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

ii. **Running title: The association between HCC and DAA**

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iv. **Authorship statement**

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iii. 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

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Conflicts of interest

Mr Mecci by LAP Research UK Grant, Professor Foster has received speaker and consultancy fees from AbbVie, Achillion, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, Idenix, Janssen, Merck, Novartis, Roche, Springbank; Professor Irving has received speaker and consultancy fees from Roche Products.

v. Structured summary

Background

Direct acting antiviral 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 that 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 multivariable analysis and Kaplan-Meier estimation.

Results

80 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. 28 patients were diagnosed with early HCC (within 6 months of therapy) and 52 presented late. Baseline non-malignant lesions (HR:1.99), thrombocytopenia (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 non-viraemic patients.

Conclusion

There is no alteration in prognosis or cancer progression following HCC development after HCV treatment. However, baseline non-malignant liver lesions, diabetes and thrombocytopaenia increases the risk of HCC and HCC is associated with a decreased SVR rate.

Keywords

Cirrhosis

Hepatitis C

Hepatocellular carcinoma

Outcomes research

Liver

vi. Main Text

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 [1]. With the advent of direct-acting antiviral (DAA) therapy for HCV, treatment options and curative rates have been transformed with high rates of sustained virological response (SVR) [2, 3]. These agents have also facilitated the treatment and cure of patients with advanced liver disease who remain at risk of HCC [4] and are therefore recommended to continue lifelong surveillance [5, 6].

There is controversy around patients with cirrhosis who have cleared virus (i.e. achieved an SVR) on DAAs and their on-going 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 [7]. Supporting this Ravi et al. found an unusually high risk (9%) of patients developing de novo HCC following DAA treatment [8]. Conversely, multiple studies have shown no increase in HCC occurrence [9] 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 [10]. These studies have suggested that alcohol consumption, diabetes mellitus, lower platelet count and higher aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio [11] 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 [12]. 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 [13]. 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 [14]. 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 non-viraemic patients.

Methods

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 [15]. 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.

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 15th 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 was collected from each of the study sites using a standardised data collection form and to ensure 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.

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-fetoprotein (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.

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 multi-disciplinary team (MDT) reports were collected centrally by the study team for the year prior to therapy and following therapy up until the study end-point. Tumour size measurements were taken from radiological reports with Barcelona clinic liver cancer (BCLC) scores [16], Liver Reporting & Data System (Li-RADS) grading [17], Milan criteria [18] and response evaluation criteria in solid tumours (RECIST) criteria [19] were generated by the study team. RECIST criteria, which take into account 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, was 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 six 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 multi-disciplinary 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 six 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 non-malignant liver lesions to HCC and the further progression of HCC.

Statistics

Baseline characteristic data are presented as the median and interquartile range (IQR) for continuous variables or as frequencies and percentages for categorical ones. Mann-Whitney U and chi-square tests were used for baseline characteristic and subsequent comparisons.

Count data for 2 group comparisons were analysed with 2 proportions tests using the normal approximation method to calculate the p-values. We have also performed odds ratio analyses using the z-score calculated as ln(OR)/SE{ln(OR)}. The odds ratio (OR), standard error and 95% confidence intervals were calculated according to Altman, 1991 [20].

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 [21]. The survival distributions were compared for equality for 2 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 values <0.05 were considered to present a statistically significant difference.

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

Results

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 8 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 antiviral 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 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). 54 (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 pre-treatment scans between those developing cancer early (22/28, 79%) vs late (41/52, 79%, p = 0.995). However, non-malignant 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, 5 had nodules, 3 had haemangiomas and 3 had "non-descript lesions", with 7 patients having more than one of the described lesions (but always of the same type). The corresponding data for the HCC patients was 6 cysts, 9 nodules, 1 haemangioma and 8 'non-descript lesions' with 9 patients having more than one of the described lesions (but again, always of the same type) (Appendix S1). Based upon the radiologist stating if a lesion had either progressed or if an HCC was diagnosed in the same anatomical region, 15 of the 24 (63%) non-malignant lesions were considered to have progressed to HCC, with 6 of these patients presenting with an early HCC and the remaining 9 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, non-malignant lesion seen on pre-treatment 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).

