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

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

iii. Ali Jibran Mecci[1], Polychronis Kemos[1], Clifford Leen[2], Adam Lawson[3], Paul Richardson[4], Salim Khakoo[5], Kosh Agarwal[6], David Mutimer[7], William R Rosenberg[8], Graham R. Foster[1] (corresponding author), William L. Irving[9] on behalf of HCV Research UK[10]

[1] Blizard Institute, Queen Mary University of London, London, UK
[2] University of Edinburgh, Edinburgh, UK
[3] Royal Derby Hospital, Derby, United Kingdom
[4] Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, UK
[5] University of Southampton, Southampton, UK
[6] King's College Hospital, London
[7] University of Birmingham, Birmingham UK
[8] University College Hospital, London, UK
[9] NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, UK
[10]HCV Research UK sites contributing to this study are listed in Appendix

iv. **Authorship statement**

i. Graham R Foster is the Guarantor for this article.

ii. 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.

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

implementation. All authors participated in data analysis and participated in the preparation of the manuscript.

Acknowledgements

The authors would like to thank the patients, their families and all participating study studies for contributing data to this study. We are also grateful to HCV Research UK, in particular, Jennifer Benselin and Sarah McDonald for collecting and collating data. HCV Research UK was supported by the Medical Research Foundation (grant reference C0365).

Financial Support

This study was supported by NHS England; Medical Research Foundation (Grant reference C0365); LAP Research UK.

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

Intro

Direct acting antiviral therapy (DAA) has transformed hepatitis C virus (HCV) care, particularly in patients with decompensated cirrhosis. However, the impact of therapy on hepatocellular carcinoma (HCC) remains unclear. We utilised the English Early Access Programme (EAP) registry to address issues around liver cancer and viral clearance.

Methods

All patients with de-novo HCC were studied and frequency matched with patients that did not develop HCC. Demographic, clinical and laboratory data were procured. Cross-sectional

imaging and multidisciplinary team reports were reviewed for date of HCC diagnosis and date of first 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 contrasted with 165 patients without HCC, treated between June 2014 and September 2015. Male predominance was found (75%) and mean follow up was 32.4 months. 28 patients were diagnosed with early HCC (within 6 months of therapy) and 52 presented after this time. Baseline non-malignant lesions, thrombocytopenia and diabetes, increased likelihood of developing HCC ($p=0.02$, OR:2.15, 95%CI:1.1-4.1). 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 life expectancy or cancer progression following successful HCV treatment. However, baseline non-malignant liver lesions and thrombocytopaenia increases HCC risk 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 thus are 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 and colleagues 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 alcohol

consumption, diabetes mellitus, lower platelet count and higher aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio [11] as 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 increased treatment failure rate of 42% for patients with an HCC present on treatment initiation, with this falling to 3% 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 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 therefore 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 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 EAP 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 +/- ribavirin at the clinician's discretion. Importantly, pre-existing HCC was an exclusion criterion.

Case Selection

The HCVRUK database was interrogated for all cases of de novo HCC diagnosed from the start of the EAP programme until 15th June 2017 regardless of diagnostic modality. patients with liver Tx or HCC diagnosis before onset of DAA therapy were excluded. A control group (two controls per case) of EAP patients with no subsequent diagnosis of HCC was then selected based on frequency matching against age, gender, Child-Turcotte-Pugh score and length of follow up. The HCVRUK database contained details of patient demographics and treatment used. To supplement these data, a standardised data collection form was sent to all sites and to ensure accuracy and data completeness sites were re-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. HCVRUK 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.

Outcome Measures

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. 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 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 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 there was diagnostic uncertainty. 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 until study end or patient death. Tumour size was calculated via radiological reports with Barcelona clinic liver cancer (BCLC) scores generated centrally along with response evaluation criteria in solid tumours (RECIST) criteria and Milan criteria. 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 EASL HCC diagnosis guidelines, as determined following local MDT 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 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, pre-defined 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 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.

To analyse the correlation of development of HCC with several variables in our dataset and investigate potential confounding factors, we have used multiple logistic regression. The model was built 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. Potential interactions were included as interaction terms in the model. The effect of each variable in the multiple model is presented with odds ratios and 95% confidence intervals. For continuous variables, the odds ratio was calculated for a clinically meaningful increment of change.

Time to event analyses were performed using the nonparametric Kaplan-Meier method (Kaplan & Meier, 1958). 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 EAP 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 EAP 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). The HCC of 45 patients was diagnosed by MRI, 26 by CT scan, 8 patients had HCC diagnosed within their explanted liver and for one patient, whilst the date of diagnosis was recorded, the mode of diagnosis was not available. The demographics of the cohort are shown in Table 1. Frequency matching provided groups with similar age, Child-Turcotte-Pugh score 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 with most patients being Child-Turcotte-Pugh stage B (63%) followed by A (22%) and C (15%). 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 EAP 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 EAP 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 also 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 taken within 12 months of EAP onset from 23/130 (18%) of the control patients, compared to scans from 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 an abnormality either progressed or if an HCC was diagnosed in the same anatomical region, 15 of the 24 (63%) non-malignant lesions 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 a univariate analysis, development of HCC for all 80 HCC patients was associated with diabetes, albumin, non-malignant lesion and platelet count. In a multivariate analysis, three of these variables, excluding albumin, remained significant (Table 2).

