Prospective association of liver function biomarkers with development of hepatobiliary cancers

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ABSTRACT

Introduction: Serum liver biomarkers (gamma-glutamyl transferase, GGT; alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP; total bilirubin) are used as indicators of liver disease, but there is currently little data on their prospective association with risk of hepatobiliary cancers.

Methods: A nested-case control study was conducted within the prospective EPIC cohort (>520,000 participants, 10 European countries). After a mean 7.5 mean years of follow-up, 121 hepatocellular carcinoma (HCC), 34 intrahepatic bile duct (IHBC) and 131 gallbladder and biliary tract (GBTC) cases were identified and matched to 2 controls each. Circulating biomarkers were measured in serum taken at recruitment into the cohort, prior to cancer diagnosis. Multivariable adjusted conditional logistic regression was used to calculate odds ratios and 95% confidence intervals (OR; 95%CI).

Results: In multivariable models, 1SD increase of each log-transformed biomarker was positively associated with HCC risk (OR(GGT)=4.23, 95%CI:2.72-6.59; OR(ALP)=3.43, 95%CI:2.31-5.10;OR(AST)=3.00, 95%CI:2.04-4.42; OR(ALT)=2.69, 95%CI:1.89-3.84; OR(Bilirubin)=2.25, 95%CI:1.58-3.20). Each liver enzyme (OR(GGT) =4.98; 95%CI:1.75-14.17; OR(AST)=3.10, 95%CI:1.04-9.30; OR(ALT)=2.86, 95%CI:1.26-6.48, OR(ALP)=2.31, 95%CI:1.10-4.86) but not bilirubin (OR(Bilirubin)=1.46,95%CI:0.85-2.51) showed a significant association with IHBC. Only ALP was significantly associated with GBTC risk (OR(ALP)=1.59, 95%CI:1.20-2.09).

Conclusion: This study shows positive associations between circulating liver biomarkers in sera collected prior to cancer diagnoses and the risks of developing HCC or IHBC, but not GBTC.

Keywords: Hepatobiliary Cancer, Liver Function Test, Biological Markers, Prospective Cohort, Nested Case-control Study

1. INTRODUCTION

Liver cancer is the sixth most commonly diagnosed cancer and the second leading cause of cancer death worldwide [1]. Hepatocellular carcinoma (HCC), the most common type of primary liver cancer, is diagnosed at late stages and characterised by a poor prognosis [2]. Established HCC risk factors are chronic hepatitis B and C virus (HBV/HCV) infection, heavy alcohol drinking leading to liver cirrhosis, smoking and dietary aflatoxin exposure [3]. Important evidence from prospective studies also supports a role for diabetes and obesity-associated non-alcoholic fatty liver disease (NAFLD) as important HCC risk factors [4,5]. The group of intrahepatic bile duct (IHBC) and biliary tract cancers (GBTC; tumours of the gall bladder and extra-hepatic bile ducts) are anatomically related to HCC. They too are often diagnosed at late stages when prognosis is poor, also with little existing information about their key determinants [6].

Liver function biomarkers (gamma-glutamyl transferase, GGT; alanine aminotransferase, ALT; aspartate aminotransferase, AST; alkaline phosphatase, ALP; total bilirubin) are used in clinical diagnosis of various disorders, including those related to liver function impairment and damage. Higher levels of specific combinations of these liver function biomarkers have been shown to be independently associated with NAFLD, liver cirrhosis, hepatitis infection, biliary obstruction [7] and diabetes risk [8,9], which is itself also associated with increased risk of HCC [10]. Previous case-control studies have found that GGT, ALT and AST are increased in approximately 90% of diagnosed HCC cases while half of the cases also showed elevated liver-specific alkaline phosphatase (ALP) or bilirubin levels [11]. For bile duct cancers, the sparse available data suggest that approximately 70% of cases have elevated levels of ALP and GGT [12]. It is thus possible that alterations in liver function biomarkers occur during the early development of hepatobiliary cancers and may relate to some of the underlying mechanisms of tumour development at these sites. In a recent systematic review GGT but not ALT was associated with increased risk of overall and liver cancer but the geographical variations were observed for ALT [13]. Existing prospective observational studies investigating the association between liver function biomarkers and liver cancers have been mostly based on Asian populations [14,15], and/or limited only to particular enzymes (either transaminases, ALT and AST, or GGT) [16,17]. A cohort study based on a mostly hepatitis negative Taiwanese subjects measured only transaminases and found that both enzymes were good independent predictors for HCC development [16]. Other studies based on hepatitis infected Asian populations have found positive HCC risk associations with many liver enzymes, but not bilirubin [14,15]. In a Swedish cohort, higher GGT levels were prospectively associated with increased risk for several cancer sites, including cancer in the liver, suggesting that this individual enzyme is not specific to disease in the liver and biliary tract [17]. However, there is currently little prospective data on this topic from additional Western populations where chronic hepatitis infections are less predominant while other HCC risk

factors such as excessive alcohol intake, obesity or diabetes are common. Very little is known about possible associations with IHBC or GBTC, particularly from prospective cohort settings.

To address this, we aimed to evaluate associations between risk of HCC, IHBC and GBTC and five commonly measured liver function biomarkers (GGT, ALT, AST, ALP and bilirubin) using a nested case-control study within the large European Prospective Investigation into Cancer and Nutrition (EPIC) cohort,.

2. MATERIALS AND METHODS

2.1 Study design

EPIC is a large multicentre prospective cohort study designed to investigate the association between diet, lifestyle and environmental factors and the incidence of various types of cancer and other chronic diseases. The rationale, study design and methods of recruitment are described in detail elsewhere [18,19]. Briefly, diet and lifestyle data were collected from approximately 520,000 men and women aged 20-85 years enrolled between 1992 and 2000 in 23 centres throughout 10 European countries: Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, the Netherlands, and United Kingdom [18]. Blood samples were collected from most participants at recruitment and are stored at the International Agency for Research on Cancer (IARC, Lyon, France) in -196°C liquid nitrogen for all countries except Denmark (-150°C, nitrogen vapour) and Sweden (-80°C, freezers). Study participants were recruited from the general population residing in a given geographical area, except for the Ragusa cohort (blood donors and their spouses), the Utrecht cohort (women attending a breast cancer screening), and the Oxford health conscious sub-cohort (mostly vegetarian and health-conscious volunteers).

All cohort members provided written informed consent. Approval for this study was obtained from the relevant ethical review boards of the participating institutions and from the IARC ethical review board (Lyon, France).

2.2 Follow-up for cancer incidence and mortality

Vital status follow-up (98.5% complete) was collected by record linkage with regional and/or national mortality registries in all countries except Germany and Greece, where follow-up was based on active follow-up through study subjects or their next-of-kin. Cancer incidence was determined through record linkage with regional cancer registries (Denmark, Italy, Netherlands, Norway, Spain, Sweden and United Kingdom; complete up to December 2006) or via a combination of methods, including the use of health insurance records, contacts with cancer and pathology registries, and active follow-up through study subjects and their next-of-kin (France, Germany, Greece; complete up to June 2010).

2.3 Case Ascertainment

According to the 10th revision of the International Statistical Classification of Diseases, Injury and Causes of Death (ICD10) HCC and IHBC were defined as C22.0 and C22.1, respectively. Biliary tract cancers (GBTC) included

tumours in the gallbladder (C23.9), extrahepatic bile ducts (C24.0), ampulla of Vater (C24.1), and biliary tract (C24.8 and C24.9). For each identified case, the histology and the methods used to diagnose the cancer were reviewed to exclude metastatic cases or other types of primary liver cancer.

2.4 Nested Case-Control Study Design

The design of the nested case-control study has been previously described in detail [20]. Briefly, 125 HCC, 35 IHBC and 137 GBTC cases were identified during the period between participants' recruitment and 2006 in EPIC. For each case, two controls were selected by incidence density sampling from all cohort members alive and free of cancer (except non-melanoma skin cancer), and matched by age at blood collection (±1 year), sex, study center, time of the day at blood collection (±3 hours), fasting status at blood collection (<3,3-6,and >6 hours); among women, additionally by menopausal status (pre-, peri-, and postmenopausal), and hormone replacement therapy use at time of blood collection (yes/no).

2.5 Biomarker measures

Hepatitis B and C virus seropositivity was detected in all case-control sets using the ARCHITECT HBsAg and anti-HCV chemiluminescent microparticle immunoassays (CMIAs) from Abbott Diagnostics (Rungis, France). C-reactive protein (CRP), AFP, GGT, ALT, AST, ALP, total bilirubin and albumin were measured on the ARCHITECT c Systems™ and the AEROSET System (Abbott Diagnostics, Rungis, France) using standard protocols. All laboratory analyses were performed at the Centre de Biologie République laboratory, Lyon, France. Participants with missing blood sample or failed laboratory assay were excluded (n=4, 1, 6 for HCC, IHBC and GBTC, respectively). Therefore, the final sample size included 568 controls and 286 cases, among which there were 121 HCC, 34 IHBC and 131 GBTC cases.

2.6 Statistical Analyses

Description of methods used for comparisons of baseline subjects characteristics, correlation and visualisation of biomarker levers over follow up time are described in **electronic supplementary material**.

Conditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) for serum individual biomarkers of interest (GGT, ALT, AST, ALP and bilirubin) in relation to HCC, IHBC and GBTC. Each main variable was included in models as categorical variables, with quartile cut-points based on sex-specific distributions among controls (for HCC and GBTC) and continuous z-standardised (Mean=0, SD=1) value of each log-transformed biomarker for all cancer sites (to enable direct comparison between the biomarkers). To test dose-responses, trend variables were assigned the sex-specific median values for overall quartiles of the biomarkers. Cubic splines were constructed to illustrate the dose-response association between biomarker levels and HCC risk. In addition, specific ratios (AST/ALT; GGT/ALP), hepatic steatosis index (HSI) and score (see **Table 1** footnotes),

and a score based on liver function biomarkers were calculated as described in more detail **in supplementary material**.

For HCC, three conditional logistic models were used as follows: model 1) a model based on matching factors only; model 2) a multivariable model incorporating additional adjustments for a priori defined confounders: smoking status (never, former, current, missing), baseline (continuous, g/d) and lifetime alcohol intake pattern (never, former, lifetime drinker and drinker only at recruitment), body mass index (BMI, continuous kg/m^2), and physical activity (active, inactive, missing); model 3) a more detailed multivariable model incorporating the above variables as well as positivity status for hepatitis B and or C infection (yes, no, missing). Additionally, models 4 and 5 with separate incorporation of prevalent diabetes status at baseline and CRP to model 2 were explored.

For IHBC and GBTC, only models 1 and 2 were considered, and a third model for GBTC with the additional adjustment for self-reported history of gallstones was run. Other potential confounders examined, but not included in the final models because their inclusion did not change the effect estimates by more than 10% were waist to hip ratio (WHR), coffee intake, and reported cardiovascular disease for HCC and IHBC or self-reported history of gallstones for GBTC.

