of HCC hinders SVR is difficult to answer without a randomised controlled trial which would be unethical.

In conclusion, we have shown the presence of baseline non-malignant lesions in addition to diabetes and a lower platelet count, to be indicative of HCC development. An absence of effect of DAA treatment on HCC progression as well as an absence of effect of viraemia on patient survival was evident.

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AUTHORSHIP

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Author contributions: The study was designed and led by GRF, WI and AJM. AJM collated the data. AJM and PK performed the data and statistical analysis. WI and GRF supervised sample collection, data management and assisted with study design and implementation. WI has received speaker and consultancy fees from Roche, Janssen Cilag, Gilead Sciences and Novartis, educational grants from Boehringer Ingelheim, Merck Sharp and Dohme and Gilead Sciences, and research grant support from GlaxoSmithKline, Pfizer, Gilead Sciences, Janssen Cilag, Abbvie and Bristol-Myers Squibb. All authors participated in data analysis and participated in the preparation of the manuscript. The study was designed and led by GRF, WI and AJM. AJM collated the data. AJM and PK performed the data and statistical analysis. WI and GRF supervised sample collection, data management and assisted with study design and implementation. All authors participated in data analysis and participated in the preparation of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

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APPENDIX

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The following were the principal investigators at HCV Research UK participating sites who contributed patients, samples and data to this study:

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