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Serum levels of total bile acids are associated with an increased risk of HCC in patients with cirrhosis

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Abstract

Background: Previous studies have reported higher circulating bile acid levels in patients with HCC compared to healthy controls. However, the association between prediagnostic bile acid levels and HCC risk among patients with cirrhosis is unclear.

Methods: We measured total BA (TBA) concentration in serum samples collected from a prospective cohort of patients with cirrhosis who were followed until the development of HCC, death, or last study date. Competing risk proportional hazard–adjusted models were used to estimate the association between tertiles of serum TBA levels and the risk of developing HCC. We quantified the incremental predictive

Abbreviations: AASLD, American Association for the Study of Liver Diseases; BA, bile acid; BMI, body mass index; TBA, total bile acid; THCCC, Texas HCC Consortium.

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value of serum bile acid when added to a previously validated clinical model.

Results: We analyzed data from 940 patients with cirrhosis, of whom 68 patients progressed to HCC during 3406 person-years of follow-up. Higher baseline serum TBA level was significantly associated with an increased risk of developing HCC with an adjusted HR of 3.69 (95% CI = 1.85–7.37) for the highest versus lowest tertile. TBA levels significantly increased predictive ability for progression to HCC at 2 years of follow-up; the c statistic increased from 0.74 to 0.80 ($p < 0.001$). There was evidence for a significant interaction between TBA level and hepatitis C ($p = 0.04$).

Conclusions: In a large prospective cohort study, the prediagnostic serum level of TBAs was associated with a significant increase in the risk of developing HCC among patients with multi-etiology cirrhosis. The TBA-associated risk was additive to that of established demographic and clinical predictors.

Keywords: alcohol, epidemiology, hepatitis C, nonalcoholic steatotic liver disease, risk stratification, liver cancer cirrhosis

BACKGROUND

Experimental studies indicate that bile acids (BAs) are involved in the development and progression of HCC.[1] BAs are involved in the pathogenesis of metabolic dysfunction–associated steatohepatitis, type 2 diabetes, and obesity by activating farnesoid X receptor and Takeda G-protein–coupled receptor-5.[2] BAs also induce the polarization of M2-like macrophages, leading to an immunosuppressive microenvironment that facilitates the immune escape of liver cancer cells. Evidence from rodent studies suggests that BA accumulation alongside suppression of farnesoid X receptor expression may act synergistically in promoting hepatic carcinogenesis.[3–5]

BAs are synthesized in hepatocytes, secreted into the biliary tree, and stored in the gallbladder. Primary BAs are dehydroxylated to secondary BAs by gut microbiota, reabsorbed in the intestine, and conjugated in the liver within an enterohepatic circulation but a small amount of BA spills over into the systemic circulation where they can be measured in the peripheral blood. Several human studies indicated elevated serum or plasma levels of total or specific BA levels in patients with HCC or those who will develop HCC compared to healthy controls.[1,6] For example, a multicenter, prospective cohort study in 23 centers throughout 10 countries in Europe (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom),[1,7] reported that elevated serum levels of glycocholic acid and chenodeoxycholic acid were associated with an increased risk of developing HCC. However, most HCC cases (80%) develop among patients with underlying cirrhosis. Few studies examined the role of BA as a possible HCC risk factor in patients with cirrhosis related to HBV or HCV infections[8,9] and arrived at conflicting findings. Wang et al,[9] in a retrospective cohort study, reported that elevated total serum BA levels were an independent risk factor for HCC in patients with HBV cirrhosis,[1,8] while other mostly cross-sectional studies reported that serum BA (chenodeoxycholic acid and glycocholic acid) was significantly decreased in patients with HCC when compared to those with HCV-related cirrhosis and no HCC.[1,10,11]

There are no longitudinal studies that have examined the role of serum total BA (TBA) as a risk factor for HCC among patients with contemporary cirrhosis, mostly related to metabolic dysfunction–associated steatotic liver disease or cured HCV infection. We therefore examined the association between baseline serum levels of TBAs in a multi-ethnic, multi-etiology prospective cohort of patients with compensated cirrhosis and the risk of developing new HCC. We examined the incremental benefit of adding serum BA as a biomarker[12] to a previously validated HCC predictive model based on demographic and clinical variables.[13] We also explored the interaction between serum BAs and major features of metabolic syndrome (diabetes and obesity) or HCV status in influencing the risk of HCC.

METHODS

Study cohort

We used data from the Texas HCC Consortium (THCCC) cohort where adult patients with cirrhosis were prospectively recruited at 7 liver clinics in 4 cities (Michael E.