In sensitivity analyses, we excluded subjects with (a) self-reported diabetes at baseline (yes/no), because of the potential for modifications in diet after diagnosis of this disease, (b) hepatitis infection, since it is an established risk factor for liver cancers, (c) subjects with follow-up of <2/4 years after blood collection to exclude possible reverse causation, d) those with abnormal value of transaminases.

For HCC, potential effect modification was studied for BMI, WHR and self-reported diabetes at baseline (since liver enzyme activity have been shown to increase in obesity and diabetes [22]), HSI score, smoking and alcohol intake (associated with liver cirrhosis [23]) and CRP, as a marker of chronic inflammation. Effect modification by these variables was tested on the multiplicative scale by including interaction terms formed by the product of modifying variable categories (see footnotes Table 1) and the value of categories of biomarkers. The statistical significance of interactions was assessed using likelihood ratio tests based on the models with and without the interaction terms. In our population only 21 cases and 12 controls within the same case-control sets had HSI below 30, a threshold for those that NAFLD can be ruled out [21], and statistical analyses were not possible in this sub-group.

Receiver operating characteristics (ROC) curves were constructed for each individual biomarker of interest and their combinations in order to assess their discriminatory performance between cases and controls, for all subjects and stratified by HSI (see **supplementary material**). All statistical tests were two-sided, and *P*-values <0.05 were considered statistically significant. All statistical analyses were conducted using SAS version 9.2 software (SAS Institute, Inc., Cary, NC, USA).

3. RESULTS

Selected baseline characteristics of cancer cases and their matched controls and correlations between variables are presented in **Table 1 and Supplementary Tables 1 and 2. Supplementary Fig. 1** illustrates differences in biomarker levels between cases and controls over time. For the description see supplementary file.

Table 1. Baseline demographic and lifestyle characteristics of HCC (N = 121), intrahepatic (N = 34) and extrahepatic (N = 131) bile duct cancer cases and their matched controls in the EPIC nested case-control study.

Characteristics	Hepatocellular Carcinoma (HCC)		Tumours of the Intrahepatic Bile Ducts (IHBC)		Tumours of the Gallbladder and Extrahepatic Bile Ducts (GBTC)	
	Cases (N = 121)	Matched controls (N = 242)	Cases (N = 34)	Matched controls (N = 67)	Cases (N=131)	Matched controls (N = 259)
Men, N (%)	82 (67.8)	165 (68.2)	18 (52.9)	35 (52.2)	58 (44.3)	112 (43.2)
Age at blood donation (y)	60.2 ± 6.5	60 ± 6.7	61.6 ± 6.3	61.6 ± 6.2	58.6 ± 7.5	58.6 ± 7.5
Follow-up from blood collection (y)	4.99 ± 2.9	-	4.15 ± 2.21	-	5 ± 2.9	-
Liver function and inflammatory biomarkers, median (5th, 95th%)						
Gamma-glutamyl transferase (GGT), U/L	84 (13, 742)	23.5 (10, 74)	44 (13, 595)	21 (11, 89)	23 (10, 99)	20 (10, 79)
Alanine aminotransferase (ALT), U/L	30 (10, 151)	17 (9, 45)	20 (12, 99)	16 (9, 32)	17 (9, 39)	17 (9, 39)
Aspartate aminotransferase (AST), U/L	43 (14, 152)	19 (13, 35)	21 (15, 83)	18 (12, 26)	19 (12, 31)	18 (13, 30)
Alkaline phosphatase (ALP), U/L	84 (44, 173)	60 (38, 100)	74 (44, 380)	66 (41, 100)	66 (39, 110)	60 (36, 91)
Total billirubin, µmol/L	10 (4, 32)	7.6 (3, 15)	7 (3, 17)	6.7 (3, 13)	7 (4, 14)	7 (3, 15)
C-reactive protein, mg/La	2 (1, 27)	2 (1, 9)	3 (1, 19)	1 (1, 9)	2 (1, 13)	1 (1, 9)
Liver function score, N (%)[b]						
0	52 (43.0)	231 (95.5)	19 (55.9)	55 (82.1)	111 (84.7)	220 (85.0)
≥1	69 (57.0)	11 (4.5)	15 (44.1)	12 (17.9)	20 (15.3)	39 (15.0)
AST/ALT ratio, N (%)						
≤2	109 (90.1)	236 (97.5)	34 (100.0)	66 (98.5)	125 (95.4)	252 (97.3)
>2	12 (9.9)	6 (2.5)	0 (0.0)	1 (1.5)	6 (4.6)	7 (2.7)
GGT/ALP ratio, N (%)						
≤2.5	104 (86.0)	238 (98.4)	33 (97.1)	67 (100.0)	130 (99.2)	255 (98.5)
>2.5	17 (14.0)	4 (1.6)	1 (2.9)	0 (0.0)	1 (0.8)	4 (1.5)
No. with diabetes (N, %) [c,d]						
No	101 (83.5)	219 (90.5)	31 (91.2)	61 (91.0)	116 (88.6)	228 (88.0)
Yes	16 (13.2)	14 (5.8)	2 (5.9)	4 (6.0)	8 (6.1)	15 (5.8)
Hepatic steatosis index (HSI; N, %)[e]score						

≤36 (unlikely NAFLD)	59 (48.8)	128 (52.9)	13 (38.2)	40 (59.7)	73 (55.7)	145 (60.0)
>36 (suspected NAFLD)	58 (47.9)	105 (43.4)	21 (61.8)	25 (37.3)	51 (37.2)	98 (37.8)
Anthropometric factors and daily dietary intake, mean ± SD						
Body mass index (BMI) [f] (kg/m^2)	28.1 (5.3)	27.0 (3.9)	28.2 (3.6)	26.4 (4.2)	26.8 (4.5)	26.4 (3.8)
Waist-to-hip ratio (WHR) [g]	0.93 (0.10)	0.91 (0.10)	0.90 (0.08)	0.89 (0.10)	0.88 (0.10)	0.87 (0.10)
Energy (kcal)	2147.5 ± 644.0	2212.3 ± 567.1	2130.2 ± 671.8	1999.6 ± 578.6	2072.1 ± 619.5	2089.3 ± 570.9
Alcohol (g)	19.7 ± 29.4	14.8 ± 18.2	14.1 ± 17.5	14.4 ± 18.1	13.4 ± 18.6	14.2 ± 18.6
Coffee consumption (g)	398.2± 445.9	449.1± 434.8	398.1± 347.6	378.2± 359.8	378.8 ± 366.6	441.9±411.3

Missing values were not excluded from percentage calculations, thus the sum of percents across sub-groups may not add up to 100%.
Categorical variables are presented as numbers (percentages).
Continuous variables are presented as mean ± standard deviations, except for liver function tests that are presented as median and (5th, 95th percentile).
[a] CRP category: ≤3>
[b] Ranges from 0 to 6; was grouped in categories as 0, 1-2, ≥3 abnormal liver function tests (ALT>55 U/L, AST>34 U/L, GGT men >64 U/L, GGT women > 36 U/L, ALP > 150 U/L, albumin < 34 g/L, total bilirubin > 20.5 μmol/L; based on the values provided by the laboratory).
[c] Self-reported.
[d] Number of cases and controls with missing variable value: HCC = 13, IHBC = 3, and GBTC = 23.
[e] Hepatic steatosis index (HSI) = 8 x ALT/AST ratio +BMI (+2, if diabetes; +2, if female). Number of cases and controls with missing variable value: HCC =13, IHBC = 3, and GBTC = 23.
[f] BMI categories: ≤ 25 normal, 25-30 overweight, ≥ 30 obese
[g] WHR category: sex-specific tertiles (men: ≤0.92, 0.92-0.97, ≥0.97; women:≤ 0.77, 0.77-0.84, ≥ 0.84).

3.1 Liver function biomarkers and the risk of liver (HCC, IHBC) and biliary tract cancers (GBTC)

In the analyses by quartiles of serum enzyme levels, for all four enzymes and total bilirubin the highest quartile was significantly positively associated with HCC risk in both models 1 and 2 (**Table 2**). For model 2 the estimates were attenuated slightly than from those for model 1; comparing the highest quartile to the lowest quartile, the findings from model 2 were as follows: $OR_{(GGT)}$=7.90, 95%CI:2.98-20.98, p_{trend}=<0.0001; $OR_{(ALT)}$=4.62, 95%CI:2.05-10.41, p_{trend}=<0.0001; $OR_{(AST)}$=5.00, 95%CI:1.95-12.86, p_{trend}=<0.0001; $OR_{(ALP)}$=6.15, 95%CI:2.32-16.31, p_{trend}=<0.0001, $OR_{(BILIRUBIN)}$= 3.22,95%CI:1.39-7.45, p_{trend}=0.0002. Further adjustment for hepatitis status slightly lowered the risk estimates for all enzymes. Only for ALP the OR was higher: $OR_{(ALP)}$=9.56, 95%CI:2.63-34.72 and for bilirubin it was no longer significant.

In continuous analyses, statistically significant positive associations were found between all liver enzymes and bilirubin and HCC (fully adjusted OR for 1 SD in log unit increase- model 2) (**Table 2**). GGT was most strongly related to HCC risk ($OR_{(GGT)}$=4.23, 95%CI:2.72-6.59), followed by ALP ($OR_{(ALP)}$=3.43, 95%CI:2.31-5.10), AST ($OR_{(ALP)}$=3.00, 95%CI:2.04-4.42), ALT($OR_{(ALT)}$=2.69, 95%CI:1.89-3.84) and bilirubin ($OR_{(BILIRUBIN)}$=2.25, 95%CI:1.58-3.20). Further adjustment for hepatitis status (model 3) made these associations weaker for most enzymes and bilirubin ($OR_{(GGT)}$=3.55, 95%CI:2.26-5.57), and stronger only for ALP ($OR_{(ALP)}$=3.17, 95% CI:2.02-4.98). No

effect on the estimates was observed after additional adjustment for diabetes status or CRP (**data not shown**). Cubic splines illustrating dose-response associations between the biomarkers and HCC risk are presented in **Fig. 1** (for GGT) and supplementary **Fig. 3**.

Table 2. The association for HCC risk with individual liver function biomarkers for quartiles and per 1 SD increase of log-transformed values.

