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Acoustic radiation force impulse predicts long-term outcomes in a large-scale cohort: High liver cancer but low comorbidity in the hepatitis B virus group

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INTRODUCTION

Chronic liver diseases are major risk factors for hepatocellular carcinoma (HCC)[1,2]. Regular screening of high-risk groups to detect HCC in the early stage can increase the chance to eradicate HCC and improve survival rates[3,4]. Liver cirrhosis is a major HCC risk factor[1-4]. In the last two decades, Fibroscan has been used to assess the HCC risk for patients with chronic liver diseases[5-9]. An alternative is the acoustic radiation force impulse (ARFI) imaging which uses ultrasound as a push pulse to measure the liver stiffness[10-12]. Both modalities are quite good in patients with chronic hepatitis C virus (HCV) infection and non-alcoholic liver diseases[13]. However, the correlation was relatively poor in patients with chronic hepatitis B virus (HBV) infection. We have been using ARFI to assess liver stiffness since 2011[12,13], which allowed the informatics department in our institute to produce a uniquely available large-scale cohort of patient data for research purposes. This would facilitate us to evaluate the ARFI's ability to assess the HCC risk using a consecutive cohort that is much larger than what has been found in prior work. The short-term correlation between ARFI and liver fibrosis is relatively poor in HBV, but the long-term effect is still not fully investigated. As well, studies into ARFI's predictive abilities of HCC diagnosis from patients with different etiologies of chronic liver disease are still rare[14]. Finally, the chronic HBV infection is characterized by an initial immune tolerance phase[15]. This could be a survival strategy[16] despite an increased risk of HCC by chronic persistent HBV infection[1,2,17,18].

Therefore, we also examine the differences in morbidities and mortalities between HBV and other etiologies in this cohort to help shed some light on this question.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board (IRB) of the Chang Gung Medical Foundation (CGMH IRB No. 201801283B0 and No. 202200758B0).

ARFI measurement

ARFI imaging was done with an Acuson S2000 system (Siemens Medical Solutions). Liver stiffness was measured with a standardized protocol at two locations of the right hepatic lobe[12,13]. The mean of the two locations was used as the final measurement. Most of the studies were done by one senior technician (HTW). Because it is not covered by the Taiwanese National Health Insurance Administration, the charge of ARFI (around 50 United States dollars) was mostly paid by the patients. The exceptions were patients undergoing a liver biopsy study or those participating in clinical trials (around 25%), where the cost was paid for by research grants. The first ARFI study was used in this analysis. The ARFI-estimated fibrosis grades will be according to the cutoff values in our previous histology proven study[13].

Patients

The data of this study were retrieved from the Chang Gung Research Database of the Chang Gung Memorial Hospital, Linkou and Taipei branches. All patients who received ARFI imaging between January 2011 and September 2018 represented as the index patients. A total population of 2405 patients were included. The Chang Gung Research Database includes the original electronic medical records, comprising health care facility information, patient information demographics, diagnosis information, drug information, procedure information, and other health digital information. All the personal identifiers were replaced by a code. We organized a team that included both hepatology and informatics departments of Chang Gung Memorial Hospital, along

with outside technical consultants, to deal with this project[19]. We excluded patients with incomplete viral markers and clinical data, dual viral hepatitis, alcoholic liver diseases, autoimmune liver diseases, toxic hepatitis, genetic liver diseases and those whose ARFI studies are with an interquartile range over median ratio > 30%, which is a recommended quality assurance criterion (Figure 1)[20]. According to the viral markers, patients were classified into three groups of HBV, HCV, and non-hepatitis B and non-hepatitis C (NBNC) patients. The NBNC group consisted mostly of non-alcoholic fatty liver disease[19]. All the patients were follow-up with liver biochemistry, alpha-fetoprotein, and liver ultrasound in 3-12-mo intervals.

