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Inflammatory and Metabolic Biomarkers and Risk of Liver and Biliary Tract Cancer

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Obesity and associated metabolic disorders have been implicated in liver carcinogenesis; however, there are little data on the role of obesity-related biomarkers on liver cancer risk. We studied prospectively the association of inflammatory and metabolic biomarkers with risks of hepatocellular carcinoma (HCC), intrahepatic bile duct (IBD), and gallbladder and biliary tract cancers outside of the liver (GBTC) in a nested case-control study within the European Prospective Investigation into Cancer and Nutrition. Over an average of 7.7 years, 296 participants developed HCC (n = 125), GBTC (n = 137), or IBD (n = 34). Using risk-set sampling, controls were selected in a 2:1 ratio and matched for recruitment center, age, sex, fasting status, and time of blood collection. Baseline serum concentrations of C-reactive protein (CRP), interleukin-6 (IL-6), C-peptide, total high-molecular-weight (HMW) adiponectin, leptin, fetuin-a, and glutamatdehydrogenase (GLDH) were measured, and incidence rate ratios (IRRs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression. After adjustment for lifestyle factors, diabetes, hepatitis infection, and adiposity measures, higher concentrations of CRP, IL-6, C-peptide, and non-HMW adiponectin were associated with higher risk of HCC (IRR per doubling of concentrations = 1.22; 95% CI = 1.02-1.46; P = 0.03; 1.90; 95% CI = 1.30-2.77; P = 0.001; 2.25; 95% CI = 1.43-3.54; P = 0.0005; and 2.09; 95% CI = 1.19-3.67; P = 0.01, respectively). CRP was associated also with risk of GBTC (IRR = 1.22; 95% CI = 1.05-1.42; P = 0.01). GLDH was associated with risks of HCC (IRR = 1.62; 95% CI = 1.25-2.11; P = 0.0003) and IBD (IRR = 10.5; 95% CI = 2.20-50.90; P = 0.003). The continuous net reclassification index was 0.63 for CRP, IL-6, C-peptide, and non-HMW adiponectin and 0.46 for GLDH, indicating good predictive ability of these biomarkers. *Conclusion:* Elevated levels of biomarkers of inflammation and hyperinsulinemia are associated with a higher risk of HCC, independent of obesity and established liver cancer risk factors. (HEPATOLOGY 2014;60:858-871)

Abbreviations: AFP, alpha-fetoprotein; anti-HCV, antibodies to hepatitis C virus; ΔAUC, area under the receiver operating characteristics curve; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; GBTC, gallbladder and biliary tract cancers outside of the liver; GLDH, glutamatdehydrogenase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus infection; HCC, hepatocellular carcinoma; HCV, hepatitis C virus infection; HMW, high molecular weight; IBD, intrahepatic bile duct cancer; ICD-10, the 10th Revision of the International Classification of Diseases; ICD-O-2, the 2nd edition of the International Classification of Diseases for Oncology; IDI, relative integrated discrimination improvement; IL-6, interleukin-6; IR, insulin resistance; IRR, incidence rate ratio; NRI, continuous net reclassification improvement; ROC, receiver operating characteristics curve; sOB-R, soluble leptin receptor; WHtR, waist-to-height ratio.

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Liver cancer is the sixth most commonly diagnosed cancer worldwide, with an estimated 749,700 new cases in 2008; it is also known as one of the most lethal tumors, with 5-year survival rates below 5%.[1] Incidence rates show substantial geographic variation, with higher rates in Southeast Asia and sub-Saharan Africa and lower rates in North America and Western Europe.[1,2] Although in recent years incidence rates have declined in many high-risk areas, they have also increased in low-risk regions.[1,2] The increasing trends of obesity and related metabolic consequences, such as diabetes mellitus, were suggested to have contributed to the higher disease rates in Western societies.[3,4] In this vein, recent estimates, based on data from the European Prospective Investigation into Cancer and Nutrition (EPIC), have suggested obesity to account for 16% of hepatocellular carcinoma (HCC),

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the predominant type of liver cancer.[5] Obesity is characterized by chronic subclinical inflammation and hyperinsulinemia, which may promote hepatocyte injury and steatohepatitis.[6,7] Thus, the adipose tissue-derived proinflammatory cytokine, interleukin-6 (IL-6),[8] which induces secretion of C-reactive protein (CRP) in the liver, may contribute to hepatocarcinogenesis.[9,10] Insulin may stimulate cell proliferation and inhibit apoptosis.[11] Fetuin-a, a plasma protein exclusively secreted by the liver in humans, is up-regulated in liver dysfunction,[12] correlates with key enzymes in glucose and lipid metabolism,[13] and thereby is possibly implicated in hepatic insulin resistance (IR) and fat accumulation.[13] Finally, the adipose tissue-derived hormones, leptin and adiponectin, which are involved in regulating insulin sensitivity and inflammation, may directly or indirectly promote fibrosis, cirrhosis, and, potentially, HCC.[14-17] Despite experimental evidence, only a few prospective epidemiological studies examined the association between inflammatory or metabolic biomarkers and risk of liver cancer in a general (mostly healthy) population.[18-20] However, such information is important because evidence on the relation between obesity-related biomarkers and risk of liver cancer may provide clues for understanding the underlying etiological mechanisms. In addition, identification of biomarkers, which quantify metabolically active adipose tissue beyond anthropometric parameters, may be a complementary approach for defining an "obesity phenotype" relevant for liver cancer. Ultimately, in the general population, these candidate biomarkers may be potentially utilized to refine cancer risk assessment and improve strategies for cancer prevention.[21]

Therefore, we studied prospectively the association of biomarkers of inflammation (CRP and IL-6), hyperinsulinemia (C-peptide), liver fat accumulation (fetuin-A), liver damage (glutamate dehydrogenase; GLDH), and circulating adipokine concentrations (adiponectin and leptin) with risk of HCC, intrahepatic bile duct cancer (IBD) and gallbladder and biliary tract cancers outside of the liver (GBTC) in a nested case-control study within the EPIC cohort.

Patients and Methods

Study Population. The EPIC study was designed to identify nutritional, lifestyle, metabolic, and genetic risk factors for cancer.[22] In brief, between 1992 and 2000 approximately 520,000 apparently healthy men and women from 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the UK), 35-75 years of age, were enrolled. For the present study, the latest dates of complete follow-up for cancer incidence and vital status in the EPIC centers ranged from 2002 to 2006.

Incident cases were defined using both the 10th Revision of the International Classification of Diseases (ICD-10)[23]and the 2nd edition of the International Classification of Diseases for Oncology (ICD-O-2).[24] Respective histologies, methods used for diagnosis of cancer, as well as alpha-fetoprotein (AFP) levels were reviewed to exclude metastatic cases or other types of liver cancers. After exclusion of cases with other types of cancer preceding the index case (n = 18), metastatic cases (n=23), or cases with ineligible histology (n = 31), 125 HCC (including 105 histologically verified cases), 35 IBD, and 137 GBTC incident cases (including 51 cases of gallbladder cancer) were identified, occurring over an average of 7.7 years (Supporting Fig. 1). HCC was defined as tumor in the liver (ICD-10 C22.0 with morphology codes ICD-O-2 "8170/3"and "8180/3"; n = 125). IBD cancer was defined as tumor in the intrahepatic bile ducts (ICD-10 C22.1; all morphology codes except ICD-O-2 "8162/3"; n = 35). GBTC cancers were defined as tumors of the gallbladder (ICD-O-2 C23.9; n = 51], ampulla of Vater (ICD-10 C24.1; n = 28), extrahepatic bile duct cancer (ICD-10 C24.0; n = 33), cancer of overlapping lesion of the biliary tract (ICD-10 C.24.8; n = 1], cancer of the biliary tract, unspecified (C24.9; n = 21), and Klatskin tumors (ICD-10 C22.1 with morphology code ICD-O-2 "8162/3"; n = 3).