	Quartile 1	Quartile 2 OR (95%CI)	Quartile 3 OR (95%CI)	Quartile 4 OR (95%CI)		Per 1 SD* log-transformed OR (95%CI)
No. of cases/ controls	11/60	10/59	10/56	90/67		121/242
GGT						
Model 1	1.00 (Ref)	1.04 (0.38, 2.84)	1.06 (0.39,2.90)	9.10 (3.93,21.10)	<*.0001*	4.58 (3.04,6.90)
Model 2	1.00 (Ref)	1.16 (0.37, 3.67)	1.12 (0.33,3.76)	7.90 (2.98,20.98)	<*.0001*	4.23 (2.72,6.59)
Model 3	1.00 (Ref)	1.18 (0.35, 4.02)	1.14 (0.33,4.03)	5.70 (1.99,16.31)	<*.0001*	3.55 (2.26,5.57)
ALT						
Model 1	1.00 (Ref)	0.97 (0.41, 2.30)	2.31 (1.03,5.21)	5.10 (2.44,10.67)	<*.0001*	2.77 (2.03,3.77)
Model 2	1.00 (Ref)	0.77 (0.28, 2.12)	1.87 (0.74,4.76)	4.62 (2.05,10.41)	<*.0001*	2.69 (1.89,3.84)
Model 3	1.00 (Ref)	2.04 (0.71, 5.85)	0.79 (0.24,2.60)	3.03 (1.14,8.09)	*0.0015*	2.04 (1.40,2.99)
AST						
Model 1	1.00 (Ref)	1.94 (0.76, 4.92)	1.15 (0.43,3.06)	6.27 (2.66,14.82)	<*.0001*	3.25 (2.31,4.59)
Model 2	1.00 (Ref)	1.96 (0.70, 5.48)	0.86 (0.28,2.60)	5.00 (1.95,12.86)	<*.0001*	3.00 (2.04,4.42)
Model 3	1.00 (Ref)	2.04 (0.71, 5.85)	0.79 (0.24,2.60)	3.03 (1.14,8.09)	*0.0028*	3.17 (2.02,4.98)
ALP						
Model 1	1.00 (Ref)	0.80 (0.30, 2.14)	1.35 (0.55,3.32)	5.97 (2.60,13.67)	<*.0001*	3.85 (2.65,5.59)
Model 2	1.00 (Ref)	1.01 (0.34, 3.02)	1.82 (0.67,4.97)	6.15 (2.32,16.31)	<*.0001*	3.43 (2.31,5.10)
Model 3	1.00 (Ref)	1.20 (0.33, 4.39)	5.85(1.44,23.81)	9.56 (2.63,34.72)	<*.0001*	2.75 (1.81,4.18)
Bilirubin						
Model 1	1.00 (Ref)	0.72 (0.35, 1.52)	1.03 (0.47,2.23)	2.89 (1.44,5.80)	<*.0001*	2.04 (1.54,2.70)
Model 2	1.00 (Ref)	0.64 (0.27, 1.49)	1.40 (0.56,3.48)	3.22 (1.39,7.45)	*0.0002*	2.25 (1.58,3.20)
Model 3	1.00 (Ref)	0.52 (0.20, 1.35)	1.04 (0.38,2.86)	2.20 (0.88,5.50)	*0.0206*	2.06 (1.40,3.03)

Model 1: matching factors: age at blood collection (±1 year), sex, study center, time of the day at blood collection (±3 hours), fasting status at blood collection (<3,3-6,and >6 hours); among women, additionally by menopausal status (pre-, peri-, and postmenopausal), and hormone replacement therapy use at time of blood collection (yes/no).

Model 2: model 1 + smoking status (never, former, current, missing), baseline (continuous, g/d) and lifetime alcohol intake pattern (never, former, lifetime drinker and drinker only at recruitment), body mass index (BMI, continuous kg/m^2), and physical activity (active, inactive, missing).

Model 3: model 2 + plus hepatitis

Cut-off values for sex-specific quartiles:

GGT- females (<14, <=14-18; >=18-26, >=26 U/L), males (<18.5, >=18.5-26.5,>=26.5- 41.5, >=41.5 U/L)
AST- females (<15, >=15-18; >=18-21, >=21 U/L), males (<16, >=16-19; >=19-24, >=24U/L)
ALP- females (<51, >=51-64; >=64-77, >=77 U/L), males (<49, >=49-58; >=58-68, >=68 U/L)
ALT- females (<11, >=11-15; >=15-20, >=20U/L), males (<15, >=15-19; >=19-26, >=26U/L)
Bilirubin- females (<5.3, >=5.3-6.7; >=6.7-8.5, >=8.5U/L), males (<6, >=6-7.75; >=7.75-10.2, >=10.2 U/L)
* $SD_{\log(GGT)} = 1.03, SD_{\log(ALT)} = 0.68, SD_{\log(AST)} = 0.61, SD_{\log(ALP)} = 0.39, SD_{\log(Bilirubin)} = 0.54$

Fig.1 Dose- response association for log-transformed GGT levels and HCC risk. Adjusted OR (solid line) and 95%CI (dashed lines) were constructed with 3 knots with the reference value set as median.

For IHBC, all of the enzymes but not bilirubin showed a significantly positive association in the multivariable continuous model (model 2: $OR_{(GGT)}$=4.98, 95%CI:1.75-14.17, $OR_{(ALT)}$=2.86, 95%CI:1.26-6.48, $OR_{(AST)}$=3.10, 95%CI:1.04-9.30, $OR_{(ALP)}$=2.31, 95%CI:1.10-4.86) (**Table 3**).

Table 3. The association of serum liver enzyme levels with IHBC risk per 1 SD increase of log-transformed values.

	Model 1 OR (95%CI)	**Model 2 OR (95%CI)**
No. of cases/controls	34/67	34/67
GGT	3.74 (1.73,8.11)	4.98 (1.75,14.17)
ALT	2.55 (1.34,4.86)	2.86 (1.26,6.48)
AST	3.18 (1.24,8.13)	3.10 (1.04,9.30)
ALP	2.24 (1.20,4.20)	2.31 (1.10,4.86)
Bilirubin	1.36 (0.87,2.13)	1.46 (0.85,2.51)

Data are log-transformed and z-standardised to 1SD and a mean of 0.
Model 1: matching factors: age at blood collection (±1 year), sex, study center, time of the day at blood collection (±3 hours), fasting status at blood collection (<3, 3-6, and >6 hours); among women, additionally by menopausal status (pre-, peri-, and postmenopausal), and hormone replacement therapy use at time of blood collection (yes/no).
Model 2: model 1 + smoking status (never, former, current, missing), baseline (continuous, g/d) and lifetime alcohol intake pattern (never, former, lifetime drinker and drinker only at recruitment), body mass index (BMI, continuous kg/m^2), and physical activity (active, inactive, missing).
* $SD_{\log(GGT)} = 0.87, SD_{\log(ALT)} = 0.56, SD_{\log(AST)} = 0.45, SD_{\log(ALP)} = 0.43, SD_{\log(Bilirubin)} = 0.48$

For GBTC, only ALP was significantly associated with higher GBTC risk (model 2: $OR_{(ALP)}$=2.80, 95%CI:1.36-5.76, p_{trend} =0.144 for the highest quartile and $OR_{(ALP)}$=1.59, 95%CI:1.20-2.09 per 1SD increase of log-transformed value) (**Table 4**).

Table 4. The association for GBTC risk with individual liver function biomarkers for quartiles and per 1 SD increase of log-transformed values.

	Quartile 1	Quartile 2 OR (95%CI)	Quartile 3 OR (95%CI)	Quartile 4 OR (95%CI)		Per 1 SD log-transformed OR (95%CI)
No. of cases/controls	29/62	30/72	28/58	44/66		131/259
GGT						
Model 1	1.00 (Ref)	0.96 (0.50,1.85)	1.08 (0.55,2.13)	1.53 (0.80,2.92)	*0.054*	1.20 (0.94,1.53)
Model 2	1.00 (Ref)	0.90 (0.46,1.76)	1.12 (0.55,2.24)	1.50 (0.76,2.98)	*0.056*	1.20 (0.93,1.56)
ALT						
Model 1	1.00 (Ref)	0.96 (0.50,1.84)	1.28 (0.67,2.43)	1.22 (0.61,2.45)	*0.361*	1.10 (0.87,1.40)
Model 2	1.00 (Ref)	0.91 (0.46,1.78)	1.28 (0.66,2.48)	1.16 (0.55,2.44)	*0.430*	1.09 (0.85,1.41)
AST						
Model 1	1.00 (Ref)	0.86 (0.46,1.63)	0.73 (0.36,1.47)	1.07 (0.53,2.16)	*0.542*	1.13 (0.90,1.43)
Model 2	1.00 (Ref)	0.87 (0.46,1.65)	0.70 (0.34,1.42)	1.12 (0.55,2.32)	*0.492*	1.17 (0.92,1.50)
ALP						
Model 1	1.00 (Ref)	1.18 (0.60,2.33)	1.72 (0.85,3.47)	2.71 (1.37,5.36)	*0.183*	1.57 (1.21,2.04)
Model 2	1.00 (Ref)	1.14 (0.56,2.35)	1.70 (0.81,3.56)	2.80 (1.36,5.76)	*0.144*	1.59 (1.20,2.09)
Bilirubin						
Model 1	1.00 (Ref)	0.74 (0.41,1.34)	0.74 (0.38,1.42)	1.05 (0.55,1.99)	*0.768*	1.01 (0.80,1.27)
Model 2	1.00 (Ref)	0.69 (0.37,1.28)	0.71 (0.35,1.44)	1.18 (0.59,2.35)	*0.474*	1.06 (0.83,1.36)

Model 1: matching factors: age at blood collection (±1 year), sex, study center, time of the day at blood collection (±3 hours), fasting status at blood collection (<3, 3-6, and >6 hours); among women, additionally by menopausal status (pre-, peri-, and postmenopausal), and hormone replacement therapy use at time of blood collection (yes/no).
Model 2: model 1 + smoking status (never, former, current, missing), baseline (continuous, g/d) and lifetime alcohol intake pattern (never, former, lifetime drinker and drinker only at recruitment), body mass index (BMI, continuous kg/m^2), and physical activity (active, inactive, missing).

Cut-off values for sex-specific quartiles:
GGT- females (<14, <=14-18; >=18-26, >=26 U/L), males (<18.5, >=18.5-26.5,>=26.5- 41.5, >=41.5 U/L)
AST- females (<15, >=15-18; >=18-21, >=21 U/L), males (<16, >=16-19; >=19-24, >=24U/L)
ALP- females (<51, >=51-64; >=64-77, >=77 U/L), males (<49, >=49-58; >=58-68, >=68 U/L)
ALT- females (<11, >=11-15; >=15-20, >=20U/L), males (<15, >=15-19; >=19-26, >=26U/L)
Bilirubin- females (<5.3, >=5.3-6.7; >=6.7-8.5, >=8.5U/L), males (<6, >=6-7.75; >=7.75-10.2, >=10.2 U/L)
* $SD_{\log(GGT)} = 0.66, SD_{\log(ALT)} = 0.45, SD_{\log(AST)} = 0.30, SD_{\log(ALP)} = 0.31, SD_{\log(Bilirubin)} = 0.42$

3.2 Interactions and sensitivity analyses

For HCC, an interaction was observed for WHR category with GGT category (p=0.013), but not for categories of BMI (p=0.605), HSI (p=0.508), CRP (p=0.079), baseline alcohol intake (p=0.413) or intake pattern (p=0.717), sex (p=0.202), diabetes (p=0.366) or smoking status (0.866). In analyses stratified by WHR category, the strongest HCC risk was observed in crude models for the highest WHR tertile (OR=5.91; 95%CI:2.78-12.55). For ALP, an

interaction was observed between the ALP quartiles and sex (p=0.0004). In the subgroup analyses, in men the highest ALP quartile exhibited a significantly higher risk of HCC (OR= 6.15; 95%CI:2.32-16.31), but this positive association was not significant for women in crude models (OR= 2.71; 95%CI:0.49-14.91). There was an interaction between AST category and alcohol drinking pattern (p=0.027). Due to low numbers of subjects, especially for controls in the former drinkers category, it was not possible to conduct subgroup analyses by alcohol intake pattern. No significant interactions existed for ALT.