Comorbidity

The comorbidities under investigation included hypertension [I10-I15], type 2 diabetes mellitus [E8-13], dyslipidemia [E78], myocardial infarction [I21-I23, I1252], atrial fibrillation [I48], Heart failure [I50], and ischemic stroke [I63-I66]. The ascertainment of these comorbidities was based on three diagnoses from the outpatient department or one diagnosis from the inpatient department.

Antiviral therapy

Patients could have undergone any of several different antiviral regimens in the study period. Similar interferon regimens had been used in both HBV and HCV groups, and such immune modulatory regimens were not uniform. Therefore, we simply recorded whether interferon therapy was given during pre-enrollment or post-enrollment.

Similarly, multiple nucleot(s)ide analogues (NA) regimens for HBV and direct antiviral agents (DAA) regimens for HCV were possibly taken in the study period. Their mechanisms are suppression or elimination of viral replication. We record such oral therapies into pre-enrollment and/or post-enrollment periods as one category.

Cancer registration data link

In addition to their medical records, study subjects were linked with the database of Cancer Registration, Chang Gung Memorial Hospital, which records information on all cancers diagnosed in this hospital since 1987. We linked this database up to June 30, 2020.

Mortality data link

This study used the national citizen identification numbers of patients to search the mortality data bank established by the Statistics Office, Department of Health, Taiwan. The mortality data bank stored death certificate data, which includes patient demographic data such as the time, place, and cause of death; and the name of the official who issued the document. Cause of death was classified using the International Classifications of Diseases, Injuries and Causes of Death (ICD-10, World Health Organization, 2015). The data was linked up to December 31, 2020.

This mortality data link will collect data for those patients lost to follow-up in this hospital.

Statistical analysis

Patient characteristics were represented as the number and percentage, or the mean ± SD, as appropriate. Continuous variables of the three independent groups were compared using one-way analysis of variance (ANOVA) with Bonferroni correction. Categorical variables were tested using the chi-square test, or the chi-square test for trend, as applicable. Logistic regression was conducted to identify independent risk factors of HCC for records before the ARFI study. Cox proportional hazards model was conducted to identify independent risk factors of HCC for records after the ARFI study. The Mantel-Cox procedure (Log Rank test) was applied to compare risk factors and the cumulative risk of HCC among different groups after ARFI study. Area under receiver operating characteristic (AUROC) curve was done by a scoring system for HCC risk prediction. The scoring system was based on the hazard ratio in the multivariate analysis. Briefly, ARFI and FIB4 are according to its values and modified by timing 0.5

or 2. Other factor were calculated as: Gender (G) (male = 2, female = 0); etiology score (E) (HBV = 3, HCV = 2, NBNC = 1); age (year) score (A) (0-35 = 0, 35-40 = 1, 40-45 = 2, 45-50 = 3, 50-55 = 4, 55-60 = 5; > 60 = 6]; platelet (10^9/L) score (A) (0-100 = 3, 100-150 = 2, >150 = 1). Statistical analyses were performed using the SPSS software (version 22; SPSS Inc., Chicago, IL, United States), and a *P* value of < 0.05 was judged as statistically significant.

RESULTS

Baseline demographic features

A total of 1962 patients met our inclusion criteria. Among them, 1064, 507, and 391 patients were in the HBV, HCV, and NBNC groups, respectively (Table 1). The HBV group had more men and lower prevalence of hypertension, diabetes mellitus, heart failure, ischemic stroke, and dyslipidemia. Patients in the HCV group were older and had lower male ratios, lower platelet counts, higher fibrosis index based on four factors (FIB4s) and mean ARFI scores. NBNC group patients had higher body mass indices (BMIs), lower prothrombin time international normalized ratios (INR), lower FIB4s, higher platelet counts, lower rates of estimated cirrhosis, and shorter durations of follow-up.

Alanine aminotransferase level grades at the enrollment

ARFI is strongly influenced by liver inflammation. This especially happens when alanine aminotransferase (ALT) levels are greater than 5 × (180 U/L) the upper limit of normal (0-36). So, we list the ALT level grades in the Supplementary Table 1. In total, 7.3% patients had ALT level greater than 180 U/L.