Virological response to DAA therapy in patients with and without HCC

143/165 (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 (i.e. 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).

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 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 2 groups. Indeed, patients with HCC developing soon after viral elimination appeared to fare slightly better, although this was not statistically significant.

Progression of liver cancer following viral clearance.

To examine the hypothesis that malignancy developing in an uninfected liver (i.e. post-SVR) may be more aggressive than cancers that develop in an HCV infected liver we examined HCC prognosis by Kaplan-Meier estimation. Figures 3a and 3b show 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 antiviral 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 – 120mm 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 non-viraemic (9.5-120mm) and viraemic (14-100mm) patients with 13 non-viraemic (28%) and 7 patients with viraemia (28%) presenting with more than 1 tumour. We assessed the cancer stage using the Milan criteria which determines 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 (i.e. circumscribed) was 61/72, following exclusion of those diagnosed on explant. 39/47 (83%) patients achieving an SVR, were within Milan criteria compared to 22/25 (88%, $p = 0.57$) patients that 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 non-viraemic 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 overall. When split into non-viraemic and viraemic we found 7 Grade 0, 25 grade A, 2 Grade B, 6 grade C, 6 grade D within the non-viraemic patients and 3 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 non-viraemic patients.

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 has 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 (i) are there any baseline features predictive of HCC development, (ii) are patients who are diagnosed with HCC during treatment less likely to achieve SVR (iii) are HCC's 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 thrombocytopaenia were associated with subsequent development of malignancy. These findings are consistent with previous studies [9, 10, 22-28] but will require formal confirmation in a larger cohort. For the 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 pre-treatment non-malignant lesions presents a challenge for hepatologists. The LI-RADS criteria were developed to try and overcome this but diagnostic uncertainty remains [17, 29]. We have shown that patients with apparently non-malignant 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 [30]. Nahon et al. found that 5/15 patients had a non-malignant 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 [24]. Alternate to this Toyoda et al. recently found no effect of previously identified Non-hypervascular 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 [31]. 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 non-characterised nodules or other lesions; however, as our follow-up period is a year longer, this suggests the progression of these nodules occurs early following DAA initiation [32]. 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 ($p = 0.009$) [12]. 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 pre-malignant/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 adopted a local, albeit none evidence-based protocol involving 3 monthly scanning of such patients for the first 12 months following completion of antiviral therapy.

The important question of whether liver cancer is more common and or more aggressive following viral clearance is difficult to answer. This would necessitate randomising patients with cirrhosis to treatment or observation and is unlikely to be popular with patients or, in our view, ethical. The use of historical controls is, to some extent, flawed as changes to treatment regimens and surveillance introduce time-dependent differences that are difficult to reconcile. 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 [2, 15]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 six months after therapy. We chose six 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 non-infected 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 matched. However, as liver function has the greatest impact on the development of hepatocellular carcinoma, we felt 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 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 was unavailable due to patient engagement or ability to gain this from the records, nevertheless the clear outcomes from the majority patients where data was 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.

