# Serum Liver-Type Fatty Acid–Binding Protein Is a Possible Prognostic Factor in Human Chronic Liver Diseases From Chronic Hepatitis to Liver Cirrhosis and Hepatocellular Carcinoma

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Liver-type fatty acid–binding protein (L-FABP) is a key regulator of fatty acid metabolism, but serum L-FABP levels are not well investigated in chronic liver diseases. We aimed to elucidate the prognostic ability of serum L-FABP in human chronic liver diseases and compare it with the albumin-bilirubin (ALBI) score. In 242 chronic liver disease patients, including chronic hepatitis (CH, n = 100), liver cirrhosis (LC, n = 142), and presence of hepatocellular carcinoma (HCC, n = 144), serum L-FABP levels were correlated with liver function ($P < 0.0001$), increased in LC compared with CH ($P < 0.01$), and correlated to ALBI score ($P < 0.0001$). Serum L-FABP levels were increased in the presence of HCC ($P < 0.0001$), correlating to des-gamma-carboxy prothrombin ($P < 0.0001$), alpha-fetoprotein ($P = 0.009$), and Barcelona-Clinic Liver Cancer stage. In the average follow-up period of 1,054 days, serum L-FABP levels were elevated ($P < 0.0001$) in patients who eventually died. The area under the curve (AUC) of serum L-FABP (0.764) was higher than that of ALB (0.709), and the patients with serum L-FABP ≤ 6.8 ng/mL had significantly longer rates of survival ($P < 0.0001$). Serum L-FABP (hazard ratio [HR] 4.0; $P < 0.001$), HCC (HR 3.7; $P = 0.001$), ALBI score (HR 2.7; $P < 0.001$), and age (HR 1.0; $P = 0.049$) were independent predictors of survival. In the subgroup who maintained liver function, the AUC of serum L-FABP (0.751) was higher than that of ALB (0.643). In this subgroup, serum L-FABP (HR 4.4; $P = 0.002$) and HCC (HR 13.9; $P < 0.001$) were independent predictors of survival. *Conclusion:* Serum L-FABP is a possible predictor of survival in chronic liver diseases from CH to LC and HCC, including any subgroup that maintains liver function. (*Hepatology Communications* 2019;3:825-837).

Chronic liver disease patients may present with myriad liver conditions, such as chronic hepatitis (CH), liver cirrhosis (LC), and liver failure, and have an elevated risk of developing hepatocellular carcinoma (HCC). For chronic liver disease patients, Child-Pugh scores (CPSs) or Model for End-Stage Liver Disease (MELD) scores generated with multiple factors and variables are used

in the assessment and prognosis of cirrhosis[1] or decompensated cirrhosis,[2] respectively. However, the predicted rate of survival is also greatly influenced by the concomitant stage of HCC, which occurs in chronic liver diseases, but is not factored into either of the two scoring methods. Recently, the albumin-bilirubin (ALBI) grade was established as an objective hepatic function reserve estimation used instead of the CPS.[3] The ALBI grade is associated with HCC progression,[4,5] but requires further investigation before mainstream clinical applicability. Therefore, we need to further develop highly precise and predictive prognosis factors for chronic liver diseases that include HCC in their overall measurement.

Liver-type fatty acid–binding protein (L-FABP) is an endogenous antioxidant protein and is expressed primarily in the liver as well as the proximal tubular epithelial cells in the kidney. Urine L-FABP levels derived from proximal tubular epithelial cells are elevated in renal tubular injury episodes and are therefore used as an established marker of several kidney diseases, including acute kidney injury and chronic kidney diseases.[6-9] In the normal liver environment, L-FABP is a key regulator of fatty acid metabolism.[10] Serum L-FABP levels are used to monitor fibrosis and hepatocellular damage during liver surgery[11] in both nonalcoholic steatohepatitis (NASH) patients[12] and patients infected with hepatitis C virus (HCV).[13] L-FABP levels are also elevated in human HCC tissues,[14] but the elevation observed within tumors does not translate into elevated levels within the blood. Furthermore, serum L-FABP levels

are associated with poor survival rates in acute liver failure caused by acetaminophen.[15] Despite all of this, the clinical significance of L-FABP as a prognostic factor has not been addressed with regard to chronic liver diseases, including HCC.

In this study, we investigated the association between serum L-FABP levels and liver function, ALBI score, and HCC, and we explored the link between L-FABP levels and the survival rate in human chronic liver diseases, including CH, LC, and HCC.

# Materials and Methods

## HUMAN SAMPLES

The study protocol was approved by the ethics committee of Mie University. This study was performed retrospectively on stored samples, and patients could opt out of their data. Patients (n = 242) were recruited by their stage of chronic liver disease. Liver cirrhosis was diagnosed based on morphologic changes of the liver such as hypertrophy of the left lateral and caudate lobes, or atrophy of the right posterior hepatic lobe on ultrasonography and through blood tests, and/or computed tomography (CT), magnetic resonance imaging (MRI), FibroScan (Echosens, French) results, and esophageal varix by endoscopy, as is the general protocol. HCC was diagnosed based on histological findings or typical imaging characteristics, and HCC patients were grouped by the Barcelona

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Clinic Liver Cancer (BCLC) staging classification.[15] Patients who had other malignancies within the past 3 years, severe hepatic failure (MELD score ≥ 30), uncontrollable infection, heart failure greater than the New York Heart Association–defined category of class II, human immunodeficiency virus infection, pregnancy, or psychiatric problems were deemed to be unsuitable for clinical study. As a general rule, the follow-up examinations included routine physical examinations and biochemical tests (1-3 monthly) and diagnostic imaging studies including ultrasonography, multiphase CT, or dynamic contrast–enhanced MRI (6 monthly).