No attenuation of the estimates was observed after excluding the first 2/4 years of follow-up. After excluding individuals with positive hepatitis status, prevalent diabetes or suspected NAFLD, the significant outcomes remained. All liver functions biomarkers but bilirubin also remained statistically significant for HCC in the subgroup of individuals with suspected NAFLD (HSI>36) (**not shown**). Interestingly for GBTC, GGT reached the significance only for HSI≤36 subgroup (OR=1.93; 95%CI=1.12-3.33). When abnormal transaminases levels were excluded (ALT <55 U/L and AST <34 U/L), the OR were: 1.85(95%CI:1.00-3.43) for GGT, 1.50(95%CI:0.82-2.74) for ALP and 1.24(95%CI:0.73-2.10) for bilirubin, but remaining numbers of cases/controls were low, i.e. 55/98.

4. DISCUSSION

In this study, all of the measured liver enzymes (GGT, ALT, AST, ALP) and total bilirubin were shown to be positively associated with HCC risk. For IHBC, increases in the enzymes, but not bilirubin, were associated with higher risk. But for GBTC, which includes cancers of gallbladder and extra-hepatic bile ducts, only ALP showed a statistically significant association. Assessment of liver function markers can provide meaningful insight into the clinical condition of the liver, including cholestasis. For this reason, such markers are commonly measured in clinical practice. But, there is on-going discussion as to whether some, such as GGT, are simply risk markers or causally involved. GGT has been related to oxidative stress due to its role in glutathione (GSH) degradation, which may create a cancer-promotive environment in the surrounding tissues [24], and in the liver [25]. There is also evidence showing an elevation of GGT in obese or diabetic patients or those with liver steatosis [26]. These disorders are related to the metabolic syndrome which has itself also been associated with increased HCC risk [5]. A main limitation of our study is the lack of information on liver diseases, such as steatosis or cirrhosis. We attempted to address this in our analyses by controlling for some conditions that may be related to these syndromes (e.g. hepatitis infection status, alcohol intake, BMI, WHR, self-reported history of diabetes or cardiovascular disease, CRP level and calculated hepatic steatosis index). However, we did not observe appreciable confounding for these factors, except for WHR. This may suggest that abdominal obesity and obesity-associated chronic inflammation and oxidative stress [27], may in large part drive this association.

Individually, liver function biomarkers lack specificity and their abnormal measures may be indicative of either hepatic [28] and/or extra-hepatic disorders, but also underlying cholestasis, biliary obstruction or bile duct inflammation [7,29]. Transaminases (AST and ALT), that we identified as positively associated with cancers located within the liver (HCC, IHBC), are mostly of hepatic origin, located in intracellular compartments and typically related to hepatic injury from either hepatitis infection, NAFLD, liver cirrhosis or other causes [29]. ALT is expressed mainly in the liver and is most specific indictor of liver injury [29]. ALP is produced in the membranes of cells lining bile ducts [28] and appears elevated in extrahepatic disease. The positive association of ALP with GBTC risk may be due to chronic inflammation in the bile ducts [29], which then affects liver function, but this requires further testing and assessment. In contrast, GGT is expressed on the cell surface and thus is released to the circulation quicker than the other enzymes in case of hepatic injury [24]. GGT is characterised by a high sensitivity but a low specificity to a particular disease of hepatobiliary tract, however in conjunction with ALP they may suggest chronic inflammation in the bile ducts of hepatic origin [29]. In our study, GGT showed the strongest association with HCC risk out of all the biomarkers assessed, consistent with previous observations on hepatitis infected subjects [14]. As for total bilirubin, although its elevated levels have been seen in hepatic failure in conjunction with other liver biomarkers [7], there is some evidence suggestive of an inverse association with colorectal, lung and breast cancer risk or mortality, likely due to its antioxidant properties [30-32] . In this study we observed a positive association with total bilirubin. The liver is involved in bilirubin metabolism, particularly its conjugation. Hence, it could be assumed that liver dysfunction may result in higher circulating bilirubin concentrations due to some underlying liver disease process [33]. It follows that observed HCC risk associations for bilirubin could then be positive. Indeed, abnormal high bilirubin levels (≥1.5 mg/dL) have been shown in previous studies to be correlated with HCC aggressiveness [34]. Elevated bilirubin levels (1.5-9 mg/dL) with all other liver enzymes being normal or in the absence of liver disease are generally indicative of benign hereditary hyperbilirubinemia (Gilbert Syndrome). This is caused by a reduced activity of the liver enzyme uridine diphosphate-glucuronosyltransferase-1 [35]. In such a case, bilirubin is proposed to have cancer-protective properties, which is currently being investigated in ongoing epidemiological studies.

It is interesting that, as illustrated by the Loess curves, all of the biomarkers were higher in the HCC cases throughout the follow up period suggesting underlying physiological changes related to liver function long before clinical diagnosis. For example, elevation of liver enzymes and bilirubin has been observed in primary sclerosing cholangitis (PSC). PSC may lead to inflammatory damage of bile ducts both inside and outside of the liver, blocking the flow of bile, causing cholestasis and, finally, leading to biliary cirrhosis and liver failure [36]. Clinical guidelines also indicate that a ratio of AST/ALT above 2 may suggest alcoholic liver disease [7], and that GGT/ALP ratio above 2.5 may indicate alcoholic liver cirrhosis [37,28]. In our study having AST/ALT above 2 was associated with

over five-fold higher HCC risk, and for subjects in the upper category for GGT/ALP (>2.5) the HCC risk was eight times higher than for the lower category. Although our observations are in line with what is known about the natural history of HCC development, it is interesting that the individual associations that we observed were maintained after adjustment for patterns of alcohol intake and levels of consumption.

A limitation of this study is a low number of cancer cases, which is a consequence of its prospective design wherein participants were recruited into the cohort prior to cancer development and followed over time, and the low incidence of the diseases studied. We also had no data on liver cirrhosis, NAFLD, NASH or steatosis that may partly mediate the observed associations. The possibility of reverse causation cannot be excluded, as is probably the case for any epidemiological study even those based on prospective designs However, we did carefully assess potential confounding and effect modification by metabolic markers that may be related to hepatic disorders (HSI, hepatitis, prevalent diabetes status, and numerous metabolic biomarkers), as well as level/patterns of alcohol consumption that may be related to development of liver cirrhosis. In sensitivity analyses, we also excluded the first two years of follow up and did not observe any change in the findings, suggesting that the observed alterations are early events. Lastly, all of the information and the biological samples were collected at baseline only and it is possible that dietary and lifestyle factors were modified during the follow up period affecting metabolic processes. The key strengths of our study include its prospective design and collection of detailed lifestyle information enabling adjustment for multiple confounders. We also evaluated the whole spectrum of enzymes and bilirubin, and had available prospectively measured hepatitis infection markers that could give a good picture of hepatic metabolic changes.

In conclusion, this study shows that all individually elevated liver enzymes and total bilirubin are good pre-diagnostic markers of cancers located within the liver (HCC, IHBC), but not in extra-hepatic compartments of hepatobiliary tract (GBTC). The study identified the most HCC-discriminant liver function biomarkers. A clinical validation study based on different patient cohorts with available markers for liver steatosis or fibrosis, i.e. in high risk individuals, would be valuable in order to test the clinical significance of this specific marker for population risk stratification and risk prediction modelling for early diagnosis.

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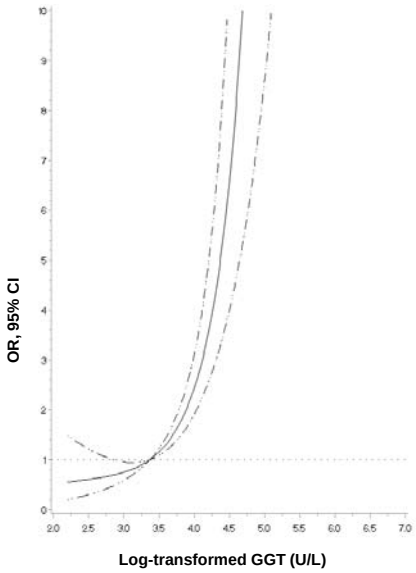

OR, 95% CI
10
9
8
7
6
5
4
3
2
1
0
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
Log-transformed GGT (U/L)

ELECTRONIC SUPPLEMENTARY MATERIAL

Additional statistical analyses

Description of the study population and biomarker levels for different follow up times for HCC risk

Comparisons of the baseline subject characteristics were done using the t-test for continuous variables and the chi-square test for categorical variables. Age-, sex-, fasting status-, hepatitis infection- and smoking-adjusted Spearman's partial correlation coefficients were used to assess the correlations between biomarkers studied (liver enzymes and bilirubin) and selected risk factors among controls. Differences in natural log-transformed biomarker levels according to different strata of variables of interest, stratified by sex, and adjusted for BMI, fasting status and country were assessed by generalized linear model (GLM). To visualize biomarker level over follow up time, Loess curves separate for cases and controls were presented for each enzyme, bilirubin and AFP by the time of follow up. For better visualization, the reference follow up time was assigned to the control subjects based on the follow up time of their corresponding cases.

Additional analyzes- biomarker ratios, liver function score and hepatic steatosis index (HSI)

The analyses for HCC and GBTC were conducted separately for men and women. Ratios of AST/ALT and GGT/ALP, used as supporting indicators of the source of liver disease, were calculated and categorised. The cut-off values for AST/ALT and GGT/ALP categories were 2 and 2.5, respectively, suggesting alcohol-related causes [24]. A liver function score (0-6) was constructed based on our data summarizing the number of abnormal values for five liver function tests (ALT>55 U/L, AST>34 U/L, GGT men>64 U/L, GGT women>36 U/L, ALP>150 U/L, bilirubin>20.5 μmol/L and albumin < 34 g/L; cut-points based directly on assay specifications). For biomarker level above the clinical threshold a mark of 1 was assigned, and 0 if the biomarker level was below this threshold. Two categories were constructed based on this score: a reference category with none biomarkers elevated (score=0) and a category of one and more elevated biomarkers (score 1-6), suggesting possible impaired liver function. Hepatic steatosis index (HSI) score was calculated based on the threshold (≤36>), which is the cut-off value for assessment of possible NAFLD. The association of liver function and HSI score and the ratios categories with risk of HCC, IHBC and GBTC was also calculated using conditional logistic regression models. Due to very low numbers of control participants with enzyme levels above the clinical threshold we were unable to obtain estimates for dichotomised variable of under- above- this threshold of each biomarker.