Nested Case-Control Study. Using risk-set sampling, 2 controls per case were selected at random from all cohort members who had donated a blood sample, were alive and free of cancer at the time of liver cancer diagnosis of the index case, and were matched to the case on study center, sex, age (±12 months), date of blood collection (±2 months), fasting status (<3, 3-6, or >6 hours), and time of the day (±3 hours) at blood collection. Women were additionally matched according to menopausal status (pre-, peri-[unknown], or postmenopausal) and exogenous hormone use (yes, no, or missing) at blood donation. After 1 IBD case and 2 respective controls were excluded because of missing information on any of the biomarkers, the current analysis was based on 125 HCC, 34 IBD, and 137 GBTC incident cases.

Laboratory Assays. As described in detail elsewhere,[25] blood samples were collected at baseline, processed, divided into heat-sealed straws, and stored in liquid nitrogen freezers (−196°C). Approval was obtained from the ethics review board of the International Agency for Research on Cancer (Lyon, France) and the local review boards pertaining to the participating institutions. Researchers were blinded to the case-control status of the samples. Measurement of biomarkers was performed at the Institute of Clinical

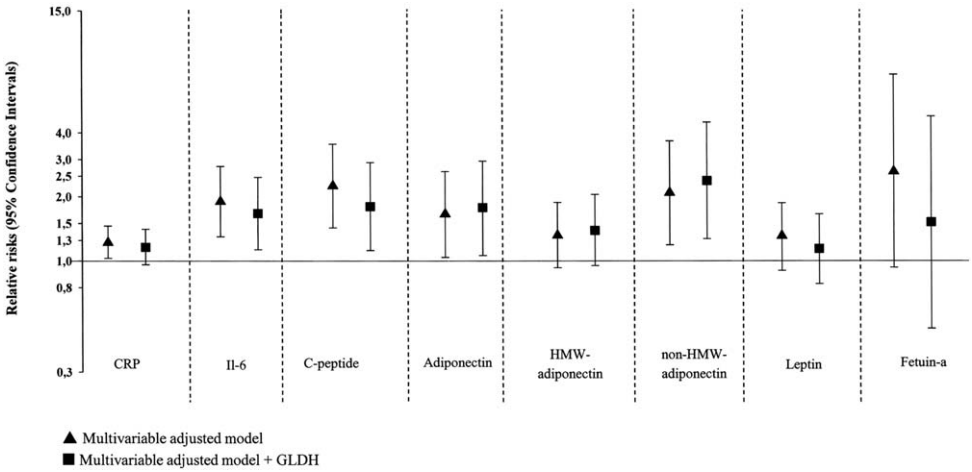

Fig. 1. Association of metabolic biomarkers (continuously per doubling of concentrations) and risk of HCC in the multivariable model[a] before and after adjustment for GLDH as a marker of liver damage. [a]Multivariable model taking into account matching factors: study center; gender; age (±12 months); date (±2 months); fasting status (<3, 3-6, or >6 hours); and time of the day (±3 hours) at blood collection. Women were additionally matched according to menopausal status (pre-, peri- [unknown], or postmenopausal) and exogenous hormone use (yes, no, or missing) at blood donation. Further adjusted for education (no school degree or primary school, secondary school, high school, or missing), smoking (never, former, current, or missing), alcohol at baseline, drinking status at baseline (nondrinker or drinker), diabetes (no, yes, or missing), coffee (g/day), HBsAg/anti-HCV (negative, positive, or missing), BMI, and WHtR adjusted for BMI. *Note:* Analyses were based on overall 293 cases and 581 controls for adiponectin, fetuin-a, and leptin, 293 cases and 577 controls for CRP and GLDH, 277 cases and 549 controls for C-peptide, and 214 cases and 419 controls for IL-6.

Chemistry, University of Magdeburg, Magdeburg, Germany. CRP was measured using a high-sensitivity assay on a Turbidimetrie Modular system (Roche, Mannheim, Germany) with reagent and calibrators from Roche. IL-6 was measured using the ECLIA Modular system (Roche). C-peptide was measured with the Immulite 2000 (Siemens AG, Erlangen, Germany). Adiponectin, leptin, and fetuin-A concentrations were measured using enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH, USA, for adiponectin; Biovendor, Heidelberg, Germany, for leptin and fetuin-a, respectively) with a minimum detectable limit of 0.04, 0.17, and 5.0 ng/mL, respectively. To quantify high-molecular-weight (HMW) adiponectin, serum samples were pretreated with a protease that specifically digests low-molecular-weight and medium-molecular-weight adiponectin. Non-HMW adiponectin was calculated by subtracting HMW adiponectin from total adiponectin. GLDH was measured on a DGKC optimized, 37°C, Modular-System (Roche). Hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (anti-HCV)were measured at the Centre de Biologie République (Lyon, France) using ARCHITECT chemiluminescent microparticle immunoassays (Abbott Diagnostics, Rungis, France), as previously described.[5] For biomarker measurements below the detection limit, we assigned half of the lower limit of detection (Supporting Table 1).

Statistical Analyses. Case-control differences were assessed using the Student paired *t* test, Wilcoxon's signed-rank test, McNemar's test, or Bowker's test of symmetry, where appropriate.[26] Spearman's partial correlation coefficients, adjusted for age at recruitment and sex, were estimated to assess correlations among biomarkers in controls.

Conditional logistic regression was used to investigate the associations between biomarkers and risk of HCC, IBD, and GBTC cancers. Incidence rate ratios (IRRs), estimated from odds ratios as derived from the risk-set sampling design[27] and 95% confidence intervals (CIs), were computed. Associations were assessed on the continuous scale by calculating the relative risks associated with an increase of log-transformed biomarker concentrations by log2, which corresponds to a doubling of the concentrations on the original scale. In addition, associations were assessed on a categorical scale according to tertiles based on the biomarker distributions among controls. *P* values for trends were calculated using median biomarker levels within tertiles among controls. Multivariable conditional logistic regression models were constructed, including *a priori*–chosen covariates, primarily based on existing evidence on liver cancer risk factors.[5] To account for potential liver injury at baseline, all multivariable models were additionally adjusted for GLDH, a marker of liver damage.[28] Multivariable models were also mutually adjusted for the different biomarkers. Restricted cubic spline regression was used to assess nonlinearity using Wald's test.[29] Models were fitted with 5th, 50th, and 95th percentile of the biomarker distribution and median biomarker concentration among the controls were used as a reference.

To assess the predictive capacity of the biomarkers beyond established liver cancer risk factors, we estimated the change in the area under the receiver operating characteristics (ROC) curve (ΔAUC), the relative integrated discrimination improvement (IDI), and the continuous net reclassification improvement (NRI).[30,31] We used SAS's "ROCCONTRAST" statement based on the nonparametric approach of DeLong et al.[32] and a "%reclassification_phreg" macro by Mühlenbruch and Bernigau extended for Cox's regression.[33] The ΔAUC is produced by taking the difference in discrimination metrics between the models with and without the new predictor variable. Similarly, IDI is defined as a difference in discrimination slopes in these models. The relative IDI is calculated as the ratio of IDI over the discrimination slope of the model without the new predictor. The continuous NRI (NRI[>0]) is obtained by the relative increase in the predicted probabilities for subjects who experienced events, compared to the decrease for subjects who did not. We considered NRI(>0) values above 0.6 to indicate strong, those around 0.4 intermediate, and those below 0.2 weak reclassification improvement.[34]

We repeated the analyses after excluding individuals with self-reported diabetes at baseline and those with positive HBsAg/anti-HCV test, high alcohol consumers, and cases that occurred during the first 2 years of follow-up. To reduce potential misclassification of cases, we also explored associations after restricting the analyses on HCC to histologically confirmed cases. We also restricted the analysis of GBTC to gallbladder cancer only. Finally, we repeated all analyses after excluding biomarker measurements, which have fallen below the detection limit (Supporting Table 1). Two-sided *P* values below 0.05 were considered to indicate statistical significance. All statistical analyses were performed using the Statistical Analysis System (SAS) (version 9.2), Enterprise Guide User Interface (version 4.3); SAS Institute, Inc., Cary, NC.