Comorbidity

There are significant differences in prevalence of hypertension, diabetes mellitus, heart failure, ischemic stroke, and dyslipidemia between HBV and the other two groups (Table 1). After adjusting for age, gender, and BMI, the prevalence of hypertension,

diabetes mellitus, ischemic stroke, and dyslipidemia were significantly lower in the HBV group than the non-HBV groups (Supplementary Table 2).

Cancers diagnosed before ARFI

We linked our index cases with cancer registration databases between 1987 and 2020. There were many cancer diagnoses prior to enrollment. These cancers were either well-treated or receiving active therapy. HBV had the highest rate of pre-enrollment HCC diagnosis (14.1%, $P < 0.001$, Table 2, upper panel). On the other hand, the NBNC group had the highest rate of non-HCC cancer (10%, $P < 0.001$), with breast cancer having the highest rate (4.3% for total or 10.8% for females alone, $P < 0.001$). Factors associated with a high risk of HCC as identified by logistic regression were male sex, high mean ARFI score, HBV, older age, higher aspartate aminotransferase (AST) levels, lower ALT levels, and lower platelet counts (Table 3). Hypertension and diabetes mellitus were associated with higher risk of pre-diagnosed HCC while dyslipidemia was associated with lower risk of pre-diagnosed HCC.

Cancers diagnosed after ARFI

After excluding patients with cancer diagnosed before enrollment, the HBV (4.0%) and NBNC (1.3%) groups exhibited the highest and lowest rates of HCC occurrence ($P = 0.033$, Table 2 middle panel). Factors associated with HCC diagnosis were male sex ($P = 0.013$), high ARFI score (< 0.001) and HBV ($P = 0.019$, Table 4). In Cox's regression analysis, male gender, HBV, platelet counts and ARFI scores were associated with higher risks of HCC.

HCC development after enrollment in different fibrosis stages

According to the cutoff values of mean ARFI determined in our previous histology proven cases[13], we divided the study population into cirrhosis, severe-fibrosis, moderate, and mild to non-fibrosis groups. The cumulative risk of HCC development was highest in the cirrhosis group, followed by the severe fibrosis group and lowest in

none to moderate fibrosis groups (Figure 2, $P < 0.001$). The 5-year risk of HCC was 5.9% and 9.8% for those patients ARFI-graded with severe fibrosis and cirrhosis, respectively. When those patients with ALT greater than 5 × upper limit normal were removed, the 5-year risk of HCC was 6.1% and 10.4% for those with severe fibrosis and cirrhosis respectively (Supplementary Figure 1). There was no difference in predictive abilities across different etiologies (Supplementary Figure 2), suggesting that high ARFI scores are a good predictor of HCC diagnosis for HBV patients, along with HCV and NBNC patients.

AUROCs of HCC prediction in different non-invasive scoring models

The AUROCs show that ARFI or FIB4 alone give similar 3- or 5-year predictions of HCC (AUROCs around 0.739-0.756) (Figure 3). After adding Gender, etiology, age, and platelet scores, the AUROCs may increase to 0.772-0.840).

Mortality during study period

No significant difference in total mortality was found across the three groups. The HBV group corresponded to a marginally higher HCC related mortality (3.3%, $P = 0.057$) (Table 2, lower panel). However, the Non-HCC related mortality was the lowest in the HBV group ($P < 0.027$). Male sex, age, lower ALT levels, higher AST levels, lower BMI, higher ARFI scores, and ischemic stroke were associated with higher risk of mortality in Cox's regression (Supplementary Table 3).