Discriminatory accuracy of the models- ROC curves

Receiver operating characteristics (ROC) curves were constructed for each individual biomarker of interest and their combinations. In order to reduce bias, the dataset was randomly split into training (60%, 70 cases and 141 controls) and testing (40%, 51 cases, 101 controls) components. Using the training component, the best discriminatory model between cases and controls based on liver function biomarkers was selected according to the area under the ROC curves (AUROC) using a stepwise selection method. In exploratory analyses we further applied the selected models

by time of follow up (cut-offs 1 and 2 years) and in hepatitis free subjects (to exclude potential effect of hepatitis infection on liver enzyme levels). Due to sample size limitation of the training and testing subgroups, the exploratory analyses were conducted on the total number of cases and controls. The models were considered ranked as excellent (AUROC greater than 90%), good (AUROC above 80%), fair (AUROC below 80%), and poor (AUROC less than 70%).

Supplementary Results

Description of the study population

More than 70% of HCC cases had at least one abnormal liver function test whereas the percentages for IHBC and GBTC cases were 46% and 15%, respectively. Observed correlations between liver biomarkers, dietary intakes and anthropometric measures among controls are presented in **Supplementary Table 2**. Liver enzymes correlated with each other, CRP and albumin, but also body fatness measures and alcohol intake. Serum liver enzyme levels according to different strata of variables of interest are presented in **Supplementary tables 3-6**.

Biomarker levels for different follow-up times for HCC

As assessed by visual inspection of Loess curves, the levels of all standardised enzymes were lower for HCC controls than the cases for different follow-up periods of their cases (**Supplementary Fig. 1**). For GGT and AST the case-control difference became more pronounced closer to the time of diagnosis, especially within 2.5 years. Similar but less clear pattern was seen for bilirubin and ALT, but not for AFP, for which the levels were higher only within the 2.5 years prior to HCC diagnosis.

Additional analyses

Adjustment for self-reported history of gallstones did not alter the findings. For both HCC and GBTC, findings were similar in men and women (**Supplementary Tables 7 and 8**). The estimates for the liver function score based on abnormal values of liver enzymes, bilirubin and albumin, as well as for enzyme ratio categories (AST/ALT and GGT/ALP) and HSI, in relation to HCC, IHBC and GBTC are presented in **Supplementary Table 9**. Significantly positive associations for subjects with at least one abnormal liver function test in multivariable model were observed for HCC and IHBC and for the ratios for HCC. No significant associations were observed for HSI in multivariable adjusted models.

Discriminatory accuracy of the models- ROC curves

ROC was used to illustrate changes in model discriminatory accuracy by comparing AUROC among individual biomarkers and their combinations. For individual enzymes in relation to HCC, AUROC was the highest 82% for GGT, AST and ALP followed by AFP (80%), ALT (75%) and bilirubin (73%). For the combination of biomarkers after stepwise selection the best performance was observed for GGT, AST and ALP (AUROC=0.88, 95%CI:0.82-0.94), suggesting that these are most HCC-specific biomarkers. Addition of ALT and/or bilirubin did not further

improve the model. Excellent predictive accuracy was observed within the 1st year of follow up. In this subset, the discriminatory accuracy based on the model including the three enzymes was 93% (AUROC=0.93, 95%CI:0.85-1.00). The exclusion of subjects diagnosed within 1or 2 years of recruitment had lower, but still good discriminatory power (82 and 83%, respectively). In subgroup analyses by HSI, discriminatory power for the combination of the 3 biomarkers was 79 and 86% for those with unlikely and suspected NAFLD, respectively, indicating its good performance in both healthy and higher HCC risk individuals. In hepatitis free individuals, as based on the full complement of cases and matched controls, the accuracy of this model showed a fair performance; model based on GGT, AST and ALP differentiated cases from controls with 78% accuracy. The prediction models performed only fair for IHBC and poor for GBTC for which weaker or no associations were observed.

A biomarker based on the sum of these biomarkers (GGT, ALT and AST) increased HCC risk by 90% (34-134%), even if hepatic, diabetic or participants with suspected NAFLD were only considered. Only exclusion of obese participants attenuated the significance (OR= 4.17; 95%CI: 0.51- 34.20). In subset of obese subjects similar risk increase to the whole cohort was observed (OR= 1.93; 95%CI: 1.41- 2.63). This biomarker could be potentially used to identify which obese individuals should be referred for screening of HCC.

Supplementary tables

Supplementary table 1: Baseline demographic and lifestyle characteristics of HCC (N = 121), intrahepatic (N = 34) and gallbladder and extrahepatic (N = 131) bile duct cancer cases and their matched controls in the EPIC nested case-control study.

Characteristics	Hepatocellular Carcinoma (HCC)		Tumours of the Intrahepatic Bile Ducts (IHBC)		Tumours of the Gallbladder and Extrahepatic Bile Ducts (GBTC)	
	Cases (N = 121)	Matched controls (N = 242)	Cases (N = 34)	Matched controls (N = 67)	Cases (N=131)	Matched controls (N = 259)
Smoking status (N, %) [a]						
Never smoker	33 27.3	104 43.0	16 47.1	29 43.3	57 43.5	123 47.5
Former smoker	39 32.2	91 37.6	9 26.5	14 20.9	38 29.0	81 31.3
Current smoker	47 38.8	46 19.0	8 23.5	20 29.9	35 26.7	53 20.5
No. with gallstones (N, %) [b]						
No	74 61.2	154 63.6	17 50	42 62.7	69 52.7	148 57.1
Yes	15 12.4	24 9.9	7 20.59	3 4.5	13 9.9	13 5.0
Total physical activity (N, %) [c]						
Inactive	11 9.1	33 13.6	5 14.7	6 9.0	20 15.3	37 14.3
Moderately inactive	37 30.6	72 29.8	11 32.4	21 31.3	43 32.8	83 32.0
Moderately active	68 56.2	129 53.3	15 44.1	36 53.7	62 47.3	127 49.0
Alcohol consumption at recruitment, g/d (N, %)						
None	30 24.8	22 9.1	7 20.6	11 16.4	21 16.0	34 13.1
0.1-6	33 27.3	77 31.8	10 29.4	24 35.8	45 34.4	86 33.2
6.1-12	12 9.9	43 17.8	3 8.8	3 4.5	23 17.6	33 12.7
12.1-24	15 12.4	49 20.2	6 17.7	14 20.9	20 15.3	62 23.9
24.1-60	19 15.7	40 16.5	8 23.5	13 19.4	14 10.7	33 12.7
>60	12 9.9	11 4.5	0 0	2 3.0	8 6.1	11 4.2
Drinking history (N, %)						
Never drinker	12 9.9	19 7.9	6 17.6	10 14.9	12 9.2	23 8.9
Former drinker	18 14.9	3 1.2	1 2.9	1 1.5	8 6.1	9 3.5
Drinker at recruitment	21 17.4	52 21.5	5 14.7	10 14.9	36 27.5	78 30.1
Lifetime drinker	70 57.9	168 69.4	22 64.7	46 68.7	75 57.3	149 57.5
Hepatitis B positive (N, %)	16 13.2	5 2.1	0 0	3 4.5	3 2.3	11 4.3
Hepatitis C positive (N,%)	24 19.8	6 2.5	0 0	1 1.5	3 2.3	3 1.2

Missing values were not excluded from percentage calculations, thus the sum of percents across sub-groups may not add up to 100%.
Categorical variables are presented as numbers and percentages.
Continuous variables are presented as mean and standard deviations, except for liver function tests that are presented as medianand5, 95 %).
[a] Number of cases and controls with missing variable value: HCC = 3, IHBC = 5, and EBD = 3.
[b] Number of cases and controls with missing variable value: HCC = 96, IHBC = 32, and EBD = 147.
[c]Total physical activity categories were sex-specific. Number of cases and controls with missing variable value: HCC = 13, IHBC = 7, and EBD = 18.

Supplementary table 2: Spearman correlations for liver biomarkers and selected potential confounders in the controls (n=722).

	GGT (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (U/L)	Albumin (g/L)	CRP (mg/dL)	BMI (kg/m2)	WHR	Alcohol intake at recruitment (g/d)	Coffee intake (g/d)	Energy intake (kcal/d)
GGT (U/L), ρ	1											
p												
AST(U/L) , ρ	0.43	1										
p	<.0001											
ALT (U/L) , ρ	0.58	0.69	1									
p	<.0001	<.0001										
ALP (U/L) , ρ	0.28	0.16	0.29	1								
p	<.0001	0.000	<.0001									
Bilirubin (g/L) , ρ	0.08	0.03	0.07	-0.03	1							
p	0.051	0.427	0.095	0.444								
Albumin (g/L) , ρ	0.13	0.17	0.17	0.08	0.17	1						
p	0.002	<.0001	<.0001	0.045	<.0001							
CRP (mg/dL) , ρ	0.21	0.11	0.18	0.27	-0.11	-0.04	1					
p	<.0001	0.009	<.0001	<.0001	0.011	0.310						
BMI (kg/m^2) , ρ	0.16	0.04	0.28	0.18	-0.08	0.00	0.26	1				
p	<.0001	0.317	<.0001	<.0001	0.064	0.935	<.0001					
WHR, ρ	0.21	0.08	0.27	0.17	-0.03	0.06	0.24	0.50	1			
p	<.0001	0.057	<.0001	<.0001	0.452	0.169	<.0001	<.0001				
Alcohol intake at recruitment (g/d) , ρ	0.24	0.17	0.07	-0.06	0.16	0.09	-0.01	-0.07	-0.03	1		
p	<.0001	<.0001	0.112	0.185	<.0001	0.029	0.820	0.111	0.456			
Coffee intake (g/d) , ρ	0.05	0.00	-0.03	-0.02	-0.08	0.05	-0.03	-0.07	-0.04	0.11	1	
p	0.240	0.954	0.412	0.605	0.060	0.220	0.454	0.099	0.322	0.006		
Energy intake (kcal/d) , ρ	0.01	0.02	0.01	-0.01	0.00	-0.16	-0.07	-0.02	-0.06	0.20	0.08	1
p	0.726	0.670	0.789	0.729	0.935	0.000	0.105	0.678	0.169	<.0001	0.061	

Spearman correlations (ρ) were conducted after adjustment for sex, age, fasting status, prevalent hepatitis infection and cigarette smoking.

Supplementary table 3: Serum gamma-glutamyltransferase (GGT) concentrations (IU/L) according to categories of various predictor variables among controls in the EPIC nested case-control study, 1992-2004 (men, n= 410 and women, n = 312).