Results

Baseline Characteristics and Demographic Data. As compared to the controls, cases of HCC were more likely to be smokers, have high alcohol and low coffee intake, be less educated, diabetics, and HBsAg/anti-HCV infection positive (Table 1). HCC cases had significantly higher body mass index (BMI), waist circumference, and waist-to-height ratio (WHtR), as well as higher concentrations of CRP, IL-6, C-peptide, adiponectin, leptin, and fetuin-A, compared to controls. GBTC cases had higher WHtR and CRP concentrations, compared to controls. IBD cases had higher BMI, waist circumference, and WHtR, as well as higher leptin and C-peptide concentrations, compared to their controls (Table 1). There was a moderate correlation among the biomarkers (Table 2). GLDH was weakly positively correlated with BMI, leptin, CRP, and C-peptide and inversely with adiponectin (Table 2).

Logistic Regression Analysis. In the final multivariable model—conditioned on matching factors and after adjustment for education, smoking, alcohol, coffee intake, diabetes, hepatitis B virus/hepatitis C virus (HBV/HCV) infection, BMI, and WHtR—higher prediagnostic concentrations of CRP, IL-6, C-peptide, and non-HMW adiponectin were associated with higher risk of HCC (IRR continuously per doubling of concentrations = 1.22; 95% CI = 1.02-1.46; $P = 0.03$; 1.90; 95% CI = 1.30-2.77; $P = 0.001$; 2.25; 95% CI = 1.43-3.54; $P = 0.0005$; and 2.09; 95% CI = 1.19-3.67; $P = 0.01$, respectively; Table 3). Higher levels of GLDH were also significantly associated with a higher risk of HCC (IRR = 1.62; 95% CI = 1.25-2.11; $P = 0.0003$; Table 3). There was no evidence for a nonlinear shape of these associations (Supporting Fig. 2). HMW adiponectin, leptin, and fetuin-A were not significantly associated with HCC risk in the multivariable-adjusted model. When additionally adjusted for GLDH, the associations remained unaltered, except for CRP, which was no longer statistically significant (Fig. 1). Mutual adjustment of biomarkers also did not substantially affect the results, with the exception of non-HMW adiponectin, which was no longer significant after IL-6 was added to the multivariable model (IRR continuously per doubling of concentrations = 1.07; 95% CI: 0.30-3.82; $P = 0.24$).

Higher CRP concentrations were associated with higher risk of GBTC (multivariable-adjusted IRR = 1.22; 95% CI = 1.05-1.42; $P = 0.01$; Table 4). This association remained statistically significant when the analyses were restricted to gallbladder cancer only (IRR = 1.55; 95% CI = 1.15-2.08; $P = 0.003$; Supporting Table 3). Higher levels of GLDH were associated with a higher risk of IBD (IRR = 10.5; 95% CI = 2.2-50.9; $P = 0.003$; Table 5), but not with GBTC (IRR = 1.15; 95% CI = 0.95-1.40; $P = 0.15$; Table 4). The remaining biomarkers were not statistically significantly related to either GBTC or IBD cancers (Tables 4 and 5).

Predictive Capacity of Biomarkers. Addition of CRP, IL-6, C-peptide, and non-HMW adiponectin to the multivariable model significantly increased the AUC for the prediction of HCC from 0.766 to 0.876, whereas addition of the liver damage marker, GLDH, to the multivariable model raised the AUC from 0.769 to 0.813 (Fig. 2). When inflammatory and metabolic biomarkers were added to the model, the IDI was 0.81 and the NRI was 0.63 ($P < 0.0001$),

Table 1. Selected Baseline Characteristics of Incident Cases of HCC, IBD, and GBTC and Their Matched Controls, the European Prospective Investigation into Cancer and Nutrition, 1992-2006

	HCC			GBTC			IBD		
Characteristic	**Cases**	**Controls**	***P* Paired***	**Cases**	**Controls**	***P* Paired***	**Cases**	**Controls**	***P* Paired***
Number	125	250		137	274		34	68	
Female sex, %	32	31.6		56.2	56.2		44.1	44.1	
Age, years, mean (SD)	60.1 (6.6)	60.1 (6.6)	0.42	58.5 (7.5)	58.5 (7.5)	0.94	61.2 (6.3)	61.2 (6.3)	
Liver cancer risk factors									
Smoking status, n (%)									
Never smoker	34 (27.2)	105 (42.0)		62 (45.2)	133 (48.5)		15 (44.2)	30 (44.1)	
Former smoker	41 (32.8)	97 (38.8)	<0.0001	38 (27.7)	84 (30.7)	0.52	10 (29.4)	15 (22.1)	0.83
Current smoker	48 (38.4)	47 (18.8)		36 (26.3)	55 (20.1)		8 (23.5)	19 (27.9)	
Education, n (%)									
No school degree or primary school	52.2	47.8		44.7	47.6		60.6	44.9	
Secondary school	29.2	30.0	0.07	38.7	35.1	0.69	30.3	28.4	0.21
High school	16.0	19.6		16.1	16.1		9.1	25.9	
BMI[†], kg/m^2, mean (SD)	28.1 (5.3)	26.9 (3.9)	0.01	26.9 (4.7)	26.4 (3.9)	0.12	28.3 (3.7)	26.4 (4.2)	0.001
Waist circumference, cm, mean (SD)	97.1 (15.2)	92.6 (11.2)	<0.0001	89.8 (14.3)	88.2 (12.6)	0.07	89.8 (14.3)	88.2 (12.6)	0.01
WHtR, mean (SD)	0.57 (0.08)	0.54 (0.06)	<0.0001	0.54 (0.08)	0.53 (0.07)	0.03	0.54 (0.09)	0.52 (0.07)	0.01
Chronic HBsAg/anti-HCV infection									
No, n (%)	82 (65.6)	231 (92.4)	<0.0001	123 (89.8)	248 (90.5)	0.45	31 (91.2)	63 (92.7)	NA
Yes, n (%)	40 (32)	13 (5.2)		10 (7.3)	16 (5.8)		3 (8.8)	4 (5.9)	
Missing, n (%)	3 (2.4)	6 (2.4)		4 (2.9)	10 (3.7)		–	1 (1.5)	
Diabetes									
No, n (%)	105 (84)	225 (90)		121 (88.3)	242 (88.3)	0.9	32 (94.1)	64 (94.1)	NA
Yes, n (%)	16 (12.8)	16 (6.4)	0.03	9 (6.6)	16 (5.8)		2 (5.9)	4 (5.9)	
Missing, n (%)	4 (3.2)	9 (3.6)		7 (5.1)	16 (5.8)		–	–	
Ethanol intake at baseline (g/day) [‡]									
None to low, n (%)	71 (56.8)	126 (50.4)		76 (55.5)	133 (48.5)	0.22	17 (50)	34 (50)	0.11
Moderate, n (%)	27 (21.6)	97 (38.8)	<0.0001	42 (30.7)	104 (37.9)		10 (29.4)	26 (38.2)	
High, n (%)	27 (21.6)	27 (10.8)		19 (13.9)	37 (13.5)		7 (20.6)	8 (11.8)	
Coffee intake, g/day									
<250	49 (39.2)	78 (31.2)	0.01	46 (33.6)	80 (29.2)	0.09	10 (29.4)	18 (26.5)	0.41
≥250	76 (60.8)			91 (66.4)	194 (70.8)		24 (70.6)	50 (73.5)	
Biomarkers									
CRP, mg/L, median (IQR)	1.6 (0.7-4.3)	1.1 (1.1-3.6)	<0.0001	1.5 (0.9-3.1)	1.0 (0.3-2.1)	0.02	23(1.0-4.5)	1.1 (0.3-3.04)	0.15
IL-6, pg/Ml, median (IQR)	3.2 (1.9-5.2)	1.7 (0.7-2.9)	<0.0001	1.7 (0.8-2.5)	1.5 (0.8-2.3)	0.59	2.9(1.6-4.0)	2.1 (0.8-3.0)	0.25
C-peptide, ng/mL, median (IQR)	2.9 (1.9-5.8)	2.16 (1.4-3.3)	<0.0001	2.1 (1.4-3.6)	2.0 (1.5-3.2)	0.98	2.1 (1.8-3.6)	1.8 (1.4-2.3)	0.0003
Total adiponectin, μg/mL, median (IQR)	5.6 (3.7-7.9)	4.7 (3.3-6.4)	<0.0001	5.2 (3.6-7.9)	5.1 (3.4-7.5)	0.19	4.3 (3.4-8.2)	5.3 (3.9-7.4)	0.42
	2.6 (1.6-4.4)	2.5 (1.6-3.9)	0.0005	2.8 (1.7-4.5)	2.6 (1.6-4.4)	0.33	2.1 (1.3-4.8)	2.7 (1.9-4.3)	0.42