DISCUSSION

Within our patient cohort of 212 patients were diagnosed with HCC prior to enrollment and 67 developed HCC after enrollment (Table 2). High ARFI scores were associated with pre-diagnosed HCC occurrences in a multivariate analysis ($P < 0.001$, Table 3) and a good predictor for HCC after enrollment using Cox's regression analysis ($P < 0.001$, Table 4). The ARFI-estimated fibrosis grades showed a severity-dependent increased risk of HCC in the post-enrollment period (Figure 2). The 5-year risk of HCC was 5.9%

and 9.8% for those patients who were ARFI-graded with severe fibrosis and cirrhosis, respectively. Addition of age, gender, and platelet factors, only a small raise of AUROC curves from 0.753 to 0.84 within 3-years and 0.742 to 0.828 within 5-year in the prediction of HCC. These results suggest that liver fibrosis is the main risk factor for HCC (Figure 3). Our finding is consistent with others in a recent study review[21] using non-invasive fibrosis diagnosis models. Those patients with grade 3 and 4 fibrosis should receive active surveillance of HCC.

ARFI-graded fibrosis predicts the HCC occurrence well among different etiologies (Supplementary Figure 2). Although the correlation between ARFI and liver histology fibrosis was relatively poorer in the HBV group[13] than with other two etiologies, HCC risk prediction was as good as the other groups. This question was not well-addressed in prior work where such investigations had much smaller clinical datasets or single etiology cohorts. Additional investigations should further pursue this link.

In terms of prior work, ARFI studies on non-HCC patients typically focused on its utility of fibrosis staging[10-12,22]. Exceptions include the work by Sun *et al*[23], who correlated ARFI with the indocyanine green test and found a positive correlation between the two. ARFI was also correlated to Child Pugh scores in that study. They suggested that ARFI imaging is a useful tool for assessing liver functional reserve. As another example, a recent meta-analysis reported that ARFI scores may be a good predictor of HCC recurrence-free survival in patients receiving radiofrequency ablation[24]. In a series of 1808 patients received ARFI, those patients with ARFI score > 1.33 cm/s showed a higher HCC development than those ≤ 1.33 cm/s[25]. Therefore, there is good evidence that ARFI can measure liver fibrosis, reflex the liver functional reserve, and predict HCC recurrence. Our study adds to these conclusions, as Cox's regression analysis confirms that high ARFI scores are a risk factor for HCC and mortality ($P < 0.001$, Table 3 and Supplementary Table 3).

In aspects of HCC diagnosis, the HBV had higher occurrences than the NBNC group (Table 2). This aligns with previous investigations, *e.g.*, the study by Chen *et al*[26] who reported higher occurrences of HCC in their HBV (4.8%) and HCV (4.7%) groups

compared to their NBNC group (0.3%). Even so, the prevalence of HCC in our NBNC group is much higher than the work, but this is because Chen *et al*[26] included healthy subjects in their NBNC group, while our NBNC cohort was a disease group. Even though NBNC patients had a lower incidence of HCC ($P < 0.001$) this was offset by higher incidences of non-HCC cancers. Consequently, the total mortality rate was similar among different groups (Table 2). This is consistent with a recent meta-analysis by Mantovani *et al*[27] that concluded that extra-hepatic cancers were increased in non-alcoholic fatty liver disease. It should be noted that such trends were only identified in the pre-enrollment period because it had a longer past history. We can record many cancers in the pre-enrollment period because the diagnosis of all cancer types has been recorded in our cancer registration database since 1987. We found that breast cancer ($P < 0.001$), colon cancer and hematologic cancers were the main cancers in the pre-enrollment period. These types of cancers respond to treatment therapy well, allowing us to include such survivors in our cohort.

There were lower rates of comorbidities in HBV than other groups (Table 1). This seems to be related to high metabolic syndrome in the HCV or NBNC group. However, after adjusting for gender, age, and BMI, such a phenomenon was still presented (Supplementary Table 2). As early as 2006, Jan *et al*[28] had reported a lower prevalence of diabetes, hypertension, obesity, hyperlipidemia, and obesity in HBV than HCV or NBNC in a population-based study in northern Taiwan. Another nationwide study by Kuo *et al*[29] that examined 1376344 diabetes patients between 2000 and 2012 also confirms these results. Before enrollment, they excluded patients with a history of myocardial infarction (2.28% in HBV group and 4.19% in non-HBV group, $P < 0.001$) and cerebrovascular diseases (15.6% in HBV group and 24.3% in non-HBV group, $P < 0.001$). The percentage of excluded patients was lower in their HBV group than non-HBV groups for both diseases. After enrollment and propensity matching, the risk of all-cause mortalities, myocardial infarction, ischemic stroke, and heart failure were higher in the non-HBV group than the HBV group ($P < 0.001$) in a mean follow-up of 5.3 year[29]. A study by Sung *et al*[30] from Korea showed similar findings. They point out