Stratum (GGT)	Women (N = 312)					Men (N = 410)				
	N	Geometric mean	95% CI		*P*-value	N	Geometric mean	95% CI		*P*-value
Total	312	20.4	18.6	22.4	---	410	29	26.4	31.7	<0.0001[a]
Age at blood collection, years										
≤55	75	18.3	15.6	21.5	0.190	115	31.9	26.8	38.0	0.533
55-60	69	21.8	18.5	25.8		105	29.7	25.1	35.0	
61-65	113	23.0	20.0	26.6		120	31.4	26.5	37.3	
>65	55	21.0	17.5	25.2		70	29.0	23.6	35.5	
Cigarette smoking [b]										
Never	188	23.7	20	28.1	0.263	123	32.7	28.2	37.8	0.729
Former	62	20.5	18.2	23.1		176	29.2	24.8	34.3	
Current	59	21.8	18.2	26.1		106	28.5	24.1	33.6	
Alcohol consumption at recruitment, g/d										
None	62	20.3	17	24.3	0.118	19	26.5	19.6	36	0.031
0.1-6	129	20.9	18.3	23.9		98	27.5	23.2	32.7	
6.1-12	46	20	16.5	24.2		57	26.6	21.8	32.5	
12.1-24	55	22.5	18.7	27		103	29.1	24.8	34	
24.1-60	20	26.0	19.4	34.7		97	37.3	31.4	44.3	
>60						36	26.5	19.6	36	
Drinking history										
Never drinker	46	21.2	17.1	26.1	0.812	12	25.9	17.3	38.8	0.411
Former drinker	14	18.3	13.2	25.4		7	26.0	15.6	43.2	
Drinker at recruitment	83	21.8	18.1	26.2		84	26.3	20.7	33.4	
Lifetime drinker	169	21.5	18.8	24.6		307	32.4	27.7	38.0	
Body mass index, kg/m^2										
≤ 25	125	19.1	16.6	21.9	0.115	136	26.1	22.4	30.5	0.008
25-30	129	23	20.2	26.3		203	32.1	27.9	37	
≥ 30	58	22.3	18.8	26.5		71	33.9	28	41.2	
Waist to hip ratio [d]										
Tertile 1	87	16.6	14.2	19.4	0.0003	103	23.8	19.9	28.5	<.0001
Tertile 2	122	19.8	17.2	22.9		171	32.1	27.1	38.1	
Tertile 3	82	24.6	21.2	28.6		128	35.2	29.3	42.3	
Coffee consumption, cups/day [e]										
None	19	21.1	16.0	28.0	0.343	21	27.9	20.6	37.8	0.620
<=1	90	22.9	19.2	27.3		110	30.8	26.1	36.4	
>1-3	86	22.6	19.2	26.6		94	34.3	28.7	41.1	
>3-5	65	18.9	15.7	22.7		81	30.5	25.0	37.2	
>5	52	19.1	15.3	24.0		104	25.4	20.8	31.1	
Prevalent diabetes [f]										
No	284	21.1	16	27.9	0.350	363	30.2	26.5	34.4	0.968
Yes	14	24.1	20.6	28.1		27	30.1	22.9	39.4	
Prevalent CVD [g]										
No	150	20.5	17.3	24.3	0.800	223	29.2	25	34.2	0.701
Yes	85	24.2	20.1	29.2		114	30.1	25.4	35.7	
Alanine aminotransferase (ALT), U/L										
≤55	307	20.6	18.6	22.8	<.0001	398	30.1	26.5	34.2	0.035

>55	5	70.6	42.1	118		12	45	30.6	66.2	
Aspartate aminotransferase (AST), U/L										
≤34	303	20.2	18.3	22.4	<.0001	385	28	24.8	31.6	<.0001
>34	9	64.4	44.1	94.2		25	72.7	56.2	94	
C-reactive protein (CRP), mg/L										
≤3	236	19.9	17.9	22.2	0.0002	313	28.1	24.5	32.2	0.002
>3	76	25.9	22	30.5		97	35.7	30.3	42	
Hepatitis B infection										
No	303	21.2	19.1	23.5	0.674	395	30.2	26.6	34.4	0.260
Yes	9	19.4	12.7	29.5		14	37.3	25.4	54.8	
Hepatitis C infection										
No	304	21.1	19	23.5	0.970	405	30.5	26.8	34.6	0.195
Yes	8	21	13.6	32.5		5	20.7	11.5	37.5	
Hepatitis B or C infection [h]										
No	296	21.2	19.1	23.5	0.640	390	30.3	26.6	34.5	0.788
Yes	16	19.7	14.3	27.1		19	31.6	22.6	44.2	

a *P*-value for comparison of ln(GGT) values between men and women, adjusted for fasting status, BMI and country

b Missing N=8

c Missing N = 164

d Missing N = 29 (women=21, men=8). Sex-specific tertiles (men: ≤0.92, 0.92-0.97, ≥0.97; women:≤ 0.77, 0.77-0.84, ≥ 0.84).

e Based on the assumption that 1 cup = 150 mL;

f Self-reported; missing N = 34

g Self-reported; missing N = 150

h The numbers of hepatitis B and C infected do not add up because one person had both, hepatitis B and C infections.

Supplementary table 4: Serum alanine aminotransferase (ALT) concentrations (IU/L) according to categories of various predictor variables among controls in the EPIC nested case-control study, 1992-2004 (men, n= 410 and women, n = 312).

Stratum (ALT)	Women (N = 312)					Men (N = 410)				
	N	Geometric mean	95% CI		*P*-value	N	Geometric mean	95% CI		*P*-value
Total	312	15.1	14.1	16.1	---	410	19.1	17.9	20.3	<0.0001[a]
Age at blood collection, years										
≤55	75	14.5	12.9	16.4	0.5463	115	20.8	18.4	23.5	0.0004
55-60	69	16.7	14.8	19.0		105	20.4	18.1	22.9	
61-65	113	16.3	14.7	18.1		120	19.6	17.4	22.1	
>65	55	14.2	12.4	16.3		70	15.5	13.4	17.9	
Cigarette smoking [b]										
Never	188	17.4	15.4	19.7	0.013	123	19.5	17.6	21.5	0.021
Former	62	14.9	13.7	16.3		176	20.6	18.4	23.0	
Current	59	13.8	12.1	15.8		106	17.5	15.6	19.6	
Alcohol consumption at recruitment, g/d										
None	62	20.3	17	24.3	0.118	19	26.5	19.6	36	0.031
0.1-6	129	20.9	18.3	23.9		98	27.5	23.2	32.7	
6.1-12	46	20	16.5	24.2		57	26.6	21.8	32.5	
12.1-24	55	22.5	18.7	27		103	29.1	24.8	34	
24.1-60	20	26.0	19.4	34.7		97	37.3	31.4	44.3	
>60						36	26.5	19.6	36	
Drinking history										
Never drinker	46	21.2	17.1	26.1	0.812	12	25.9	17.3	38.8	0.411
Former drinker	14	18.3	13.2	25.4		7	26.0	15.6	43.2	
Drinker at recruitment	83	21.8	18.1	26.2		84	26.3	20.7	33.4	
Lifetime drinker	169	21.5	18.8	24.6		307	32.4	27.7	38.0	
Body mass index, kg/m2										
≤ 25	125	13.1	11.9	14.5	<.0001	136	16.1	14.5	18.0	<.0001
25-30	129	16.7	15.2	18.4		203	19.9	18.1	22.0	
≥ 30	58	17.7	15.6	20.0		71	23.4	20.5	26.8	
Waist to hip ratio d										
Tertile 1	87	14.5	12.9	16.2	0.0016	103	23.8	19.9	28.5	<.0001
Tertile 2	122	15.7	14.1	17.4		171	32.1	27.1	38.1	
Tertile 3	82	17.1	15.3	19.1		128	35.2	29.3	42.3	
Coffee consumption, cups/day e										
None	19	16.0	13.1	19.7	0.3729	21	21.8	17.7	26.9	0.0933
<=1	111	15.8	13.9	18.0		144	19.2	17.1	21.5	
>1-3	68	15.3	13.6	17.3		66	19.9	17.6	22.5	
>3-5	114	14.7	12.8	16.8		179	19.1	16.7	21.9	
>5	52	14.6	12.4	17.2		104	17.4	15.2	20.0	
Prevalent diabetes f										
No	284	15.1	13.9	16.3	0.972	363	19.0	17.4	20.8	0.284
Yes	14	15.2	11.9	19.3		27	20.9	17.4	25.1	
Prevalent CVD g										
No	150	15.2	13.4	17.1	0.071	223	18.8	16.9	20.9	0.563
Yes	85	17.1	15.0	19.6		114	19.4	17.3	21.8	
Gamma glutamyltransferase (GGT), U/L										
≤36/64	247	13.9	12.9	14.9	<.0001	228	16.1	14.8	17.6	<.0001

>36/64	65	21.7	19.4	24.2		182	24.0	21.9	26.3	
C-reactive protein (CRP), mg/L										
≤3	236	19.9	17.9	22.2	0.0002	313	28.1	24.5	32.2	0.002
>3	76	25.9	22	30.5		97	35.7	30.3	42	
Hepatitis B infection										
No	303	15.2	14.1	16.5	0.573	395	19.1	17.5	20.9	0.162
Yes	9	16.6	12.2	22.6		14	22.9	17.6	29.7	
Hepatitis C infection										
No	304	21.1	19	23.5	0.970	405	30.5	26.8	34.6	0.195
Yes	8	21	13.6	32.5		5	20.7	11.5	37.5	
Hepatitis B or C infection [h]										
No	296	15.2	14.1	16.4	0.606	390	19.1	17.5	20.9	0.275
Yes	16	16.2	12.8	20.4		19	21.6	17.2	27.1	

a *P*-value for comparison of ln(ALT) values between men and women, adjusted for fasting status, BMI and country

b Missing N=8

c Missing N = 164

d Missing N = 29 (women=21, men=8). Sex-specific tertiles (men: ≤0.92, 0.92-0.97, ≥0.97; women:≤ 0.77, 0.77-0.84, ≥ 0.84).

e Based on the assumption that 1 cup = 150 mL;

f Self-reported; missing N = 34

g Self-reported; missing N = 150

h The numbers of hepatitis B and C infected do not add up because one person had both, hepatitis B and C infections.

Supplementary table 5: Serum aspartate aminotransferase (AST) concentrations (IU/L) according to categories of various predictor variables among controls in the EPIC nested case-control study, 1992-2004 (men, n= 410 and women, n = 312).