Table 1. *Continued*

Characteristic	HCC			GBTC			IBD		
	Cases	Controls	*P* Paired*	Cases	Controls	*P* Paired*	Cases	Controls	*P* Paired*
HMW adiponectin, μg/mL, median (IQR)	2.7 (2.0-3.6)	2.3 (1.8-2.9)	<0.0001	2.4 (1.8-3.4)	2.4 (1.8-3.1)	0.28	2.2 (1.7-3.0)	2.6 (2.0-3.4)	0.19
Non-HMW adiponectin, μg/mL, median (IQR)	9.2 (5.1-14.6)	6.7 (3.5-14.2)	0.004	8.9 (5.0-161)	9.4 (5.0-17.2)	0.80	9.5 (5.5-20.2)	6.8 (4.1-17.8)	0.02
Leptin, ng/mL, median (IQR)	207.6 (176.0-237.3)	200.6 (175.3-227.3)	0.0006	206.8 (179.9-242.1)	202.6 (175.8-235.1)	0.16	232.9 (188.0-260.2)	217.6 (182.6-249.4)	0.12
Fetuin-a, μg/mL, median (IQR)	124.0 (53.0-206.0)	55.0 (35.5-94.5)	<0.0001	59.0 (36.0-105.0)	50.0 (32.0-88.0)	0.02	84.0 (60.0-188.0)	48.0 (32.0-78.0)	<0.0001
GLDH, μmol/sec/L, median (IQR)									

The analyses were based on overall 293 cases and 581 controls for adiponectin, fetuin-a, and leptin, 293 cases and 577 controls for CRP and GLDH, 277 cases and 549 controls for C-peptide, and 214 cases and 419 controls for IL-6.

**P* values for the difference between cases and controls were determined by the Student paired *t* test for variables expressed as means, Wilcoxon's signed-rank test for variables expressed as medians, and McNemar's test and Bowker's test of symmetry for variables expressed as percentages.

†HBsAg positive when ≥0.05 IU/mL; HCV positive when the ratio of sample relative light units to cut-off relative light units ≥1 in two measurements. There were 17 HCC cases and 7 controls, 4 extrahepatic bile duct cases and 11 controls, and 1 IBD case and 3 controls who were HBsAg positive and 27 HCC cases and 7 controls, 6 extrahepatic bile duct case and 5 controls, and 2 IBD case and 1 controls who were HCV positive.

‡Low intake: men (0 to <10 g/day), women (0 to < 5 g/day); moderate: men (10 to <40 g/day), women (5 to < 20 g/day); high: men (≥40 g/day), women (≥20 g/day).

Abbreviations: SD, standard deviation; IQR, interquartile range; NA, not available.

Table 2. Spearman's Partial* Correlations Among Biomarkers in Control Population (*P* Values in Parentheses)

Obesity Measures and Biomarkers	CRP	IL-6	C-peptide	Adiponectin	HMW Adiponectin	Non-HMW Adiponectin	Leptin	Fetuin-A	GLDH
BMI	0.27 (<0.0001)	0.20 (<0.0001)	0.23 (<0.0001)	−0.27 (<0.0001)	−0.27 (<0.0001)	−0.24 (<0.0001)	0.62 (<0.0001)	0.11 (0.0007)	0.12 (0.002)
WHtR	0.17 (<0.0001)	0.15 (0.002)	0.03 (0.46)	−0.19 (<0.0001)	−0.18 (<0.0001)	−0.19 (<0.0001)	0.25 (<0.0001)	0.15 (0.0004)	0.060.13
CRP	1.00	0.42 (<0.0001)	0.12 (0.006)	−0.24 (<0.0001)	−0.21 (0.0002)	−0.24 (<0.0001)	0.27 (0.002)	0.01 (0.96)	0.17 (<0.0001)
Il-6		1.00	0.05 (0.43)	−0.18 (0.002)	−0.14 (0.002)	−0.21 (<0.0001)	0.22 (0.002)	0.06 (0.21)	0.03 (0.55)
C-peptide			1.00	−0.20 (<0.0001)	−0.20 (<0.0001)	−0.22 (<0.0001)	0.37 (<0.0001)	0.17 (0.0001)	0.13 (0.003)
Adiponectin				1.00	0.95 (<0.0001)	0.87 (<0.0001)	−0.18 (<0.0001)	−0.05 (0.28)	−0.10 (0.01)
HMW adiponectin					1.00	0.70 (<0.0001)	−0.14 (0.001)	−0.05 (0.25)	−0.10 (0.02)
Non-HMW adiponectin						1.00	−0.15 (<0.0001)	−0.06 (0.12)	−0.10 (0.02)
Leptin							1.00	0.14 (0.001)	0.23 (<0.0001)
Fetuin-A								1.00	0.05 (0.19)
GLDH									1.00

Analyses were based on overall 581 controls for adiponectin, fetuin-A, and leptin, 577 controls for CRP and GLDH, 549 controls for C-peptide, and 419 controls for IL-6.

*Adjusted for age at study recruitment and sex.

Table 3. Relative Risks (95% Confidence Intervals) of HCC Across Tertiles of Prediagnostic Biomarker Concentrations in the European Prospective Investigation into Cancer and Nutrition Cohort, 1992-2006