vii. References

1. Chung, R.T. and T.F. Baumert, *Curing chronic hepatitis C--the arc of a medical triumph.* N Engl J Med, 2014. **370**(17): p. 1576-8.
2. Cheung, M.C., et al., *Outcomes after successful direct-acting antiviral therapy for patients with chronic hepatitis C and decompensated cirrhosis.* J Hepatol, 2016. **65**(4): p. 741-7.
3. Foster, G.R., et al., *Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection.* N Engl J Med, 2015. **373**(27): p. 2608-17.
4. van der Meer, A.J., et al., *Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication.* J Hepatol, 2017. **66**(3): p. 485-493.
5. *EASL Recommendations on Treatment of Hepatitis C 2016.* J Hepatol, 2017. **66**(1): p. 153-194.
6. *AASLD-IDSA. Recommendations for testing, managing, and treating hepatitis C.* 2017; Available from: http://www.hcvguidelines.org/node/141.
7. Conti, F., et al., *Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals.* J Hepatol, 2016. **65**(4): p. 727-733.
8. Ravi, S., et al., *Unusually High Rates of Hepatocellular Carcinoma After Treatment With Direct-Acting Antiviral Therapy for Hepatitis C Related Cirrhosis.* Gastroenterology, 2017. **152**(4): p. 911-912.
9. Waziry, R., et al., *Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression.* J Hepatol, 2017. **67**(6): p. 1204-1212.
10. Ioannou, G.N., P.K. Green, and K. Berry, *HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma.* J Hepatol, 2017.
11. van der Meer, A.J., et al., *Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis.* Jama, 2012. **308**(24): p. 2584-93.
12. Prenner, S.B., et al., *Hepatocellular carcinoma decreases the chance of successful hepatitis C virus therapy with direct-acting antivirals.* J Hepatol, 2017. **66**(6): p. 1173-1181.
13. Alavi, M., et al., *Trends in hepatocellular carcinoma incidence and survival among people with hepatitis C: An international study.* J Viral Hepat, 2017.
14. Llovet, J.M., et al., *Sorafenib in advanced hepatocellular carcinoma.* N Engl J Med, 2008. **359**(4): p. 378-90.
15. Foster, G.R., et al., *Impact of direct acting antiviral therapy in patients with chronic hepatitis C and decompensated cirrhosis.* J Hepatol, 2016. **64**(6): p. 1224-31.
16. Forner, A., et al., *Current strategy for staging and treatment: the BCLC update and future prospects.* Semin Liver Dis, 2010. **30**(1): p. 61-74.
17. Kielar, A.Z., et al., *LI-RADS 2017: An update.* J Magn Reson Imaging, 2018.
18. Mazzaferro, V., et al., *Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis.* N Engl J Med, 1996. **334**(11): p. 693-9.
19. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.
20. Altman, D., *Practical statistics for medical research*. 1991, London: Chapman & Hall.
21. Kaplan, E.L. and P. Maier, *Nonparametric Estimation from Incomplete Observations.* Journal of the American Statistical Association, 1958. **53**(282): p. 457-481.
22. Kanwal, F., et al., *Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents.* Gastroenterology, 2017. **153**(4): p. 996-1005.e1.
23. Calvaruso, V., et al., *Incidence of Hepatocellular Carcinoma in Patients with HCV-associated Cirrhosis Treated with Direct-Acting Antiviral Agents.* Gastroenterology, 2018.
24. Nahon, P., et al., *Incidence of Hepatocellular Carcinoma After Direct Antiviral Therapy for HCV in Patients With Cirrhosis Included in Surveillance Programs.* Gastroenterology, 2018. **155**(5): p. 1436-1450.e6.
25. Singer, A.W., et al., *Direct-acting antiviral treatment for hepatitis C virus infection and risk of incident liver cancer: a retrospective cohort study.* Aliment Pharmacol Ther, 2018. **47**(9): p. 1278-1287.
26. Huang, Y.W., et al., *Increased risk of hepatocellular carcinoma in chronic hepatitis C patients with new onset diabetes: a nation-wide cohort study.* Aliment Pharmacol Ther, 2015. **42**(7): p. 902-11.
27. Ogawa, E., et al., *Short-term risk of hepatocellular carcinoma after hepatitis C virus eradication following direct-acting anti-viral treatment.* Aliment Pharmacol Ther, 2018. **47**(1): p. 104-113.

28. Ioannou, G.N., et al., *Development of models estimating the risk of hepatocellular carcinoma after antiviral treatment for hepatitis C.* J Hepatol, 2018. **69**(5): p. 1088-1098.
29. Alessandro, F. and B.A. A., *Problematic lesions in cirrhosis.* Clinical Liver Disease, 2018. **11**(2): p. 43-47.
30. Scott, R.A., et al., *Pretreatment Lesions on Magnetic Resonance Imaging in Patients With Hepatitis C Virus Infection Diagnosed With Hepatocellular Carcinoma After Initiating Direct-Acting Antiviral Therapy.* Gastroenterology, 2018. **154**(6): p. 1848-1850.
31. Toyoda, H., et al., *The impact of HCV eradication by direct-acting antivirals on the transition of precancerous hepatic nodules to HCC: A prospective observational study.* Liver Int, 2019. **39**(3): p. 448-454.
32. Marino, Z., et al., *Time association between hepatitis C therapy and hepatocellular carcinoma emergence in cirrhosis: Relevance of non-characterized nodules.* J Hepatol, 2019. **70**(5): p. 874-884.