that the difference was more profound in HBV with liver dysfunction than those without liver dysfunction. They suggest that the HBV-related proinflammatory effect may be the reason for decreased risk of comorbidity.

Previous studies did not discuss the reason for these low comorbidities in the HBV patients. A recent review suggests chronic HBV infection may protect infected subjects from the development of metabolic syndrome and hepatic steatosis[31]. The immune system is a double-edged sword. Its efforts against microorganisms may induce host tissue damage[32]. Chronic persistent HBV infection is characterized by an initial immune tolerance phase that allows active HBV replication without immune-mediated inflammation to liver tissue[33]. Liver inflammation occurs only when the immune system is triggered to attack HBV carried hepatocytes[34]. This contrasts with HCV and NBNC groups with persistently mild inflammation in the liver.

There is a longer HBV-related immune tolerance phase in East Asian than in the African which could be related to genetic polymorphism in human leukocyte antigen (HLA)-DP and -DQ loci[16,35]. Such HBV-related gene variants decrease antigen presentation to avoid fatal immune response, but also establish an environment that is suitable for chronic persistent HBV infection[16]. Our recent study indicated that those HBV-related single nucleotide polymorphisms (SNP)s in HLA-DP and -DQ loci were associated with high viral load in the HCC family[36]. Such patients would be more likely to be associated with liver dysfunction as those mentioned in Sung's series[30]. From the above clues, we suspect that the low comorbidity trend in the HBV group may be partly associated with a low antigen presentation strategy.

One of our limitations is that Life-long disease consequences are not easy to examine. The mean follow-up duration after enrollment could be considered as relatively short (3.13 years to 4.59 years). We may need longer and more specific studies to explore the link between HBV infection and comorbidity. It should be noted that HBV-related single nucleotide polymorphism at HLA-DP and -DQ loci in East Asians are quite different from other regions[16]. The low comorbidity in the HBV group may be limited to East Asian. Another limitation is that the contribution of therapy to morbidity and

mortality were difficult to evaluate. We notice that the post-enrollment interferon therapy was associated with lower pretreatment HCC and post-enrollment oral antivirus therapy was associated with lower post-enrollment HCC. However, these findings may be due to a relatively better condition of chronic liver disease, which could have made the pre-enrollment therapy unnecessary. Similar situations concerned about therapeutic response in HCC-related mortality could be presented. With the success of checkpoint inhibitors in HCC therapy[37], future predictive biomarker study will be needed to clarify the difference in mortality among groups[38].

CONCLUSION

We conclude that even ARFI fibrosis prediction in the HBV group is relatively poorer than other groups, its performance or clinical significance in predicting HCC or mortality is as good as with other etiologies. The HBV group had the highest risk of HCC and the NBNC group had the highest risk of non-HCC tumors, especially breast cancer. Low comorbidities in the HBV group were found, which may be a consequence of low metabolic syndrome and low antigen presentation strategy.

Figure 1 Patient flow chart. HCC: Hepatocellular carcinoma; ARFI: Acoustic radiation force impulse; HBV: Hepatitis B virus; NBNC: Non- hepatitis B, hepatitis C.