Biomarkers	Tertiles			*P* Value for Linear Trend	Continuously Per Doubling of Biomarker Concentrations	
	T1	T2	T3		RR (95% CI)	*P* Value
Median CRP, mg/L	0.3	1.1	3.2			
Number, cases/controls	33/89	32/68	60/86			
Crude model*	1.00 (Reference)	1.32 (0.74-2.35)	1.98 (1.19-3.28)	0.02	1.25 (1.10-1.42)	0.0007
Multivariable model†	1.00 (Reference)	1.12 (0.54-2.36)	1.41 (0.67-2.96)	0.05	1.22 (1.02-1.46)	0.03
Median IL-6, pg/Ml	0.8	1.8	3.1			
Number, cases/controls	20/73	8/37	64/68			
Crude model*	1.00 (Reference)	1.04 (0.37-2.91)	4.65 (2.05-10.54)	<0.0001	1.99 (1.48-2.66)	<0.0001
Multivariable model†	1.00 (Reference)	0.73 (0.17-3.10)	3.85 (1.31-11.38)	0.004	1.90 (1.30-2.77)	0.001
Median C-peptide, ng/mL	1.2	2.1	3.9			
Number, cases/controls	16/72	32/75	70/83			
Crude model*	1.00 (Reference)	2.10 (1.03-4.22)	5.74 (2.64-12.45)	<0.0001	2.49 (1.77-3.50)	<0.0001
Multivariable model†	1.00 (Reference)	1.30 (0.52-3.24))	3.13 (1.20-8.12)	0.009	2.25 (1.43-3.54)	0.0005
Median total adiponectin, μg/mL	2.9	4.9	8.3			
Number, cases/controls	41/94	33/78	51/74			
Crude model*	1.00 (Reference)	1.06 (0.61-1.82)	1.84 (1.02-3.30)	0.03	1.76 (1.23-2.51)	0.001
Multivariable model†	1.00 (Reference)	1.12 (0.55-2.26)	1.50 (0.69-3.28)	0.29	1.66 (1.04-2.63)	0.03
Median HMW adiponectin, μg/mL	1.3	2.5	4.9			
Number, cases/controls	38/100	39/72	48/74			
Crude model*	1.00 (Reference)	1.44 (0.86-2.42)	1.94 (1.08-3.48)	0.03	1.42 (1.09-1.85)	0.009
Multivariable model†	1.00 (Reference)	1.01 (0.51-1.98)	1.74 (0.78-3.88)	0.15	1.32 (0.93-1.88)	0.12
Median non-HMW adiponectin, μg/mL	1.6	2.4	3.5			
Number, cases/controls	31/89	38/84	56/73			
Crude model*	1.00 (Reference)	1.37 (0.75-2.48)	2.77 (1.49-5.16)	0.001	2.30 (1.45-3.64)	0.0004
Multivariable model†	1.00 (Reference)	1.63 (0.79-3.36)	2.62 (1.17-5.89)	0.02	2.09 (1.19-3.67)	0.01
Median leptin, ng/mL	3.0	7.9	19.8			
Number, cases/controls	36/99	46/76	43/71			
Crude model*	1.00 (Reference)	1.70 (1.00-2.89)	1.92 (1.02-3.63)	0.08	1.35 (1.11-1.64)	0.003
Multivariable model†	1.00 (Reference)	1.46 (0.72-2.95)	1.18 (0.43-3.26)	0.94	1.31 (0.92-1.86)	0.13
Median fetuin-A, μg/mL	164.6	203.3	245.8			
Number, cases/controls	40/83	38/92	47/71			
Crude model*	1.00 (Reference)	0.82 (0.46-1.43)	1.51 (0.83-2.73)	0.18	2.38 (1.05-5.42)	0.03
Multivariable model†	1.00 (Reference)	1.22 (0.59-2.52)	1.54 (0.75-3.14)	0.23	2.63 (0.93-7.49)	0.07
Median GLDH (μmol/sec/L)	27	52.5	118			
Number, cases/controls	20/72	18/81	87/91			
Crude model*	1.00 (Reference)	0.73 (0.34-1.55)	3.84 (2.07-7.13)	<0.0001	1.88 (1.52-2.33)	<0.0001
Multivariable model†	1.00 (Reference)	0.86 (0.34-2.17)	2.83 (1.32-6.08)	0.002	1.62 (1.25-2.11)	0.0003

*The crude model is based on conditional logistic regression, taking into account matching factors: study center; gender; age (±12 months); date (±2 months); fasting status (<3, 3-6, or >6 hours); and time of the day (±3 hours) at blood collection. Women were additionally matched according to menopausal status (pre-, peri- [unknown], or postmenopausal) and exogenous hormone use (yes, no, or missing) at blood donation.

†The multivariable model takes into account matching factors with additional adjustment for education (no school degree or primary school, secondary school, high school, or missing), smoking (never, former, current, or missing), alcohol at baseline, drinking status at baseline (nondrinker or drinker), diabetes (no, yes, or missing), coffee (g/day), HBsAg/anti-HCV (negative, positive, or missing), BMI, and WHtR adjusted for BMI. *P* values for trends were calculated using median biomarker levels within tertiles among controls.

indicating strong reclassification improvement, whereas when GLDH was added to the model, the IDI was 0.24 and the NRI was 0.46 ($P = 0.07$), indicating moderate improvement. Addition of CRP, IL-6, C-peptide, and non-HMW adiponectin to the multivariable model that additionally included AFP significantly increased the AUC for the prediction of HCC from 0.777 to 0.855; GLDH increased the AUC from 0.803 to 0.836 (Fig. 3). When inflammatory and metabolic biomarkers were added to the model, the IDI was 0.43, and NRI(>0) was 0.44 ($P = 0.0004$), indicating moderate reclassification improvement; when GLDH was added to the model, the IDI was 0.10 and the NRI(>0) was 0.21 ($P = 0.29$), indicating weak improvement (Fig. 3).

Sensitivity Analyses. After exclusion of cases that occurred during the first 2 years of follow-up, the associations of the biomarkers with HCC were not substantially changed, except for CRP and non-HMW adiponectin, which were no longer statistically significant (IRR, 1.10; 95% CI 0.88-1.37; $P = 0.12$; and 1.63; 95% CI: 0.86-3.05; $P = 0.12$; Supporting Table 2). The association with CRP was also attenuated and lost statistical significance after excluding cases with

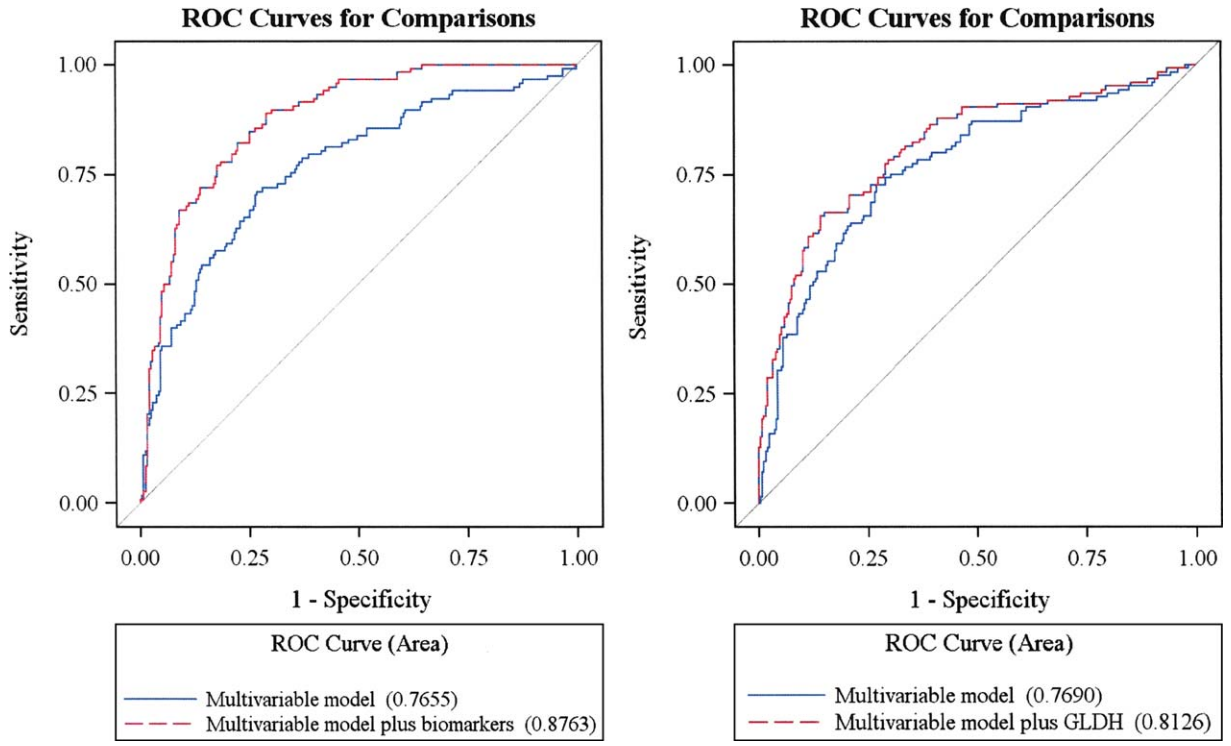

Fig. 2. Predictive ability of inflammatory and metabolic biomarkers[a] and GLDH beyond the multivariable adjusted model[b]. [a]The biomarkers included in the model have been associated with HCC risk. These include CRP, Il-6, C-peptide, and non-HMW adiponectin. [b]Multivariable model taking into account matching factors: study center; gender; age (±12 months); date (±2 months), fasting status (<3, 3-6, or >6 hours); and time of the day (±3 hours) at blood collection. Women were additionally matched according to menopausal status (pre-, peri- [unknown], or postmenopausal) and exogenous hormone use (yes, no, or missing) at blood donation. Further adjusted for education (no school degree or primary school, secondary school, high school, or missing), smoking (never, former, current, or missing), alcohol at baseline, drinking status at baseline (nondrinker or drinker), diabetes (no, yes, or missing), coffee (g/day), HBsAg/anti-HCV (negative, positive, or missing), BMI, and WHtR adjusted for BMI. *Note:* Analyses were based on overall 293 cases and 581 controls for adiponectin, 293 cases and 577 controls for CRP and GLDH, and 277 cases and 549 controls for C-peptide. For this analysis, missing values for IL-6 (33 cases and 72 controls) were substituted with sex- and case-control–specific median values.