DeBakey veterans affairs Medical Center and Baylor St. Luke's Medical Center in Houston; University of Texas Southwestern Medical Center, Parkland Health, Baylor Scott & White Hospitals in Dallas and Fort Worth; Doctor's Hospital at Renaissance in McAllen; and Texas Liver Institute in San Antonio).[14,15] Blood samples were collected at each study visit, and processed locally into serum aliquots which were batch shipped to a central laboratory for long-term storage. We analyzed samples from the first 940 unique subjects with BA samples consecutively recruited between December 2016 and February 2020 who were followed through December 31, 2023. The research was conducted in accordance with both the Declarations of Helsinki and Istanbul. Research was approved by the IRB at Baylor College of Medicine, and written consent was given by all patients to conduct this research.

We followed the PRoBE (prospective-specimen collection, retrospective-blinded-evaluation) guidance for conducting biomarker studies.[16] Cirrhosis diagnosis was based on predefined criteria for liver histology, radiology, elastography, or serum biomarkers.[11] Patients with uncontrolled hepatic decompensation, history of HCC, or nonhepatic cancers were excluded. All participants had a negative liver imaging at baseline, and those with current or prior diagnosis of liver cancer were excluded. Our primary outcome was incident HCC, defined as tumors occurring at least 1 month after the index visit to minimize the risk of prevalent HCC, but we also conducted a sensitivity analysis excluding patients diagnosed with HCC within the first 6 months of follow-up. HCC was defined according to AASLD criteria,[17,18] including histological or radiological diagnosis using characteristic appearance (arterial enhancement and delayed washout of contrast) on triple-phase computerized tomography or MRI (LI-RAD 5) or those with suspicious lesions (LI-RAD 4) that were reviewed in multidisciplinary tumor boards and treated as HCC. We included 7 patients with LI-RADS 4 (suspicious for HCC) but not LI-RADS 3 (intermediate probability of HCC), given the high risk of prevalent HCC in the former but not the latter.[19,20]

The underlying etiological risk factors for cirrhosis were defined using the treating physician's determination and lab tests. Hepatitis C was defined by the presence of a positive result of HCV RNA or hepatitis C–related treatment. While some patients fell into multiple etiological categories, we categorized them for the purpose of this analysis, into mutually exclusive categories in the following order: hepatitis C (active/cured), alcohol-associated liver disease, autoimmune disorders, metabolic dysfunction–associated steatotic liver disease, and Others.

Patient serum samples were thawed on ice and then diluted in ddH_2O. TBA concentration in μmol/L was measured using Abcam's Total Bile Acids Assay Kit (ab239702). Reagents were prepared according to the manufacturer's protocol. Diluted samples and a standard curve were added in duplicates to a 96-well flat-bottom plate. ddH_2O was used for background wells. TBA probe mix was added and then plates were incubated at 37°C for 10 minutes. TBA reaction mix (cycling buffer, enzyme mix, and NADH) was added to each well, except for the background which received only cycling buffer and NADH. The absorbance was read at 405 nm, every 10 minutes for 60 minutes while keeping the plate at 37°C. All samples were tested at multiple dilutions in duplicates. We estimated the inter-coefficient of variance by testing 2 aliquots of the same sample in 50 randomly selected samples.

Statistical methods

We used Fine-Gray competing risk models to estimate HRs and their corresponding 95% CIs for associations with HCC risk. These models accounted for the competing risks of liver transplantation and death in assessing HCC risk.[21] The main exposure variable was TBA examined as tertiles (lowest tertile as the referent group). We ran univariate and multivariate models, with variables included in the final multivariable model if they were statistically significantly associated (ie, $p < 0.05$) with HCC risk in the univariate analysis. We examined the association between BA level and risk of HCC overall, and stratified by HCV status (HCV active or cured vs. non-HCV), obesity (< 30 vs. ≥ 30 kg/m^2), and diabetes (yes vs. no). Potential interactions were assessed by fitting the interaction term between TBA and HCV, obesity, diabetes, and gender into the model. The significance of the interaction terms was assessed using its associated p value in the model. We then explored the joint association of TBA level and those 3 factors with the risk of developing HCC. All analyses were conducted using SAS version 9.4.

The incremental predictive value of TBA was evaluated by comparing the predictive performance of a model that added TBA to that of the previously validated base model. The base model was developed using 2 prospective cirrhosis cohort studies, THCCC and Houston Veterans Administration Cirrhosis Surveillance Cohort,[13,22] and externally validated in a separate prospective veterans affairs cohort. The risk index computed by the base model is:

$0.0399 \times \text{age} + 0.5617 \times \text{gender (male} = 1) + 0.9023 \times \log10 \text{ alpha-fetoprotein} + (-1.2631) \times \log10 \text{ platelets} + (-0.3357) \times \log10 \text{ ALT} + (-0.5859) \times \text{albumin} + 0.0252 \times \text{BMI} + 0.2816 \times \text{smoking (past/current} = 1) + 0.2446 \times \text{alcohol (current heavy} = 1) + (0.3596) \times \text{alcohol (other} = 1) + 0.4611 \times \text{etiology (HCV active/cured} = 1)$. We used the C-index to evaluate the model performance of the additional factor, specifically TBA, in comparison to the base model above for HCC risk at 1 and 2 years of follow-up.