Figure 2 Cumulative risk of hepatocellular carcinoma after enrollment in different acoustic radiation force impulse-fibrosis grades. Higher risk of hepatocellular carcinoma (HCC) was found in acoustic radiation force impulse (ARFI)-severe fibrosis and -cirrhosis grades than none to moderate fibrosis grades. The 5-year risk of HCC was 9.8 % for those ARFI fibrosis graded as cirrhosis; 5.9% for those graded as severe fibrosis; and only 1.7%-2.0% for those lower or equal to moderate fibrosis. Purple: cirrhosis; yellow: severe fibrosis; green: moderate fibrosis, blue: none or mild fibrosis [Log rank test: $P = 0.026$ (3 *vs* 4); $P = 0.015$ (3 *vs* 1+2); $P < 0.001$ (4 *vs* 1+2)]. HCC: Hepatocellular carcinoma; ARFI: Acoustic radiation force impulse.

Figure 3 The area under receiver operating characteristics of hepatocellular carcinoma prediction in different non-invasive scoring models. A: Within 3-year period after enrollment; B: Within 5-year period after enrollment. The area under receiver operating characteristic (AUROC) show that acoustic radiation force impulse (ARFI) or four factors (FIB4) alone give similar 3- or 5-year predictions of hepatocellular carcinoma (HCC) (AUROC around 0.739-0.756). After adding G (gender score), (etiology score), A (age score) and P (platelet score), the AUROC may increase to 0.772-0.840. Both ARFI and FIB4 models predict 3- or 5-year HCC quite satisfactory suggesting that fibrosis is the main risk factor for HCC. ARFI: Acoustic radiation force impulse; FIB4: Fibrosis index based on four factors; G: Gender score, male = 2, female = 0; E: Etiology score, hepatitis B virus (HBV) = 3, HCV = 2, NBNC = 1; A: Age score (year), 0-35 = 0, 35-40 = 1, 40-45 = 2, 45-50 = 3, 50-55 = 4, 55-60 = 5; > 60 = 6; P: Platelet score (10^9), 0-100 = 3, 100-150 = 2, > 150 = 1; Sen: Sensitivity; Spe: specificity.

Table 1 Baseline demographic features of the cohort

	HBV (*n* = 1064)	HCV (*n* = 507)	NBNC (*n* = 391)	*P* value	Missing rate
Demographics					
Age (yr)	52.05 ± 10.90	58.69 ± 10.84	51.97 ± 13.10	< 0.0001 (2 & 3, 1 & 2)	0.00%
Male sex, *n* (%)	825 (77.5)	273 (53.8)	233 (59.6)	< 0.0001	0.00%
Weight (kg)	68.36 ± 12.30	64.53 ± 12.17	70.94 ± 14.09	< 0.0001 (2 & 3, 1 & 2, 1 & 3)	0.97%
Height (cm)	166.34 ± 7.62	161.67 ± 8.57	164.18 ± 8.75	< 0.0001 (2 & 3, 1 & 2, 1 & 3)	2.80%
BMI (kg/m^2)	24.66 ± 3.62	24.58 ± 3.75	26.25 ± 4.23	< 0.0001 (2 & 3, 1 & 3)	3.47%
Lab data at ARFI study					
Spleen Index (cm^2)	31.97 ± 14.47	33.63 ± 16.72	34.07 ± 16.05	0.0640 (1 & 3)	0.03%
Albumin (mg/dL)	4.345 ± 0.51	4.29 ± 0.51	4.43 ± 0.48	0.0040 (2 & 3)	42.15%
AST (U/L)	57.50 ± 102.16	62.46 ± 58.08	58.40 ± 50.72		1.12%
ALT (U/L)	75.05 ± 162.88	71.52 ± 69.61	83.46 ± 78.32		1.33%
Bilirubin (mg/dL)	0.93 ± 1.23	0.91 ± 1.19	0.92 ± 1.21		12.79%
Prothrombin time (INR)	1.10 ± 0.13	1.11 ± 0.21	1.06 ± 0.13	0.0040 (2 & 3, 1 & 3)	42.15%
Platelet (10^9/L)	177.86 ± 61.86	170.13 ± 60.19	218.96 ± 76.08	< 0.0001 (2 & 3, 1 & 3)	13.25%
FIB4	2.454 ± 2.720	3.31 ± 3.04	2.06 ± 2.13	< 0.0001 (2 & 3, 1 & 2)	23.35%
Mean ARFI (m/s)[1]	1.40 ± 0.46	1.60 ± 0.61	1.42 ± 0.62	< 0.0001 (2 & 3, 1 & 2)	0.00%