underlying HBsAg/anti-HCV infection (IRR, 1.17; 95% CI: 0.95-1.45; $P = 0.12$; Supporting Table 2). After excluding individuals with high alcohol consumption, the main results remained essentially unaltered. Similarly, no substantial changes in risk estimates were seen after exclusion of cases with prevalent diabetes, with the exception of the estimated risk of fetuin-a and HCC, which became statistically significant (IRR, 5.64; 95% CI: 1.60-19.89; Supporting Table 2). Because of the small number of cases, these analyses should be interpreted with caution. Finally, the associations were also not altered when we restricted the analyses on HCC to histologically confirmed cases.

Discussion

In this prospective, nested, case-control study, higher-circulating concentrations of IL-6, CRP, C-peptide, non-HMW adiponectin, and GLDH were significantly associated with higher risk of HCC, independent of established liver cancer risk factors and obesity parameters. Furthermore, our data suggest these biomarkers to be able to improve the risk assessment of HCC, beyond established liver cancer risk factors, therefore suggesting their potential application for identification of individuals at high risk of cancer.

In animal models, it was shown that obesity may promote HCC development through elevated production of tumor necrosis factor and IL-6.[35] In clinical studies, higher levels of IL-6 and CRP have been found among patients with HCC, when compared to controls.[36,37] Chronic inflammation is associated with persistent liver injury and consecutive regeneration, potentially leading to fibrosis and cirrhosis and, consequently, to the development of HCC.[38] Chronic inflammation may also originate from hepatotropic viruses, toxins, or impaired autoimmunity.[39] Mechanisms that link inflammation and liver cancer are not completely understood, but transcription factors of the nuclear factor kappa B family and signal transducer and activator of transcription 3, cytokines such as IL-6, and ligands of the epidermal growth factor receptor family are pivotal players.[39,40] In line with our findings, a recent case-control study nested in a Japanese cohort with 188 HCC cases and 605 controls reported

Table 4. Relative Risks (95% Confidence Intervals) of GBTC Across Tertiles of Prediagnostic Biomarker Concentrations in the European Prospective Investigation Into Cancer and Nutrition Cohort, 1992-2006

	Tertiles				Continuously Per Doubling of Biomarker Concentrations	
Biomarkers	T1	T2	T3	*P* Value for Linear Trend	RR (95% CI)	*P* Value
Median CRP, mg/L	0.3	1.1	3.2			
Number, cases/controls	29/93	47/93	58/81			
Crude model*	1.00 (Reference)	1.61 (0.95-2.74)	2.29 (1.35-3.89)	0.03	1.24 (1.08-1.42)	0.002
Multivariable model†	1.00 (Reference)	1.57 (0.89-2.76)	2.26 (1.26-4.07)	0.009	1.22 (1.05-1.42)	0.01
Median IL-6 (pg/Ml)	0.8	1.8	3.1			
Number, cases/controls	37/96	30/51	32/54			
Crude model*	1.00 (Reference)	1.71 (0.88-3.31)	1.72 (0.83-3.55)	0.15	1.28 (0.97-1.68)	0.08
Multivariable model†	1.00 (Reference)	1.69 (0.81-3.54)	1.19 (0.54-2.62)	0.68	1.15 (0.85-1.56)	0.35
Median C-peptide, ng/mL	1.2	2.1	3.9			
Number, cases/controls	46/86	37/83	44/86			
Crude model*	1.00 (Reference)	0.84 (0.46-1.50)	0.92 (0.50-1.70)	0.96	1.10 (0.82-1.48)	0.50
Multivariable model†	1.00 (Reference)	0.77 (0.41-1.44)	0.77 (0.39-1.52)	0.58	1.09 (0.79-1.51)	0.59
Median total adiponectin, μg/mL	2.9	4.9	8.3			
Number, cases/controls	41/82	36/91	57/95			
Crude model*	1.00 (Reference)	0.50 (0.45-1.41)	1.32 (0.72-2.42)	0.25	1.18 (0.84-1.65)	0.34
Multivariable model†	1.00 (Reference)	0.87 (0.48-1.58)	1.82 (0.93-3.53)	0.04	1.43 (0.98-2.10)	0.07
Median HMW adiponectin, μg/mL	1.3	2.5	4.9			
Number, cases/controls	36/81	39/89	59/98			
Crude model*	1.00 (Reference)	1.00 (0.57-1.77)	1.53 (0.84-2.82)	0.11	1.10 (0.85-1.43)	0.48
Multivariable model†	1.00 (Reference)	1.21 (0.65-2.23)	2.39 (1.20-4.76)	0.009	1.27 (0.94-1.72)	0.12
Median non-HMW adiponectin, μg/mL	1.6	2.4	3.5			
Number, cases/controls	44/89	36/85	54/94			
Crude model*	1.00 (Reference)	0.80 (0.45-1.45)	1.23 (0.67-2.24)	0.41	1.26 (0.83-1.89)	0.28
Multivariable model†	1.00 (Reference)	0.98 (0.52-1.87)	1.75 (0.89-3.42)	0.08	1.54 (0.98-2.42)	0.06
Median leptin, ng/mL	3.0	7.9	19.8			
Number, cases/controls	35/73	52/88	47/107			
Crude model*	1.00 (Reference)	1.25 (0.78-2.16)	0.84 (0.45-1.57)	0.37	0.99 (0.82-1.20)	0.91
Multivariable model†	1.00 (Reference)	1.00 (0.56-1.68)	0.52 (0.24-1.13)	0.05	0.89 (0.70-1.13)	0.35
Median fetuin-A (μg/mL)	164.6	203.3	245.8			
Number, cases/controls	36/92	45/85	53/91			
Crude model*	1.00 (Reference)	1.47 (0.83-2.56)	1.67 (0.93-3.03)	0.09	1.80 (0.79-4.14)	0.16
Multivariable model†	1.00 (Reference)	1.49 (0.83-2.69)	1.42 (0.74-2.70)	0.30	1.41 (0.55-3.60)	0.47
Median GLDH, μmol/sec/L	27	52.5	118			
Number, cases/controls	38/97	44/85	52/84			
Crude model*	1.00 (Reference)	1.41 (0.82-2.42)	1.81 (1.03-3.17)	0.05	1.22 (1.02-1.48)	0.03
Multivariable model†	1.00 (Reference)	1.32 (0.75-2.33)	1.55 (0.86-2.78)	0.17	1.15 (0.95-1.40)	0.15

*The crude model is based on conditional logistic regression, taking into account matching factors: study center; gender; age (±12 months); date (±2 months); fasting status (<3, 3-6, or >6 hours); and time of the day (±3 hours) at blood collection. Women were additionally matched according to menopausal status (pre-, peri- [unknown], or postmenopausal) and exogenous hormone use (yes, no, or missing) at blood donation.