RESULTS

We analyzed data from 940 patients with cirrhosis, of whom 68 patients progressed to develop incident HCC during 3406 person-years of follow-up (annual HCC incidence rate, 2.0%). The mean age of the cohort was 60.3 years (SD: 9.6); 34.5% were women, 51.4% non-Hispanic White, 25.1% Hispanic, and 21.2% non-Hispanic Black (Table 1). The underlying etiology for cirrhosis was metabolic dysfunction–associated steatotic liver disease (278, 29.57%), active HCV (136, 14.47%), cured HCV (276, 29.36%), alcohol-associated liver disease (142, 15.11%), or HBV (12, 1.28%). The majority had a Child-Pugh class A (71.44%), followed by Child-Pugh class B (25.33%) and Child-Pugh class C (3.22%). At baseline, about 30.5% of the patients used furosemide or spironolactone; 26.3% had controlled ascites; 40.3% had varices; and 17.5% had controlled encephalopathy.

For the overall study cohort, the median TBA serum level was 14.58 μmol/L (IQR: 4.24–42.88 μmol/L). Most patients had TBA value <100 μmol/L (91.2%). The inter-coefficient of variance for TBA assay was 0.97. The median (IQR) levels of TBA in patients who developed HCC was 33.9 μmol/L (10–55 μmol/L) compared with 13.4 μmol/L (4.0–42.1 μmol/L) in patients who did not develop HCC ($p = 0.001$).

Baseline serum levels of TBA were associated with an increase in HCC risk in both unadjusted and adjusted models (Table 1). The adjusted models (Supplemental Table S1, http://links.lww.com/HC9/B53) contained variables with $p < 0.05$ in the unadjusted analyses (race/ethnicity, smoking status, alcohol drinking status, diabetes, BMI, and alpha-fetoprotein). Compared with the lowest tertile, the highest tertile of TBA was associated with almost 4-fold significantly increased risk for HCC (adjusted HR = 3.69; 95% CI = 1.85–7.37).

TBA for HCC risk prediction

TBA modeled as tertiles had a significant additive effect on the total discrimination of the predictive model for 1- and 2-year HCC risk as measured by c-index over other demographic and clinical variables (Table 4). Adding TBA to the base HCC risk predictive model[13] increased the c-index for predicting 1-year risk for HCC from 0.76 (0.59–0.93) to 0.83 (0.72–0.95) ($p = 0.03$). Similarly, adding TBA to the base risk model increased the c-index for predicting 2-year risk for HCC from 0.74 (0.62–0.85) to 0.80 (0.71–0.89) ($p = 0.001$).

Stratified analyses

There was a significant interaction between TBA level and HCV status (p value = 0.044). In analyses stratified by HCV status, the highest tertile of TBA was associated with almost 6-fold higher risk for HCC compared with the lowest tertile (adjusted HR = 5.92; 95% CI = 2.27–15.44) Table 2. The magnitude of this association was lower among patients with cirrhosis without HCV (tertile 3 vs. tertile 1, adjusted HR = 2.61; 95% = 0.94–7.23). Among patients with obesity (BMI ≥ 30), the highest tertile of TBA was associated with almost 4-fold higher risk for HCC (adjusted HR = 3.85; 95% = 1.62–9.15). The magnitude of this association was lower among patients with cirrhosis with BMI <30 (adjusted HR = 2.75; 95% = 0.86–8.83). Conversely, the association between TBA and HCC was stronger among patients with cirrhosis without diabetes (tertile 3 vs. tertile 1; adjusted HR = 6.05; 95% = 1.41–25.92) than among those with diabetes (tertile 3 vs. tertile 1; adjusted HR = 3.00; 95% = 1.35–6.70) (Table 2). However, despite the suggested differences in point estimates, the tests for interactions between TBA level and diabetes, obesity, or gender were not significant (p values = 0.83, 0.51, and 0.99, respectively).

Joint associations

In analyses examining joint associations and testing for interaction, we found suggestive evidence between TBA and HCV status with respect to HCC risk (Tables 3 and 4). Patients with both high TBA level (tertile 3) and HCV had over 5 times the risk of developing HCC compared to those with a low level of TBA (tertile 1) and no HCV (adjusted HR = 5.85, 95% CI = 2.00–17.08). No other groups had statistically significantly different HCC risk compared to those with a low level of TBA (tertile 1) and no HCV (Table 3).