Stiffness status[1]				< 0.0001
Cirrhosis	331 (31.1)	163 (32.1)	78 (19.9)	
Severe fibrosis	138 (13.0)	110 (21.7)	61 (15.6)	
Moderate fibrosis	114 (10.7)	68 (13.4)	29 (7.4)	
Mild or non-fibrosis	481(45.2)	166(32.7)	223(57.0)	
Comorbidities				
Hypertension	181 (17.0)	147 (29.0)	115 (29.4)	< 0.0001
Diabetes	119 (11.2)	103 (20.3)	76 (19.4)	< 0.0001
Heart failure	14 (1.3)	17 (3.4)	7 (1.8)	0.023
Atrial fibrillation	9 (0.8)	11 (2.2)	5 (1.3)	0.091
Myocardial infraction	12 (1.1)	5 (1.0)	2 (0.5)	0.567
Ischemic stroke	6 (0.6)	17 (3.4)	8 (2.0)	< 0.0001
Dyslipidemia	170 (16.0)	95 (18.7)	125 (32.0)	< 0.0001
Follow-up (year)				
Mean ± SD	4.59 ± 2.22	4.34 ± 2.35	3.13 ± 2.15	< 0.0001 (1 & 3, 2 & 3)
Median/IQR	4.54/3.59	3.97/3.88	2.61/3.23	

[1]Cutoff values according to reference 11.

ARFI: Acoustic radiation force impulse; FIB4: Fibrosis-4 index; BMI: Body mass index; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNC: Non-hepatitis B, non-hepatitis C; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 2 Cancers and mortality in different groups

	HBV	HCV	NBNC	*P* value
Total cases	1064	507	391	
Female	239 (22.5)	234 (46.2)	158 (40.4)	< 0.027
Follow years before enrollment	-2.65 ± 3.98	-2.78 ± 5.56	-3.30 ± 6.25	< 0.001 (3 & 1, 3 & 2)
Cancers, pre-enrollment				
HCC	150 (14.1)	42 (8.3)	20 (5.1)	< 0.001
Non-HCC cancer	36 (3.4)	30 (5.9)	39 (10.0)	< 0.001
Colon cancer	9 (0.8)	3 (0.6)	6 (1.5)	NS
Breast cancer	1 (0.1)	9 (1.8)	17 (4.3)	< 0.001[1]
Hematology cancers	3 (0.3)	4 (0.8)	2 (0.5)	NS
Follow years before enrollment	-2.76 ± 4.55	-3.57 ± 5.18	-3.22 ± 4.40	NS
Cancers, post-enrollment				
HCC	43 (4.0)	19 (3.7)	5 (1.3)	0.033

Non-HCC tumor	21 (2.2)	14 (2.8)	12 (3.1)	NS
Colon cancer	2 (0.1)	3 (0.6)	1 (0.3)	NS
Breast cancer	2 (0.2)	0 (0.0)	1 (0.3)	NS
Hematology cancers	6 (0.6)	0 (0.0)	1 (0.5)	NS
Prostate cancer	2 (0.1)	1 (0.2)	1 (0.3)	NS
Follow years after enrollment	2.43 ± 1.86	2.51 ± 1.86	2.25 ± 2.00	NS
Mortality total	72 (6.8)	41 (8.1)	21 (5.4)	NS
HCC	35 (3.3)	13 (2.6)	4 (1.0)[2]	0.057
Non-HCC cancers	16 (1.5)	8 (1.6)	7 (1.8)[2]	NS
Liver disease	6 (0.6)	7 (1.4)	6 (1.5)	NS
Non-liver disease	15 (1.4)	13 (2.6)	4 (1.0)	NS
Follow years after enrollment	2.59 ± 1.96	2.69 ± 1.99	2.00 ± 1.87	NS

[1]Female only;

[2]$P = 0.027$.