Stratum (AST)	Women (N = 312)					Men (N = 410)				
	N	Geometric mean	95% CI		*P*-value	N	Geometric mean	95% CI		*P*-value
Total	312	18.0	17.2	18.8		410	19.8	18.9	20.7	0.0001[a]
Age at blood collection, years										
≤55	75	17.5	16.3	18.8	0.1381	115	20.0	18.3	21.8	0.4559
55-60	69	17.8	16.6	19.2		105	21.0	19.3	22.8	
61-65	113	19.0	17.8	20.3		120	19.8	18.2	21.6	
>65	55	18.6	17.1	20.2		70	19.3	17.5	21.4	
Cigarette smoking [b]										
Never	188	20.0	18.5	21.6	0.011	123	20.8	19.3	22.4	0.012
Former	62	18.1	17.2	19.1		176	20.8	19.2	22.6	
Current	59	17.3	16.0	18.8		106	18.6	17.2	20.2	
Alcohol consumption at recruitment, g/d										
None	62	17.7	16.3	19.1	0.3045	19	18.2	15.6	21.2	0.0133
0.1-6	129	18.4	17.3	19.6		98	19.6	17.9	21.3	
6.1-12	46	18.5	17.0	20.2		57	19.6	17.7	21.6	
12.1-24	55	18.3	16.9	19.9		103	19.8	18.3	21.4	
24.1-60	20	19.2	16.8	22.0		97	22.4	20.6	24.4	
>60						36	21.0	18.5	23.7	
Drinking history										
Never drinker	46	17.5	15.9	19.2	0.378	12	18.2	14.9	22.2	0.394
Former drinker	14	17.6	15.2	20.4		7	17.7	13.7	22.8	
Drinker at recruitment	83	17.7	16.2	19.2		84	19.4	17.2	21.8	
Lifetime drinker	169	18.9	17.8	20.1		307	20.7	19.1	22.3	
Body mass index, kg/m2										
≤ 25	125	18.0	16.9	19.1	0.7783	136	19.0	17.6	20.6	0.0052
25-30	129	18.7	17.6	19.8		203	20.4	19.0	21.9	
≥ 30	58	18.2	16.8	19.7		71	21.8	19.8	24.0	
Waist to hip ratio d										
Tertile 1	87	18.1	16.8	19.5	0.4143	103	20.1	18.4	22.1	0.0433
Tertile 2	122	18.3	17.1	19.5		171	19.5	17.9	21.3	
Tertile 3	82	19.1	17.8	20.5		128	21.2	19.3	23.4	
Coffee consumption, cups/day e										
None	19	18.1	15.9	20.6	0.5022	21	22.5	19.3	26.1	0.0153
<=1	111	19.3	17.8	20.9		144	20.6	18.9	22.3	
>1-3	68	18.3	17.0	19.7		66	20.7	18.9	22.6	
>3-5	114	17.5	16.1	19.0		179	19.7	17.9	21.7	
>5		17.8	16.1	19.8			18.1	16.4	20.0	
Prevalent diabetes f										
No	284	18.4	17.5	19.4	0.018	363	20.2	18.9	21.6	0.575
Yes	14	15.3	13.2	17.8		27	19.5	17.0	22.3	
Prevalent CVD g										
No	150	17.4	16.1	18.8	0.051	223	19.8	18.3	21.4	0.415
Yes	85	18.9	17.4	20.6		114	20.4	18.8	22.2	
Gamma glutamyltransferase (GGT), U/L										

≤36/64	247	17.3	16.5	18.1	<.0001	228	18.2	17.1	19.4	<.0001
>36/64	65	22.5	21.0	24.1		182	23.1	21.5	24.7	
C-reactive protein (CRP), mg/L										
≤3	236	18.0	17.1	18.9	0.042	313	19.9	18.6	21.3	0.274
>3	76	19.4	18.0	20.9		97	20.8	19.1	22.5	
Hepatitis B infection										
No	303	18.3	17.4	19.2	0.058	395	20.2	18.9	21.5	0.442
Yes	9	21.9	18.1	26.5		14	21.7	17.9	26.2	
Hepatitis C infection										
No	304	18.3	17.4	19.2	0.371	405	20.2	18.9	21.5	0.667
Yes	8	20.0	16.4	24.4		5	21.5	16.0	28.9	
Hepatitis B or C infection [h]										
No	296	18.3	17.4	19.2	0.458	390	20.2	18.9	21.5	0.369
Yes	16	19.3	16.7	22.3		19	21.7	18.3	25.6	

a *P*-value for comparison of ln(GGT) values between men and women, adjusted for fasting status, BMI and country

b Missing N=8

c Missing N = 164

d Missing N = 29 (women=21, men=8). Sex-specific tertiles (men: ≤0.92, 0.92-0.97, ≥0.97; women:≤ 0.77, 0.77-0.84, ≥ 0.84).

e Based on the assumption that 1 cup = 150 mL;

f Self-reported; missing N = 34

g Self-reported; missing N = 150

h The numbers of hepatitis B and C infected do not add up because one person had both, hepatitis B and C infections.

Supplementry table 6: Serum Alkaline phosphatase (ALP) concentrations (IU/L) according to categories of various predictor variables among controls in the EPIC nested case-control study, 1992-2004 (men, n= 410 and women, n = 312).

Stratum (ALP)	Women (N = 312)					Men (N = 410)				
	N	Geometric mean	95% CI		*P*-value	N	Geometric mean	95% CI		*P*-value
Total	312	64.1	61.3	66.9	---	410	59.3	56.9	61.9	0.0011
Age at blood collection, years										
≤55	75	55.9	51.1	61.2	0.0047	115	59.9	56.0	64.2	0.9699
55-60	69	66.6	60.7	73.1		105	61.0	57.1	65.1	
61-65	113	70.0	64.6	75.8		120	59.0	55.2	63.0	
>65	55	67.1	60.5	74.3		70	60.7	56.0	65.7	
Cigarette smoking [b]										
Never	188	64.1	58.2	70.5	0.276	123	58.7	55.4	62.2	0.079
Former	62	63.6	59.4	68.0		176	60.0	56.3	63.9	
Current	59	69.0	62.3	76.4		106	63.1	59.1	67.3	
Alcohol consumption at recruitment, g/d										
None	62	67.9	61.4	75.1	0.447	19	65.5	58.0	74.0	0.0232
0.1-6	129	64.7	60.0	69.7		98	62.3	58.1	66.7	
6.1-12	46	62.5	56.0	69.6		57	62.3	57.5	67.4	
12.1-24	55	64.0	57.7	71.0		103	59.0	55.4	62.8	
24.1-60	20	63.3	53.7	74.6		97	57.7	53.9	61.8	
>60						36	60.3	54.6	66.5	
Drinking history										
Never drinker	46	71.1	63.1	80.2	0.400	12	62.8	53.7	73.5	0.500
Former drinker	14	63.0	52.4	75.8		7	69.2	56.7	84.3	
Drinker at recruitment	83	67.2	60.5	74.7		84	60.2	54.9	66.1	
Lifetime drinker	169	63.4	58.7	68.4		307	59.8	56.2	63.6	
Body mass index, kg/m^2										
≤ 25	125	59.3	54.9	64.1	0.0004	136	60.3	56.8	64.1	0.2926
25-30	129	67.3	62.5	72.4		203	59.4	56.2	62.8	
≥ 30	58	72.6	65.8	80.0		71	62.8	58.2	67.7	
Waist to hip ratio [d]										
Tertile 1	87	56.3	51.7	61.4	<.0001	103	58.4	54.3	62.9	0.1524
Tertile 2	122	62.9	58.2	68.0		171	59.5	55.6	63.8	
Tertile 3	82	68.6	63.2	74.4		128	61.4	56.9	66.4	
Coffee consumption, cups/day [e]										
None	19	67.3	57.5	78.7	0.9154	21	61.2	54.4	68.8	0.1906
<=1	111	61.2	55.5	67.6		144	61.1	57.3	65.2	
>1-3	68	68.9	62.9	75.5		66	60.9	56.8	65.2	
>3-5	114	61.7	55.7	68.4		179	63.2	58.6	68.3	
>5		67.9	59.9	76.9			54.4	50.3	58.8	
Prevalent diabetes [f]										
No	284	63.8	60.0	67.9	0.009	363	60.1	57.1	63.2	0.804
Yes	14	82.3	68.2	99.4		27	60.8	54.7	67.6	
Prevalent CVD [g]										
150	62.8	62.8	57.2	69.0	0.023	223	59.4	55.9	63.2	0.415
85	70.7	70.7	63.7	78.4		114	59.8	55.9	63.9	
Gamma glutamyltransferase (GGT), U/L										
≤36/64	247	62.3	58.6	66.2	0.0001	228	57.2	54.2	60.4	<0.0001

>36/64	65	74.5	67.9	81.6		182	64.5	61.0	68.3	
C-reactive protein (CRP), mg/L										
≤3	236	62.5	58.8	66.5	0.001	313	57.9	54.9	61.0	<0.0001
>3	76	72.8	66.4	79.7		97	65.6	61.6	69.8	
Hepatitis B infection										
No	303	64.7	61.0	68.6	0.729	395	60.3	57.4	63.5	0.469
Yes	9	67.4	53.2	85.4		14	57.2	49.2	66.5	
Hepatitis C infection										
No	304	64.8	61.1	68.8	0.672	405	60.4	57.4	63.5	0.370
Yes	8	61.5	48.1	78.6		5	54.4	43.1	68.6	
Hepatitis B or C infection [h]										
No	296	64.7	61.0	68.6	0.855	390	60.4	57.5	63.5	0.266
Yes	16	65.8	55.0	78.7		19	56.3	49.4	64.2	

a *P*-value for comparison of ln(GGT) values between men and women, adjusted for fasting status, BMI and country

b Missing N=8

c Missing N = 164

d Missing N = 29 (women=21, men=8). Sex-specific tertiles (men: ≤0.92, 0.92-0.97, ≥0.97; women:≤ 0.77, 0.77-0.84, ≥ 0.84).

e Based on the assumption that 1 cup = 150 mL;

f Self-reported; missing N = 34

g Self-reported; missing N = 150

h The numbers of hepatitis B and C infected do not add up because one person had both, hepatitis B and C infections.

Supplementary table 7. Association for liver function biomarkers (per 1SD logarithm transformed values) and liver function score with HCC risk separate for men and women.

HCC (n_{cases}=121)	**Model 1**		**Model 2**		**Model 3**	
Men (n_{cases}=82)	OR	95% CI	OR	95% CI	OR	95% CI
GGT	6.09	3.38, 10.94	5.84	3.01,11.33	4.57	2.36,8.84
ALT	2.88	1.97, 4.21	3.01	1.87,4.84	2.10	1.28,3.46
AST	4.08	2.60, 6.41	4.26	2.42,7.50	3.25	1.80,5.88
ALP	4.46	2.71,7.35	4.39	2.33,8.27	4.86	2.21,10.70
Bilirubin	2.12	1.50,2.98	2.59	1.61,4.18	2.13	1.29,3.52
Liver function score[a]	4.25	2.55,7.08	4.00	2.32,6.92	3.35	1.91,5.87
Women (n_{cases}=37)						
GGT	3.03	1.70,5.40	3.03	1.70,5.40	3.68	1.39,9.73
ALT	2.55	1.51,4.32	2.55	1.51,4.32	2.20	0.98,4.92
AST	3.33	1.73,6.43	3.33	1.73,6.43	2.81	1.12,7.01
ALP	2.09	1.30,3.35	2.09	1.30,3.35	2.89	1.25, 6.65
Bilirubin	1.89	1.15,3.09	1.89	1.15,3.09	2.01	0.94,4.32
Liver function score	2.56	1.55,4.23	3.27	1.50,7.15	2.67	1.13,6.32

Model 1: matching factors
Model 2: model 1 adjusted for BMI continuous alcohol at recruitment continuous, drinking history, smoking status, physical activity

Model 3: model 2 + plus hepatitis

[a] Ranges from 0 to 6; based on abnormal liver function tests (ALT>55 U/L, AST>34 U/L, GGT men >64 U/L, GGT women > 36 U/L, ALP > 150 U/L, albumin < 34 g/L, total bilirubin > 20.5 μmol/L; values were provided by the laboratory).