We also observed suggestive evidence of joint associations between TBA and obesity and TBA and diabetes with respect to HCC risk (Table 3). Compared to those with a low level of TBA (tertile 1) and without obesity, individuals with the highest TBA tertile and obesity had an 8-fold higher risk of HCC (adjusted HR = 8.32, 95% CI = 2.75–25.12). Similarly, compared to individuals with a low level of TBA (tertile 1) and no diabetes, those with the highest TBA tertile with diabetes had ~18-fold higher risk of developing HCC (adjusted HR = 17.64, 95% CI = 4.14–75.20).

In an exploratory analysis, we calculated the Youden index to determine the optimal cutoff point of TBA for predicting HCC development. The optimal cutoff was determined to be 29 μmol/L for the whole study population (n = 940), which was very close to the cutoff for the upper tertile category presented in the main analysis (32 μmol/L). The optimal cutoff was 68 μmol/L in the non-HCV group (n = 528).

DISCUSSION

In this prospective study, we found that higher prediagnostic serum levels of TBA were associated with a

TABLE 1 Baseline demographic, lifestyle, and clinical variables for the study cohort (n = 940)

Variable	Categories	Controls (n = 872)	Cases (n = 68)	*p* value of chi-square test
Total bile acids (μmol/L)	Tertile 1 (0.03–6.38)	303 (34.75)	11 (16.18)	0.0005
	Tertile 2 (6.44–32.20)	292 (33.49)	21 (30.88)	
	Tertile 3 (32.44–237.79)	277 (31.77)	36 (52.94)	
Age	< 55	211 (24.2)	10 (14.71)	0.2021
	55– < 65	373 (42.78)	32 (47.06)	
	65+	288 (33.03)	26 (38.24)	
Gender	Male	569 (65.25)	47 (69.12)	0.5183
	Female	303 (34.75)	21 (30.88)	
Race and ethnicity	NH-White	438 (50.23)	45 (66.18)	0.0547
	NH-Black	190 (21.79)	9 (13.24)	
	Hispanic	222 (25.46)	14 (20.59)	
	Others	22 (2.52)	—	
Smoking	Never	334 (38.3)	23 (33.82)	0.5012[a]
	Former	346 (39.68)	25 (36.76)	
	Current	187 (21.44)	20 (29.41)	
	Missing	5 (0.57)	—	
Alcohol drinking	Never	241 (27.64)	18 (26.47)	0.2426[a]
	Others (past [no, current not heavy, or declined])	577 (66.17)	42 (61.76)	
	Current heavy	49 (5.62)	8 (11.76)	
	Missing	5 (0.57)	—	
Hypertension	Yes	437 (50.11)	35 (51.47)	0.8295
Dyslipidemia	Yes	281 (32.22)	27 (39.71)	0.2055
Diabetes	Yes	372 (42.66)	42 (61.76)	0.0022
Body mass index (kg/m^2)	< 30	453 (51.95)	25 (36.76)	0.0368
	≥ 30	413 (47.36)	43 (63.24)	
	Missing	6 (0.69)	—	
AFP (ng/ml)	< 10	785 (90.02)	55 (80.88)	0.0194[a]
	10–20	60 (6.88)	6 (8.82)	
	> 20	24 (2.75)	7 (10.29)	
	Missing	3 (0.34)	—	
Etiology	No HCV	495 (56.77)	33 (48.53)	0.1874
	HCV (active or cured)	377 (43.23)	35 (51.47)	
Child class	A	598 (68.58)	45 (66.18)	0.4392[a]
	B	207 (23.74)	21 (30.88)	
	C	28 (3.21)	1 (1.47)	
	Missing	39 (4.47)	1 (1.47)	
Bilirubin	Tertile 1	268 (30.73)	21 (30.88)	0.0450[a]
	Tertile 2	317 (36.35)	15 (22.06)	
	Tertile 3	286 (32.8)	32 (47.06)	
	Missing	1 (0.11)	—	
MELD	Tertile 1	277 (31.77)	16 (23.53)	0.4346
	Tertile 2	313 (35.89)	30 (44.12)	
	Tertile 3	266 (30.5)	21 (30.88)	
	Missing	16 (1.83)	1 (1.47)	

[a]Fisher exact test.
Abbreviations: AFP, alpha-fetoprotein; NH, non-Hispanic.

TABLE 2 Unadjusted and adjusted HR (95% CI) for associations with BA and risk of HCC, both overall and stratified by diabetes, obesity, and HCV