Remark: Different cancers on the same patient were count separately. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNC: Non-hepatitis B, non-hepatitis C.

Table 3 Logistic regression for hepatocellular carcinoma diagnosed before enrollment

	Univariate	Multivariate analysis		95%CI	
	P value	*P* value	Hazard ratio	Lower	Upper
Male	< 0.001	0.000	3.399	2.150	5.373
Etiology	< 0.001	0.000	-	-	-
HBV		0.000	4.009	2.219	7.241
HCV		0.169	1.644	0.810	3.335
Age, yr	< 0.001	0.000	1.047	1.029	1.066
ALT	0.243	0.001	0.993	0.989	0.997
AST	0.010	0.000	1.012	1.007	1.017
Bilirubin	0.170	0.729	0.980	0.875	1.098
Platelet	0.138	0.035	1.003	1.000	1.005
Spleen index	0.060	0.811	1.001	0.990	1.013
BMI	0.015	0.076	0.956	0.910	1.005
ARFI	< 0.001	0.010	1.556	1.110	2.181
Interferon therapy	0.011	0.012			
Pre-enrollment		0.618	1.204	0.581	2.496

Post-enrollment		0.004	0.211	0.073	0.617
Oral anti-virus agents	0.041	0.181			
Pre-enrollment		0.168	1.721	0.795	3.728
Post-enrollment		0.156	0.667	0.381	1.167
Pre- and post-enrollment		0.527	1.534	0.408	5.773
Hypertension	< 0.001	0.000	2.551	1.702	3.824
Diabetes mellitus	< 0.001	0.031	1.618	1.044	2.508
Dyslipidemia	< 0.001	0.000	0.358	0.207	0.620
Ischemic stroke	0.341	0.884	1.097	0.317	3.794

1481/1962 (75.5%) with complete data; cases (hepatocellular carcinoma), n = 189. HBV: Hepatitis B virus; HCV: Hepatitis C virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; ARFI: Acoustic radiation force impulse.

Table 4 Cox's regression for hepatocellular carcinoma occurred after enrollment

	Univariate	Multivariate analysis		95%CI	
	P value	*P* value	Hazard ratio	Lower	Upper
Male	0.005	0.009	20.796	1.293	6.045
Etiology	0.019	0.057			
HBV		0.023	40.473	1.232	16.245
HCV		0.150	20.670	0.701	10.173
Age (yr)	< 0.001	0.094	10.028	0.995	1.061
ALT (U/L)	0.188	0.211	0.994	0.985	1.003
AST (U/L)	0.605	0.449	10.005	0.993	1.017
Bilirubin (mg/dL)	0.962	0.326	0.772	0.460	1.295
Platelet (10^9/L)	< 0.001	0.041	0.993	0.986	1.000
Spleen index (cm^2)	0.019	0.169	10.012	0.995	1.030
BMI	0.334	0.524	0.973	0.894	1.059
ARFI (m/s)	< 0.001	0.000	20.775	10.624	4.742
Interferon therapy	0.814	0.943			
Pre-enrollment		0.734	0.772	0.174	3.435

Post-enrollment		0.914	0.949	0.366	2.459
Oral anti-virus therapy	0.0174	0.086			
Pre-enrollment		0.977	10.031	0.134	7.948
Post-enrollment		0.029	20.105	10.080	4.102
Pre- and post-enrollment		0.110	30.362	0.758	14.903
Hypertension	0.001	0.211	10.554	0.778	3.104
Diabetes mellitus	0.002	0.304	10.460	0.710	3.006
Ischemic stroke	0.305	0.971	00.000	0.000	1.46E+269
Dyslipidemia	0.233	0.065	10.920	0.960	3.840

1274/1749 (72.8%) with complete data, 49 cases (hepatocellular carcinoma). HBV: Hepatitis B virus; HCV: Hepatitis C virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; ARFI: Acoustic radiation force impulse.

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