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). 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) and 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 within the Milan criteria (i.e. circumscribed) was 61/72, following exclusion of those diagnosed on explant. 39/47 (83%) patients that achieved SVR, were within Milan criteria compared to 22/25 (88%, $p = 0.57$) patients that did not achieve SVR.

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 EAP 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 produced 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 and thrombocytopaenia were associated with subsequent development of malignancy. These findings are consistent with previous studies [9, 10, 16, 17] 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 [18, 19]. We have shown that patients with apparently non-malignant lesions in scans taken within 12 months of 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 [20]. Vigilance is clearly indicated in patients with pre-existing liver lesions.

We find that patients diagnosed with HCC within 6 months of 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 when started on treatments with a combination of sofosbuvir, lediposvir, simepravir, 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 that small pre-existing liver cancers may harbour virus that is either resistant to therapy or, more likely, can not be accessed by antiviral drugs. However, in our study, we also detected a lower SVR12 rate (65%) in patients who were diagnosed with HCC more than 6 months after onset of therapy. This suggests that either 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 and our policy is to conduct intense surveillance on such patients to allow early detection of malignancy.

The important question of whether liver cancer is more common and or more aggressive following viral clearance is difficult to answer – the ideal study randomising patients with cirrhosis to treatment or observation is unlikely to be popular with patients and is, in our view, unethical. 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 EAP 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 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 times 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 study of decompensated cirrhotic patients. The patient selection provided a sampling from most major cities and the standard of data collection was high throughout the study and carried out to a clinical trial standard, although not formally audited. With the use of information available at sites including scan reports and MDT outcomes, this study is readily translatable to the real world everyday patient care.

Although our study is one of the most extensive studies in this cohort we nevertheless only have 80 HCC patients treated with DAAs and this may be underpowered to detect small yet significant differences in populations and is compounded as the follow-up time is relatively short. 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 but were not otherwise matched. 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. 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 changes of platelets to be indicative of HCC production. An absence of effect of DAA treatment on HCC 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. 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.
17. Calvaruso, V., et al., *Incidence of Hepatocellular Carcinoma in Patients with HCV-associated Cirrhosis Treated with Direct-Acting Antiviral Agents.* Gastroenterology, 2018.
18. Kielar, A.Z., et al., *LI-RADS 2017: An update.* J Magn Reson Imaging, 2018.
19. Alessandro, F. and B.A. A., *Problematic lesions in cirrhosis.* Clinical Liver Disease, 2018. **11**(2): p. 43-47.
20. 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.

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

		Non-HCC		All HCC		Early HCC (<6 months)		Late HCC (>6 months)	
		Number	%/(IQR)	Number	%/(IQR)	Number	%/(IQR)	Number	%/(IQR)
	Total (245)	165	67	80	33	28	11	52	21.6
Age, med yrs. †		57	(52.9-61.9)	57	(51.8-60.9)	55	(50-60.9)	57.2	(54.2-61.4)
Males, n †		123	75	61	76	22	79	39	75
CPT score †		B	62	B	65	B	53.6	B	71
Length of FU med 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	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 Ca (non-HCC), n		17		5		2		3	
Prev. Tx failure, n		102	62	50	63	19	70	31	60
Type of treatment, n	Sof/Led	6	3	1	1	1	3	0	0
	Sof/Led/Rib	115	70	59	74	22	79	37	71
	Sof/Dac	3	2	0	0	0	0	0	0
	Sof/Dac/Rib	41	25	20	25	5	18	15	29
SVR achieved		143	87	54	68	20	71	34	65
Albumin, med		29.0	(26-34)	27.0*	(23-32)	28.0	(23-32)	27.0*	(22.5-31)
Alpha-fetoprotein, med		7.0	(5-15.1)	7.0	(4-16.5)	9	(5.6-25)	6.1	(3.6-12.3)
Alkaline Phosphatase, med		148	(108-202)	121	(101-186)	111	(90-154)	139	(105-189)
Bilirubin, med		34.0	(22-49)	38.0	(23-52.75)	32.0	(20-52)	39.0	(25-53.5)
INR, med		1.3	(1.2-1.4)	1.0	(1.2-1.5)	1.3	(1.2-1.5)	1.4	(1.2-1.5)
Platelet, med		74.0	(53-98)	63*	(44-85.5)	68.0	(44-95)	59.0*	(43.5-80)
Sodium, med		136.0	(134-139)	136.0	(132-138)	137.0	(133-140)	136.0	(131.5-137)
BMI, med		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. *denotes p<0.05.

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

Variable	Effect	P - value
Platelets (Change of $50x10^9$/L)	OR: 0.89, 95% CI: 0.8-0.9	0.006
Diabetes	OR: 2.1, 95% CI: 1.1-4.0	0.025
Baseline lesions	OR: 2.4, 95% CI – 1.1-5.0	0.031
Albumin	n/a	n.s

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.