Total bile acids (μmol/L)			
Overall[a]	Tertile 1 (0.025–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	1.94 (0.94–4.02)	1.78 (0.83–3.81)
	Tertile 3 (32.440–237.794)	3.59 (1.84–7.00)	3.69 (1.85–7.37)
Diabetes[b]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	1.46 (0.61–3.50)	1.29 (0.51–3.29)
	Tertile 3 (32.44–237.79)	3.49 (1.62–7.49)	3.00 (1.35–6.70)
No diabetes[b]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	4.57 (1.00–20.82)	4.23 (0.94–19.09)
	Tertile 3 (32.44–237.79)	6.32 (1.46–27.44)	6.05 (1.41–25.92)
Obesity[c]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	1.21 (0.46–3.17)	1.20 (0.45–3.17)
	Tertile 3 (32.44–237.79)	3.36 (1.49–7.59)	3.85 (1.62–9.15)
No obesity[c]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	3.29 (1.05–10.33)	2.85 (0.86–9.43)
	Tertile 3 (32.44–237.79)	3.24 (1.02–10.27)	2.75 (0.86–8.83)
HCV[a]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	2.46 (0.91–6.62)	2.00 (0.68–5.88)
	Tertile 3 (32.44–237.79)	7.45 (2.99–18.54)	5.92 (2.27–15.44)
No HCV[a]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	1.46 (0.50–4.26)	1.57 (0.50–4.99)
	Tertile 3 (32.44–237.79)	2.11 (0.79–5.66)	2.61 (0.94–7.23)
Male[a]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	1.67 (0.75–3.75)	1.41 (0.60–3.35)
	Tertile 3 (32.44–237.79)	3.69 (1.78–7.66)	3.48 (1.59–7.59)
Female[a]	Tertile 1 (0.03–6.38)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.44–32.20)	4.73 (0.58–38.83)	5.08 (0.56–46.21)
	Tertile 3 (32.44–237.79)	6.51 (0.85–50.20)	5.97 (0.77–46.11)

[a]Adjusted for race/ethnicity, smoking, alcohol drinking, diabetes, obesity, and AFP.
[b]Adjusted for race/ethnicity, smoking, alcohol drinking, obesity, and AFP.
[c]Adjusted for race/ethnicity, smoking, alcohol drinking, diabetes, and AFP.
Abbreviation: AFP, alpha-fetoprotein.

significant increase in the risk of developing HCC among patients with multi-etiology cirrhosis. This effect was more pronounced among patients who also had HCV. Serum TBA levels also had a significant additive effect on the total discrimination value of a previously validated HCC predictive model that includes alpha-fetoprotein and other key demographic and clinical variables.

Although TBA was associated with HCC risk overall, we found that the magnitude of risk was not uniform among all patients with cirrhosis. In particular, a high level of TBA may act synergistically with established HCC risk factors, including HCV, obesity, or diabetes, to confer an especially high risk of HCC. For all analyses of joint associations, we found that HCC risk was highest among those jointly exposed to both high TBA levels and the other risk factor. For HCV, HCC risk among patients with cirrhosis with HCV and high TBA was greater than the summed individual effects of each of these factors, suggesting biological interaction. Although in healthy individuals, fasting plasma concentrations of individual BAs and TBAs were reported to be higher in men than in women,[23,24] we did not observe an interaction between TBA and gender.

The findings help to clarify the association between BA and HCC risk in patients with cirrhosis. Previous studies examining the association between BA and HCC were mostly cross-sectional in design and/or compared BA between patients with HCC and healthy controls.[8,25–30] Cross-sectional studies could discern the temporal association between BA levels and HCC occurrence. Comparison to health controls is less relevant to HCC prevention or biomarker development because most cases of HCC develop in cirrhosis.

Our findings are suggestive of an etiological link between TBA and HCC and possible utility as a predictive biomarker for better risk stratification. It is possible that elevated TBA is part of the metabolic abnormalities that underlie the severity of these

TABLE 3 Unadjusted and adjusted HR for independent and joint associations with TBA on the risk of HCC