†The multivariable model takes into account matching factors with additional adjustment for education (no school degree or primary school, secondary school, high school, or missing), smoking (never, former, current, or missing), alcohol at baseline, drinking status at baseline (nondrinker or drinker), diabetes (no, yes, or missing), coffee (g/day), HBsAg/anti-HCV (negative, positive, or missing), BMI, and WHtR adjusted for BMI. *P* values for trends were calculated using median biomarker levels within tertiles among controls.

relative risks (95% CI) of 1.94 (0.72-5.51) for CRP and 5.12 (1.54-20.1) for Il-6 for the highest tertile of biomarker distribution versus the lowest after multivariable adjustment.[41] Interestingly, a recent study observed a lower risk of HCC among aspirin users, providing additional means for cancer prevention.[42]

Hyperinsulinemia is often present in patients with chronic hepatitis C and is associated with more advanced HCV-related hepatic fibrosis.[43] Clinical studies suggested that IR is significantly associated with HCC development in patients with chronic HCV infection.[44,45] Our data suggest that C-peptide, as a marker of hyperinsulinemia, is strongly positively associated with risk of HCC and IBD cancer, even after adjusting for HBV/HCV infection and inflammation, giving support to the hypothesis that hyperinsulinemia may increase risk of HCC and IBD cancer. High insulin levels may directly promote cell proliferation and survival through the phosphoinositide 3-kinase/protein kinase B and Ras/mitogen-activated protein kinase pathways.[46,47] Insulin may also interact with leptin and adiponectin (see below).

Adiponectin is involved in the regulation of energy homeostasis, vascular reactivity, inflammation, cell proliferation, and tissue remodeling.[48,49] It primarily acts

Table 5. Relative Risks (95% CIs) of IBD Across Tertiles of Prediagnostic Biomarker Concentrations in the European Prospective Investigation into Cancer and Nutrition Cohort, 1992-2006

	Tertiles			*P* Value for Linear Trend	Continuously Per Doubling of Biomarker Concentrations	
Biomarkers	T1	T2	T3		RR (95% CI)	*P* Value
Median CRP, mg/L	0.3	1.1	3.2			
Number, cases/controls	6/20	7/22	21/25			
Crude model*	1.00 (Reference)	0.81 (0.22-2.96)	3.29 (1.00-10.77)	0.02	1.31 (1.00-1.71)	0.05
Multivariable model†	1.00 (Reference)	0.86 (0.15-5.10)	3.92 (0.78-19.68)	0.05	1.43 (0.97-2.11)	0.07
Median IL-6, pg/Ml	0.8	1.8	3.1			
Number, cases/controls	5/11	3/11	15/18			
Crude model*	1.00 (Reference)	0.47 (0.07-3.29)	1.87 (0.43-8.12)	0.22	1.38 (0.75-2.52)	0.30
Multivariable model†	1.00 (Reference)	NA	NA	NA	3.81 (0.42-34.50?)	0.23
Median C-peptide, ng/mL	1.2	2.1	3.9			
Number, cases/controls	5/24	14/26	12/14			
Crude model*	1.00 (Reference)	2.05 (0.66-6.41)	5.52 (1.24-24.54)	0.03	1.96 (0.94-4.11)	0.07
Multivariable model†	1.00 (Reference)	1.38 (0.36-5.30)	9.89 (1.21-80.45)	0.03	1.86 (0.78-4.42)	0.16
Median total adiponectin, μg/mL	2.9	4.9	8.3			
Number, cases/controls	15/16	8/26	11/25			
Crude model*	1.00 (Reference)	0.32 (0.10-1.01)	0.47 (0.16-1.37)	0.25	0.67 (0.35-1.25)	0.20
Multivariable model†	1.00 (Reference)	0.44 (0.11-1.76)	0.42 (0.11-1.29)	0.23	0.62 (0.27-1.41)	0.25
Median HMW adiponectin, μg/mL	1.3	2.5	4.9			
Number, cases/controls	13/12	10/34	11/21			
Crude model*	1.00 (Reference)	0.32 (0.12-0.89)	0.54 (0.18-1.62)	0.55	0.75 (0.46-1.21)	0.24
Multivariable model†	1.00 (Reference)	0.45 (0.12-1.58)	0.55 (0.14-2.12)	0.52	0.74 (0.41-1.35)	0.32
Median non-HMW adiponectin, μg/mL	1.6	2.4	3.5			
Number, cases/controls	11/15	14/25	9/27			
Crude model*	1.00 (Reference)	0.78 (0.27-2.27)	0.43 (0.13-1.41)	0.15	0.45 (0.14-1.48)	0.19
Multivariable model†	1.00 (Reference)	0.65 (0.17-2.47)	0.32 (0.07-1.42)	0.13	0.52 (0.18-1.50)	0.22
Median leptin, ng/mL	3.0	7.9	19.8			
Number, cases/controls	8/21	11/30	15/16			
Crude model*	1.00 (Reference)	1.25 (0.38-4.07)	3.81 (0.94-15.42)	0.03	1.61 (1.03-2.50)	0.03
Multivariable model†	1.00 (Reference)	1.19 (0.19-7.39)	3.73 (0.36-38.47)	0.14	1.52 (0.75-3.08)	0.25
Median fetuin-A, μg/mL	164.6	203.3	245.8			
Number, cases/controls	8/19	7/16	19/32			
Crude model*	1.00 (Reference)	1.05 (0.32-3.46)	1.50 (0.50-4.53)	0.43	2.29 (0.47-11.23)	0.31
Multivariable model†	1.00 (Reference)	0.43 (0.06-3.13)	1.75 (0.36-8.50)	0.23	2.74 (0.34-22.26)	0.34
Median GLDH, μmol/sec/L	27	52.5	118			
Number, cases/controls	4/22	11/26	19/19			
Crude model*	1.00 (Reference)	4.07 (0.79-20.78)	22.96 (3.08-171.40)	0.002	4.92 (2.01-12.0)	0.001
Multivariable model†	1.00 (Reference)	4.62 (0.62-34.50)	30.70 (2.19-429.60)	0.01	10.5 (2.20-50.90)	0.003

*The crude model is based on conditional logistic regression, taking into account matching factors: study center; gender; age (±12 months); date (±2 months); fasting status (<3, 3-6, or >6 hours); and time of the day (±3 hours) at blood collection. Women were additionally matched according to menopausal status (pre-, peri- [unknown], or postmenopausal) and exogenous hormone use (yes, no, or missing) at blood donation.

†The multivariable model takes into account matching factors with additional adjustment for education (no school degree or primary school, secondary school, high school, or missing), smoking (never, former, current, or missing), alcohol at baseline, drinking status at baseline (nondrinker or drinker), diabetes (no, yes, or missing), coffee (g/day), HBsAg/anti-HCV (negative, positive, or missing), BMI, and WHtR adjusted for BMI. *P* values for trends were calculated using median biomarker levels within tertiles among controls.

Abbreviation: NA, not available.

as an insulin-sensitizing agent,[50] but may also inhibit cancer cell growth,[51] induce apoptosis,[52] and thus be directly implicated in cancer.[53] High adiponectin concentrations have been found to be associated with lower risks of prostate, breast, endometrial, colorectal,[54] and pancreatic cancer.[55] In contrast, in our study, higher adiponectin levels were associated with higher risk of HCC. Whereas this may be surprising, given the beneficial aspects attributed to adiponectin, this is in line with previous studies that found adiponectin positively correlated with hepatic inflammation in patients with chronic liver disease[56] and with HCV-related HCC.[57] We also observed that non-HMW adiponectin, but not HMW adiponectin, was significantly associated with risk of HCC. Furthermore, the association between non-HMW adiponectin and HCC risk was statistically largely accounted for by IL-6. Because low-molecular forms of adiponectin are more closely associated with inflammation compared to high-molecular forms,[58] we speculate whether IL-6 may act as a mediator in these associations.