Supplementary table 8. Association for liver function biomarkers (per 1SD logarithm transformed values) and liver function score with GBTC risk separate for men and women.

GBTC (n_{cases}=131)	**Model 1**		**Model 2**	
Men (n_{cases}=58)	OR	95% CI	OR	95% CI
GGT	1.54	0.93,2.54	1.43	0.95,2.13
ALT	1.07	0.49,2.33	0.96	0.64,1.45
AST	1.43	0.52,3.92	1.09	0.77,1.53
ALP	14.84	3.16,69.80	2.33	1.31,4.17
Bilirubin	0.78	0.35,1.72	0.95	0.65,1.40
Liver function score[a]	1.12	0.64,1.95	1.17	0.62,2.20
Women (n_{cases}=73)				
GGT	1.11	0.65,1.91	1.00	0.68,1.47
ALT	1.40	0.69,2.86	1.19	0.84,1.69
AST	1.61	0.49,5.30	1.20	0.82,1.74
ALP	2.21	0.83,5.87	1.33	0.96,1.85
Bilirubin	1.32	0.62,2.83	1.17	0.83,1.66
Liver function score	1.05	0.57,1.95	1.02	0.53,1.94

Model 1: matching factors

Model 2: model 1 adjusted for BMI continuous alcohol at recruitment continuous, drinking history, smoking status, physical activity

[a] Ranges from 0 to 6; based on abnormal liver function tests (ALT>55 U/L, AST>34 U/L, GGT men >64 U/L, GGT women > 36 U/L, ALP > 150 U/L, albumin < 34 g/L, total bilirubin > 20.5 μmol/L; values were provided by the laboratory).

Supplementary table 9. HCC, IHBC and GBTC risk by categories of HSI, liver function score, AST/ALT and GGT/ALP ratios.

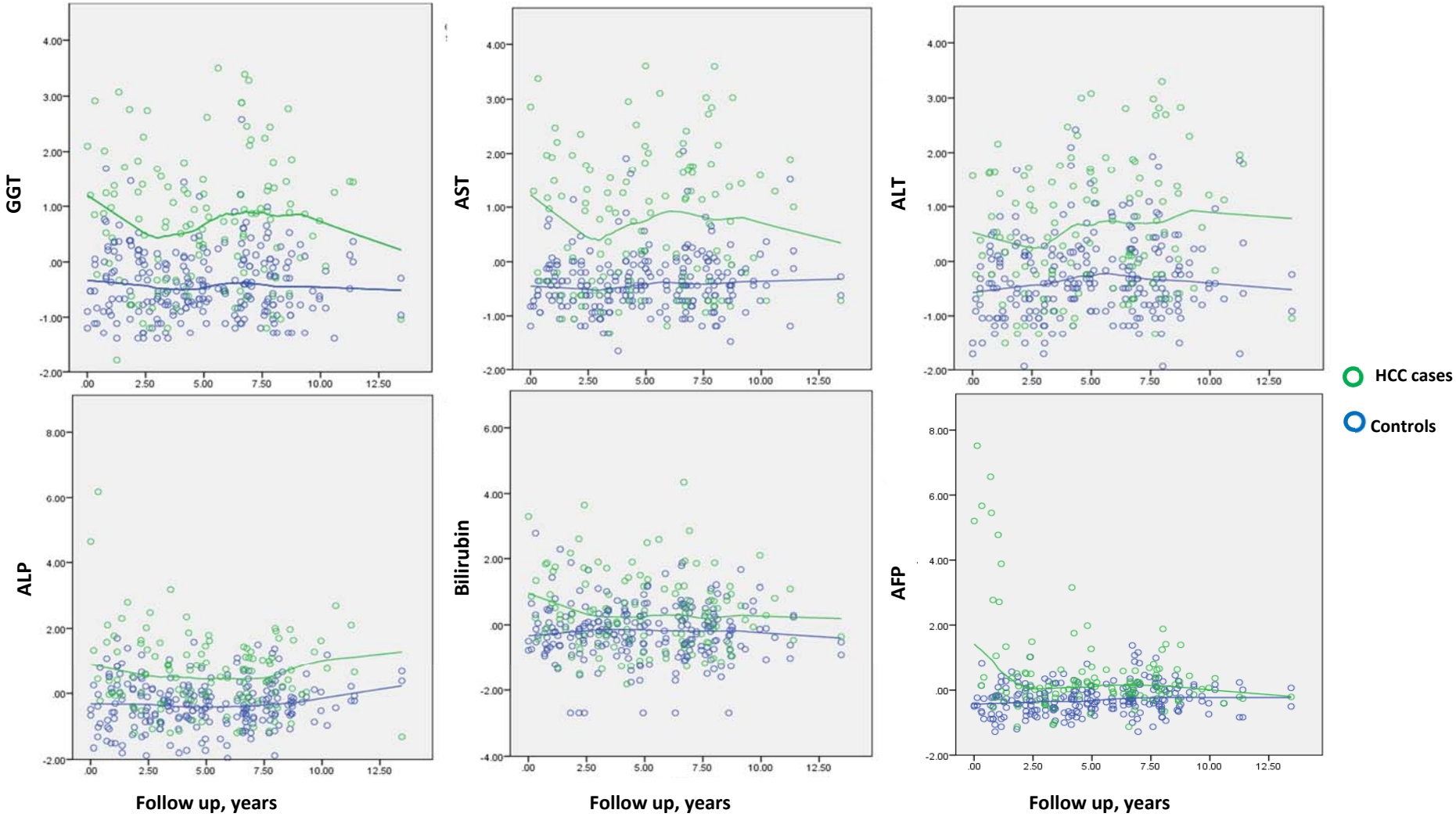
		HSI category [a]		**Liver function score category [b]**		**AST/ALT ratio category [c]**		**GGT/ALP ratio category [d]**	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
HCC (n_{cases}=121)	Model 1	1.20	0.78, 1.86	9.35	5.27, 16.58	4.53	1.59,12.94	10.86	3.17,37.15
	Model 2	0.92	0.46, 1.84	8.61	4.50,16.46	5.36	1.49,19.35	8.68	2.25,33.50
IHBC[d] (n_{cases}=35)	Model 1	2.95	1.18, 7.33	3.53	1.33,9.40	-	-	-	-
	Model 2	2.76	0.60, 12.79	4.45	1.17,16.98	-	-	-	-
GBTC[e] (n_{cases}=131)	Model 1	1.00	0,64, 1.59	0.97	0.52,1.80	1.70	0.54,5.39	0.50	0.06,4.47
	Model 2	0.72	0.37, 140	0.92	0.49,1.73	2.07	0.61,7.01	0.59	0.06,5.79

HCC, hepatocellular carcinoma; IHBC, intrahepatic bile duct cancer; GBTC, gallbladder and biliary tract cancer

[a] Hepatic steatosis index (HSI) = 8 x ALT/AST ratio +BMI (+2, if diabetes; +2; if female), HSI<36 (reference; liver steatosis unlikely) and ≥36 (suspected liver steatosis)
[b] Liver function score is based on abnormal liver function tests (ALT>55 U/L, AST>34 U/L, GGT men >64 U/L, GGT women > 36 U/L, ALP > 150 U/L, albumin < 34 g/L, total bilirubin > 20.5 µmol/L; values were provided by the laboratory). The liver function score was grouped into two categories: 0 (reference) or 1 -6 abnormal liver function tests.
[c] AST/ALT ratio categories: ≤2 (reference) and >2; ratio > 2 may signalise alcoholic liver disease
[d] AST/ALT ratio categories: ≤2.5 (reference) and >2.5; ratio >2.5 may indicate liver cirrhosis
[e] not enough subjects in the upper category for AST/ALP ratio (n=2) and GGT/ALP ratio (n=1)

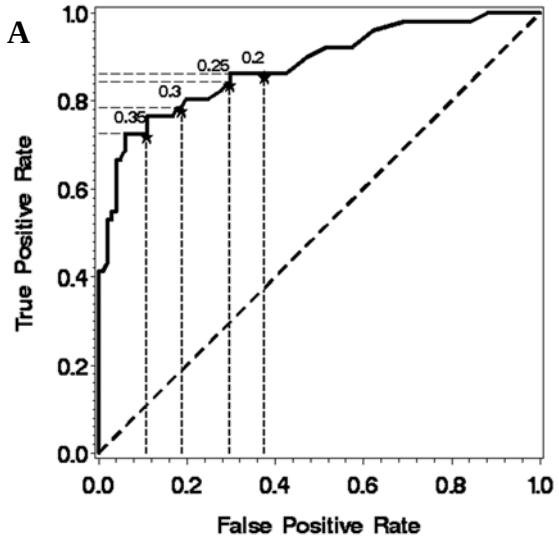
SUPPLEMENTARY FIGURES

Supplementary Fig. 1*:* Loess curves for HCC of log-transformed standardised levels (rescaled to have a mean of 0 and a SD of 1) of liver biomarkers (GGT, AST, ALT, ALP, bilirubin and AFP) by the time of follow up. Follow up time length of the HCC cases was assigned to their controls for better visualization of the data.



Supplementary Fig. 2. Area under receiver operating characteristic curve (AUROC) based only on stepwise selection of liver function biomarkers and corresponding sensitivity and specificity in validation data.

A



B

	AUROC (95% CI)	Sensitivity (true positive rate)	Specificity (false positive rate)
GGT, AST, ALT	0.88 (0.82, 0.94)	0.78	0.81

AUROC displays the discriminatory accuracy for predicting the development of HCC. Independent liver function biomarkers were selected with a stepwise selection method based on c-statistic. Sensitivity indicates the probability of correctly selecting prospective HCC cases. Specificity indicates the probability of correctly selecting controls. Red points indicate the threshold probabilities for which sensitivity and specificity points were selected based on the shortest distance for a perfect marker.

Supplementary Fig. 3 Cubic splines for the dose-response associations for log-transformed levels of ALT, ALP, AST and Bilirubin and HCC risk. Adjusted OR and 95%CI (dashed lines) were constructed with 3 knots with the reference value set as median.