Variable	Categories	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
TBA (μmol/L)[a]	Tertile 1 (0.025–6.383)	1.00 (Reference)	1.00 (Reference)
	Tertile 2 (6.440–32.198)	1.94 (0.94–4.02)	1.78 (0.83–3.81)
	Tertile 3 (32.440–237.794)	3.59 (1.84–7.00)	3.69 (1.85–7.37)
TBA and BMI[b]	Tertile 1, not obese	1.00 (Reference)	1.00 (Reference)
	Tertile 1, obese	2.42 (0.71–8.24)	2.36 (0.68–8.17)
	Tertile 2, not obese	3.30 (1.06–10.34)	3.14 (0.99–9.98)
	Tertile 2, obese	2.92 (0.91–9.38)	2.70 (0.80–9.07)
	Tertile 3, not obese	3.23 (1.02–10.28)	3.58 (1.13–11.36)
	Tertile 3, obese	8.24 (2.89–23.53)	8.32 (2.75–25.12)
TBA and diabetes[c]	Tertile 1, no diabetes	1.00 (Reference)	1.00 (Reference)
	Tertile 1, diabetes	4.77 (1.04–21.95)	5.10 (1.09–23.89)
	Tertile 2, no diabetes	4.48 (0.98–20.42)	3.61 (0.79–16.53)
	Tertile 2, diabetes	6.91 (1.54–31.06)	6.44 (1.40–29.71)
	Tertile 3, no diabetes	6.33 (1.45–27.66)	5.08 (1.17–22.01)
	Tertile 3, diabetes	16.53 (3.91–69.89)	17.64 (4.14–75.20)
TBA and HCV[a]	Tertile 1, no HCV	1.00 (Reference)	1.00 (Reference)
	Tertile 1, HCV	0.78 (0.24–2.53)	0.98 (0.29–3.33)
	Tertile 2, no HCV	1.51 (0.52–4.40)	1.65 (0.56–4.89)
	Tertile 2, HCV	1.88 (0.65–5.40)	1.94 (0.65–5.78)
	Tertile 3, no HCV	2.13 (0.80–5.70)	2.65 (0.99–7.06)
	Tertile 3, HCV	5.76 (2.14–15.49)	5.85 (2.00–17.08)
TBA and gender[a]	Tertile 1, Female	1.00 (Reference)	1.00 (Reference)
	Tertile 1, Male	2.90 (0.37–22.71)	2.66 (0.33–21.59)
	Tertile 2, Female	4.83 (0.59–39.47)	4.97 (0.60–41.28)
	Tertile 2, Male	4.77 (0.62–36.62)	3.73 (0.47–29.67)
	Tertile 3, Female	6.73 (0.88–51.63)	6.64 (0.88–50.06)
	Tertile 3, Male	10.80 (1.45–80.74)	9.84 (1.29–75.43)

[a]Adjusted for race/ethnicity, smoking, alcohol drinking, diabetes, obesity, and AFP.
[b]Adjusted for race/ethnicity, smoking, alcohol drinking, diabetes, and AFP.
[c]Adjusted for race/ethnicity, smoking, alcohol drinking, obesity, and AFP.
Abbreviations: AFP, alpha-fetoprotein; BMI, body mass index; TBA, total bile acid.

etiological risk factors. Elevated TBA may also reflect the severity of the underlying liver disease; however, in our study, they added to the HCC prediction conferred by conventional measures of liver disease severity (eg, serum levels of platelets and albumin). Therefore, TBA may contribute an independent predictive effect related to dimensions of pathophysiology not captured by other variables. This effect may have clinical implications, especially if our results are confirmed in other cohorts. We expect that progress in HCC risk stratification will occur from adding biomarkers reflecting different biological domains (eg, genomics and radiomics) in progression to HCC. Biomarkers that require additional cost and testing need to significantly add to the base model to maintain the eventual applicability and cost-effectiveness. Furthermore, to establish a TBA clinical test, reference standardization would be important, and the range in our study was large.

TABLE 4 Performance characteristics of predictive models for HCC risk stratification in patients with cirrhosis

	Overall, N = 868 (63 HCC)		1-year, N = 868 (11 HCC)		2-year, N = 868 (26 HCC)	
	C-index	*p*	C-index	*p*	C-index	*p*
Risk score[a]	0.70 (0.62–0.77)		0.76 (0.59–0.93)		0.74 (0.62–0.85)	
Bile acid (3 groups: tertile) + risk score	0.74 (0.67–0.81)	0.05	0.83 (0.72–0.95)	0.03	0.80 (0.71–0.89)	0.001

[a]Contains the following predictors: age, gender, AFP, platelets, ALT, albumin, BMI, smoking, alcohol, and HCV status.
Abbreviations: AFP, alpha-fetoprotein; BMI, body mass index.

TBA can be a potential biomarker for HCC risk stratification. The incremental gain in discrimination over a robust externally validated model containing demographic and clinical variables was significant. However, progress in risk stratification is likely to occur in small steps, from adding biomarkers reflecting different biological domains (eg, genomics and transcriptomics) in progression to HCC.[22,31] A guiding principle is that variables that typically require additional cost and testing need to significantly add to the base model to maintain the eventual applicability and cost-effectiveness.

The study had several limitations. We did not fractionate TBA into individual components, and it is possible that values or ratios of these components would convey different associations with HCC risk. Despite the large cohort examined, the number of HCC was relatively limited (n = 68). Lastly, we did not examine serial changes in TBA, and it is possible that repeat measurements of TBA would have conveyed different information.

Our study also has several strengths and the use of the THCCC cohort as its data source, including the prospective recruitment and data collection, the longitudinal follow-up, the accurate and complete definition of cirrhosis and HCC, and the contemporary profile of underlying risk factors (mostly alcohol and metabolic dysfunction–associated liver disease, and low proportions of active HCV and HBV[12]).

In summary, we found in a prospective cohort study that higher prediagnostic serum TBA levels were associated with an increased future risk of developing HCC among patients with cirrhosis. Furthermore, the TBA-associated risk was incremental to that of established demographic and clinical predictors. Further studies should examine serum BA as biomarkers for HCC risk stratification.