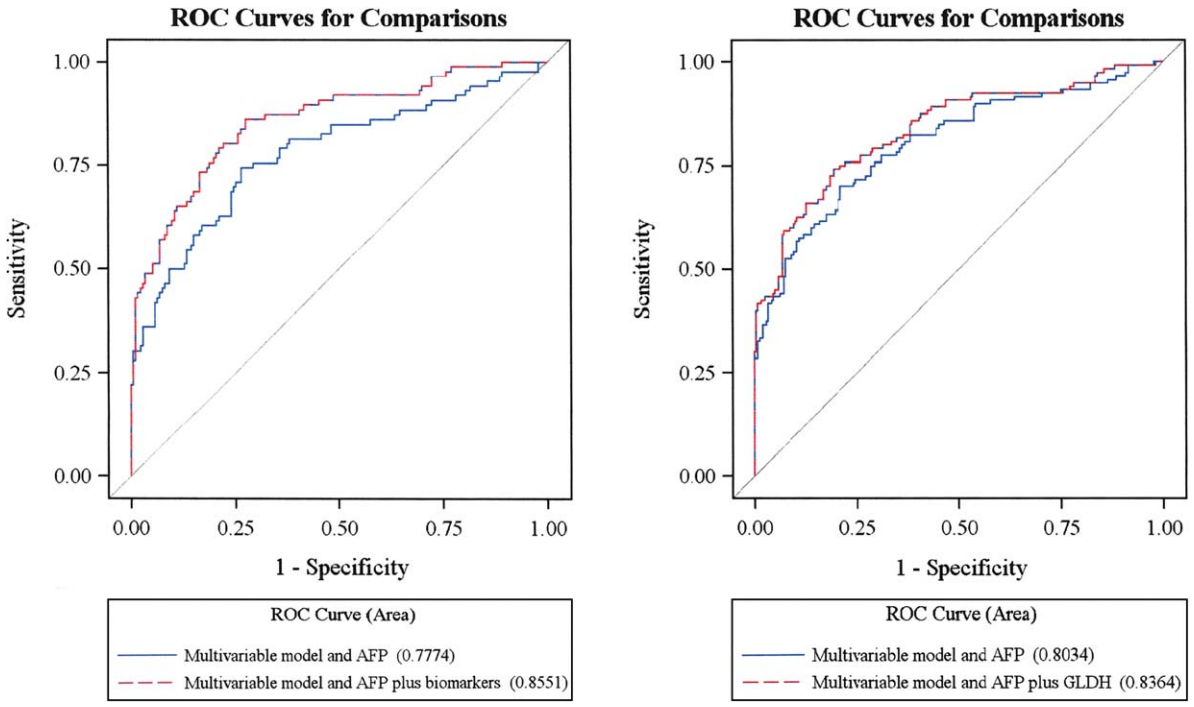

Fig. 3. Predictive ability of inflammatory and metabolic biomarkers and GLDH beyond the multivariable adjusted model and AFP levels. [a]The biomarkers included in the model have been associated with HCC risk. These include CRP, Il-6, C-peptide, and non-HMW adiponectin. [b]Multivariable model taking into account matching factors: study center; gender; age (±12 months); date (±2 months); fasting status (<3, 3-6, or >6 hours); and time of the day (±3 hours) at blood collection. Women were additionally matched according to menopausal status (pre-, peri-[unknown], or postmenopausal) and exogenous hormone use (yes, no, or missing) at blood donation. Further adjusted for education (no school degree or primary school, secondary school, high school, or missing), smoking (never, former, current, or missing), alcohol at baseline, drinking status at baseline (nondrinker or drinker), diabetes (no, yes, or missing), coffee (g/day), HBsAg/anti-HCV (negative, positive, or missing), BMI, and WHtR adjusted for BMI. *Note:* Analyses were based on overall 293 cases and 581 controls for adiponectin, 293 cases and 577 controls for CRP and GLDH, and 277 cases and 549 controls for C-peptide. For this analysis, missing values for IL-6 (33 cases and 72 controls) were substituted with sex- and case-control–specific median values.

Leptin has angiogenic properties, promotes cell proliferation and migration, and interacts with growth factors, all of which could promote tumor growth.[59] Evidence on the role of leptin in non-alcoholic fatty liver disease and cancer risk is controversial, with some studies showing positive associations and others showing null results.[60,61] Our study does not support the hypothesis that leptin levels are associated with liver cancer risk. On the basis of the mechanistic evidence obtained with cultured cells and tumor specimens, we speculate that local, rather than systemic, leptin concentrations may be important for tumor progression. In addition, leptin concentrations in plasma may be affected by the soluble leptin receptor (sOB-R), a marker related to diabetes and cancer risk[62]; however, future studies are warranted to examine whether sOB-R may be specifically related to liver cancer.

Fetuin-a is suggested to provide a link between fatty liver and IR,[63,64] thereby being potentially relevant for liver cancer. In our data, a significant association of fetuin-A with HCC risk was observed only after exclusion of participants with prevalent diabetes at baseline. Although these results may be the outcome of a chance finding, we also speculate on whether mechanisms other than insulin sensitivity may be more relevant here.

High serum GLDH levels occur in liver diseases with hepatocyte necrosis as the predominant event, such as toxic liver damage or hypoxic liver disease, and they have been useful in clinical practice in distinguishing between acute viral hepatitis and acute toxic liver necrosis or acute hypoxic liver disease.[65] In our analysis, higher prediagnostic concentrations of GLDH were associated with higher risks of HCC and IBD. These data suggest that GLDH may be used as a marker of hepatic injury in liver cancer pathogenesis among ostensibly healthy subjects. Interestingly, in our analysis, the associations for IL-6, C-peptide, and non-HMW adiponectin with HCC risk remained statistically significant after adjustment for GLDH, suggesting that prevalent undiagnosed liver injury may not account for these associations.

Strengths of our study include the prospective design and the ability to control for established and putative

liver cancer risk factors and for a variety of circulating metabolic biomarkers. Anthropometric data were mostly measured, rather than self-reported, which reduces the possibility of residual confounding by obesity. Limitations of our study include a relatively small number of incident cases, particularly for the analyses of the inflammatory biomarkers, which limited the possibility to perform detailed stratified and sensitivity analyses. The duration of follow-up was relatively short, and concentrations of biomarkers may have been influenced by preexisting undiagnosed disease. However, our risk estimates did not appreciably change after exclusion of patients who were diagnosed within the first 2 years of follow-up. Because most of our study participants were HBV/HCV negative, our findings are largely valid for HCC of nonviral etiology. Because histologically confirmed and probable HCC cases were included in the analyses, a potential misclassification of liver cancer cases may have occurred. However, when we performed analyses only with histologically confirmed HCC cases, the results did not change. Additionally, because the distal part of the extrahepatic bile duct runs through the head of the pancreas, some of the cancers classified as GBTC may, in fact, be cancers of the pancreas and *vice versa*. Our results are based on single assessments of exposure variables within participants, and biomarkers may be susceptible to short-term variation, which would bias the results toward the null; however, most biomarkers have shown relatively high reliability over time.[66] Because of the low prevalence of established risk factors (i.e. HBV/HCV infection, diabetes, and alcohol consumption) in this study population, we were not able to evaluate whether biomarkers are specifically related to risk among persons with known risk factors, which may be a question of relevance to the clinical practice. We adjusted our analysis for a number of potential risk factors of liver cancer. Nevertheless, we cannot rule out the possibility of residual confounding. Furthermore, given its observational nature, our study does necessarily prove causation.

In conclusion, higher-circulating concentrations of IL-6, CRP, C-peptide, non-HMW adiponectin, and GLDH were significantly associated with higher risk of HCC, independent of established liver cancer risk factors and obesity parameters. Further studies are warranted to investigate the role of these inflammatory and metabolic biomarkers as mediators of the relation between obesity and liver cancer, as well as to explore their potential applications for cancer prevention.

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