Appendix: HCV Research UK

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

K. Agarwal, King's College Hospital, London; M Aldersley, St James' University Hospital, Leeds; A Ali, Frimley Park Hospital, Surrey; S Aravamuthan, Lincoln County Hospital, Lincoln; R Aspinall, Queen Alexandra Hospital, Portsmouth; E Barnes, John Radcliffe Hospital, Oxford; A Brown, St Mary's Hospital, London; C. Ch'ng, Singleton Hospital, Swansea; L Corless, Hull and East Yorkshire Hospital, Hull; M Cramp, Derriford Hospital, Plymouth; D Forton, St George's Hospital, London; GR Foster, Royal London and St Bart's Hospitals, London; M Foxton, Charing Cross Hospital, London; W. Gelson, Addenbrooke's Hospital, Cambridge; D Gorard, Wycombe Hospital, Wycombe; F Gordon, Bristol Royal Infirmary; SI Khakoo, Southampton General Hospital; A Lawson, Royal Derby Hospital, Derby; C Leen, Western General Hospital, Edinburgh; S McPherson, Freeman Hospital, Newcastle; S Moreea, Bradford Royal infirmary, Bradford; D Mutimer, Queen Elizabeth Hospital, Birmingham; M Prince, Manchester Royal Infirmary, Manchester; P Richardson, Royal Liverpool and Broadgreen University Hospital, Liverpool; WR Rosenberg, University College Hospital, London; SD Ryder, Queen's Medical Centre, Nottingham; B Stone, Royal Hallamshire Hospital, Sheffield; A Ustianowski, North Manchester General Hospital; S Verma, Royal Sussex County Hospital; M Wiselka, Leicester Royal infirmary, Leicester.

viii. Tables

Table 1 Baseline characteristics of HCC and non-HCC patients

Characteristic	**Non-HCC (n=165)**	**All HCC (n=80)**	**Early HCC (<6 months) (n=28)**	**Late HCC (>6 months) (n=52)**
Median age, (IQR), yrs. †	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), mths. †	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-fetoprotein, (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), $x10^9/L$	74 (53-98)	63 (44-85.5)*	68 (44-95)	59 (43.5-80)*
Median sodium, (IQR), mmol/L	136.0 (134-139)	136.0 (132-138)	137.0 (133-140)	136.0 (131.5-137)
Median BMI, (IQR), kg/m^2	27.6 (24.6-32.3)	27.0 (24.7-31.4)	27.5 (24.3-33)	27.1 (25.3-30.5)

†Frequency matching criteria. P-values generated via a chi-squared test for categorical values and Mann-Whitney U test for continuous variables. Unknown values were excluded where unknown values existed. CTP: Childs-Turcotte-Pugh, n: number, IQR: Inter-quartile range, yrs.: years, mths.: months, HCC: hepatocellular carcinoma, SVR: Sustained viral response, Sof: Sofosbuvir, Dac: Daclatasvir, Riba: Ribavirin, BMI: body mass index. Standardised units supplied where appropriate

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
Non-malignant 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

P-value significant <0.05, OR: Odds ratio, HR: Hazards ratio, CI: confidence interval, n.s: Not significant

Table 3 Description of tumours split by viraemic and non-viraemic, excluding those found on explant

	All (n=72)	**Non-viraemic (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)

Li-RADS: Liver Imaging Reporting and Data System, n: number, Standardised units supplied where appropriate

ix. Figure legends

Figure 1 – Flowchart for baseline non-malignant lesions

Figure 2a – 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.

Figure 2b – Time from HCC diagnosis to death split by early vs late HCC. Kaplan-Meier estimation depicted. Mantel-Cox comparison test p = 0.12.

Figure 3a – 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.

Figure 3b – 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

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.