AUTHOR CONTRIBUTIONS

Study concept and design: Hashem B. El-Serag, Salma Kaochar, and Fasiha Kanwal. Acquisition of data: Hashem B. El-Serag, Aaron P. Thrift, Saira Khaderi, Amit G. Singal, Sumeet K. Asrani, Jorge A. Marrero, Michelle Luster, Abeer Al-Sarraj, Emad Salem, Michael Scheurer, and Fasiha Kanwal. Analysis and interpretation of data: Hashem B. El-Serag, Aaron P. Thrift, Hao Duong, Jing Ning, Hannah Powell, Kinza Rizwan, Omar Najjar, Christopher Amos, Michael Scheurer, Salma Kaochar, and Fasiha Kanwal. Drafting of manuscript: Hashem B. El-Serag, Aaron P. Thrift, Hao Duong, Michelle Luster, Salma Kaochar, and Fasiha Kanwal. Critical revision of the manuscript: Hashem B. El-Serag, Aaron P. Thrift, Hao Duong, Jing Ning, Saira Khaderi, Amit G. Singal, Sumeet K. Asrani, Jorge A. Marrero, Hannah Powell, Kinza Rizwan, Omar Najjar, Christopher Amos, Michelle Luster, Abeer Al-Sarraj, Emad Salem, Michael Scheurer, Jagpreet Chhatwal, Salma Kaochar, and Fasiha Kanwal. Statistical analysis: Hashem B. El-Serag, Aaron P. Thrift, Hao Duong, Jing Ning, Christopher Amos, Salma Kaochar, and Fasiha Kanwal. Study supervision: Hashem B. El-Serag, Michael Scheurer, Salma Kaochar, and Fasiha Kanwal. Final approval for publication: Hashem B. El-Serag.

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CONFLICTS OF INTEREST

Saira Khaderi consults for AstraZeneca. Amit G. Singal consults for Genentech/Roche, AstraZeneca, Bayer, Eisai, Merck, Exelixis, FujiFilm Sciences, Glycotest, Exact Sciences, and Boston Scientific. Jorge A. Marrero received grants from Glycotest. Jagpreet Chhatwal owns stock in Value Analytics Labs. The remaining authors have no conflicts to report.

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REFERENCES

1. Colosimo S, Tomlinson JW. Bile acids as drivers and biomarkers of hepatocellular carcinoma. World J Hepatol. 2022;14:1730–8.
2. Sun L, Cai J, Gonzalez FJ. The role of farnesoid X receptor in metabolic diseases, and gastrointestinal and liver cancer. Nat Rev Gastroenterol Hepatol. 2021;18:335–47.
3. Kim I, Morimura K, Shah Y, Yang Q, Ward JM, Gonzalez FJ. Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. Carcinogenesis. 2007;28:940–6.
4. Kong B, Zhu Y, Li G, Williams JA, Buckley K, Tawfik O, et al. Mice with hepatocyte-specific FXR deficiency are resistant to spontaneous but susceptible to cholic acid-induced hepatocarcinogenesis. Am J Physiol Gastrointest Liver Physiol. 2016;310: G295–302.
5. Attia YM, Tawfiq RA, Gibriel AA, Ali AA, Kassem DH, Hammam OA, et al. Activation of FXR modulates SOCS3/Jak2/STAT3 signaling axis in a NASH-dependent hepatocellular carcinoma animal model. Biochem Pharmacol. 2021;186:114497.
6. Hsu JK, Lin CL, Liu CJ, Huang CJ, Yu MW. Identification of serum metabolite profiles associated with the risk of developing hepatitis B-related hepatocellular carcinoma using metabolomics. J Hepatol. 2017;66:S247.
7. Stepien M, Keski-Rahkonen P, Kiss A, Robinot N, Duarte-Salles T, Murphy N, et al. Metabolic perturbations prior to hepatocellular carcinoma diagnosis: Findings from a prospective observational cohort study. Int J Cancer. 2021;148:609–25.
8. Thomas CE, Luu HN, Wang R, Xie G, Adams-Haduch J, Jin A, et al. Association between pre-diagnostic serum bile acids and hepatocellular carcinoma: The Singapore Chinese Health Study. Cancers (Basel). 2021;13:2648.
9. Wang H, Shang X, Wan X, Xiang X, Mao Q, Deng G, et al. Increased hepatocellular carcinoma risk in chronic hepatitis B patients with persistently elevated serum total bile acid: A retrospective cohort study. Sci Rep. 2016;6:38180.
10. Xiao JF, Varghese RS, Zhou B, Nezami Ranjbar MR, Zhao Y, Tsai TH, et al. LC-MS based serum metabolomics for identification of hepatocellular carcinoma biomarkers in Egyptian cohort. J Proteome Res. 2012;11:5914–23.
11. Ressom HW, Xiao JF, Tuli L, Varghese RS, Zhou B, Tsai TH, et al. Utilization of metabolomics to identify serum biomarkers for hepatocellular carcinoma in patients with liver cirrhosis. Anal Chim Acta. 2012;743:90–100.
12. Qi L, Chen Y. Circulating bile acids as biomarkers for disease diagnosis and prevention. J Clin Endocrinol Metab. 2023;108: 251–70.
13. Kanwal F, Khaderi S, Singal AG, Marrero JA, Asrani SK, Amos CI, et al. Risk stratification model for hepatocellular cancer in patients with cirrhosis. Clin Gastroenterol Hepatol. 2023;21: 3296–304.
14. Feng Z, Marrero JA, Khaderi S, Singal AG, Kanwal F, Loo N, et al. Design of the Texas Hepatocellular Carcinoma Consortium Cohort Study. Am J Gastroenterol. 2019;114:530–2.
15. El-Serag HB, Kanwal F, Feng Z, Marrero JA, Khaderi S, Singal AG. Risk factors for cirrhosis in contemporary hepatology practices—Findings from the Texas Hepatocellular Carcinoma Consortium Cohort. Gastroenterology. 2020;159:376–7.
16. Pepe MS, Feng Z, Janes H, Bossuyt PM, Potter JD. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: Standards for study design. J Natl Cancer Inst. 2008;100:1432–8.
17. Singal AG, Llovet JM, Yarchoan M, Mehta N, Heimbach JK, Dawson LA, et al. AASLD practice guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma. Hepatology. 2023;78:1922–65.
18. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology (Baltimore, Md). 2018;68:723–50.
19. Kanneganti M, Marrero JA, Parikh ND, Kanwal F, Yokoo T, Mendiratta-Lala M, et al. Clinical outcomes of patients with Liver Imaging Reporting and Data System 3 or Liver Imaging Reporting and Data System 4 observations in patients with cirrhosis: A systematic review. Liver Transpl. 2022;28:1865–75.
20. Dunn C, Lin B, Rich NE, Patel MS, Gopal P, Singal AG. Correlation of LI-RADS 3 or 4 observations with histopathologic diagnosis in patients with cirrhosis. Clin Gastroenterol Hepatol. 2023;21:1351–53.e2.
21. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94: 496–509.
22. El-Serag H, Kanwal F, Ning J, Powell H, Khaderi S, Singal AG, et al. Serum biomarker signature is predictive of the risk of hepatocellular cancer in patients with cirrhosis. Gut. 2024;73: 1000–7.
23. Xiang X, Backman JT, Neuvonen PJ, Niemi M. Gender, but not CYP7A1 or SLCO1B1 polymorphism, affects the fasting plasma concentrations of bile acids in human beings. Basic Clin Pharmacol Toxicol. 2012;110:245–52.
24. Bennion LJ, Drobny E, Knowler WC, Ginsberg RL, Garnick MB, Adler RD, et al. Sex differences in the size of bile acid pools. Metabolism. 1978;27:961–9.
25. Han J, Qin W, Li Z, Xu A, Xing H, Wu H, et al. Tissue and serum metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. Clin Chim Acta. 2019;488:68–75.
26. Sun Y, Zhu M, Zhao H, Ni X, Chang R, Su J, et al. Serum fibroblast growth factor 19 and total bile acid concentrations are potential biomarkers of hepatocellular carcinoma in patients with type 2 diabetes mellitus. Biomed Res Int. 2020;2020:1751989.
27. Li H, Zhao H, Li Q, Meng D, Li Z. Analysis of glycocholic acid in human plasma and urine from hepatocellular carcinoma patients. Chromatographia. 2017;80:209–15.
28. Ikegami T, Honda A, Miyazaki T, Yara S, Matsuzaki Y. Characteristic features of serum bile acids profile in patients with nonalcoholic steatohepatitis with hepatocellular carcinoma. Mol Cancer Res. 2016;14:B72.
29. Banales JM, Iñarrairaegui M, Arbelaiz A, Milkiewicz P, Muntané J, Muñoz-Bellvis L, et al. Serum metabolites as diagnostic biomarkers for cholangiocarcinoma, hepatocellular carcinoma, and primary sclerosing cholangitis. Hepatology. 2019;70: 547–62.
30. Stepien M, Lopez-Nogueroles M, Lahoz A, Kühn T, Perlemuter G, Voican C, et al. Prediagnostic alterations in circulating bile acid profiles in the development of hepatocellular carcinoma. Int J Cancer. 2022;150:1255–68.
31. Thrift AP, Kanwal F, Lim H, Duong H, Liu Y, Singal AG, et al. PNPLA3, obesity and heavy alcohol use in cirrhosis patients may exert a synergistic increase hepatocellular carcinoma risk. Clin Gastroenterol Hepatol. 2024;22:1858–66.

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