## BLOOD PREPARATION

Blood samples were collected when patients showed up at the hospital, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), gamma-glutamyltransferase (γ-GT), total bilirubin (T-Bil), sodium (Na), platelet (PLT) count, prothrombin time (PT), cholinesterase (ChE), alpha-fetoprotein (AFP), and des-gamma-carboxy pro-thrombin (DCP) were measured. Blood samples were kept at –80 days until L-FABP measurement, using a high-sensitivity human L-FABP enzyme-linked immunosorbent assay kit (CMIC Holdings Co., Ltd, Tokyo, Japan) according to manufacturer's instruction. The fibrosis index based on 4 factors (FIB-4)[17] and ALBI score[3] were calculated.

## STATISTICAL ANALYSES

All data are expressed as mean ± SD. Data were analyzed using Manny-Whitney U test in two groups and one-way analysis of variance for comparison of continuous variables. Correlation was determined using single regression analysis or Spearman rank–sum test. For each continuous variable, the optimal cutoff value that maximized the sum of sensitivity and specificity was selected using receiver operating characteristic [curve] (ROC) analysis for survival. The cumulative survival rates were estimated with the Kaplan-Meier method and compared between groups by the log-rank test. The statistical analyses were performed using Prism (GraphPad Software, Inc., La Jolla, CA) for comparison of continuous variables and SPSS version 22 (SPSS Japan, Inc., Tokyo, Japan)

for univariate and multivariate Cox regression analyses. Differences were considered to be significant at $P < 0.05$.

# Results

## SERUM L-FABP LEVELS CORRELATE WITH LIVER ENZYME LEVELS, LIVER FUNCTION, AND ALBI SCORE

We investigated whether serum L-FABP levels can be used as a prognostic factor in human chronic liver diseases, including CH, LC, and HCC. We recruited 242 patients (100 CH and 142 LC), 153 males and 89 females, with a mean age of 67.3 ± 11.0 years. The cohort of study patients were admitted to our investigation based on a variety of causative agents: 23 hepatitis B virus (HBV), 114 hepatitis C virus (HCV), 26 NASH, 33 patients with alcoholism, 15 primary biliary cholangitis/autoimmune hepatitis, and 31 others (Table 1). We identified 144 of 242 patients who presented with HCC in addition to their underlying chronic liver disease (Table 1). Within our cohort, we had subgroups of patients who were receiving drugs for the treatment of other underlying conditions: 33 patients taking lipid-lowering agents, 63 patients taking antihyperglycemic agents, and 100 patients taking antihypertensive drugs. Serum L-FABP levels were significantly correlated with ALT ($r = 0.269$; $P < 0.0001$); AST ($r = 0.526$; $P < 0.0001$); ALB ($r = -0.411$; $P < 0.0001$); ALP ($r = 0.298$; $P < 0.0001$); γ-GT ($r = 0.151$; $P < 0.05$); ChE ($r = -0.384$; $P < 0.0001$); Na ($r = -0.311$; $P < 0.0001$); and PT ($r = -0.239$; $P < 0.001$) (Fig. 1A), suggesting a predictive ability inherent in serum L-FABP levels for liver damage. There were no significant correlations between L-FABP levels and age, T-Bil ($P = 0.066$) or PLT count, body mass index, the presence of type 2 diabetes mellitus, or the parallel administration of medications (lipid-lowering agents, antihyperglycemic agents, and anti-hypertensive drugs) in select patients. When we divided our cohort into four groups, CH without HCC (CH-non-HCC), CH with HCC (CH-HCC), LC without HCC (LC-non-HCC), and LC with HCC (LC-HCC) (Table 1),

**TABLE 1. PATIENT CHARACTERIZATION**

| Variables | All (n = 242) | CH-non-HCC (n = 62) | CH-HCC (n = 38) | LC-non-HCC (n = 36) | LC-HCC (n = 106) |
|---|---|---|---|---|---|
| Gender (male/female) | 153/89 | 24/38 | 31/7 | 20/16 | 78/28 |
| Age (years) | 67.3 ± 11.0 | 60.7 ± 11.5 | 72.9 ± 8.2 | 63.7 ± 10.0 | 70.3 ± 9.6 |
| Etiology | | | | | |
| (HBV/HCV/NASH/ALD/AIH-PBC/others) | 24/113/27/32/15/31 | 2/25/23/4/5/3 | 7/14/0/6/0/11 | 2/14/1/8/6/5 | 13/60/3/14/4/12 |
| HCC (+/−) | 144/98 | 0/62 | 38/0 | 0/36 | 106/0 |
| ALB (g/dL) | 3.8 ± 0.68 | 4.3 ± 0.3 | 4.1 ± 0.6 | 3.3 ± 0.6 | 3.5 ± 0.6 |
| ALT (IU/L) | 39.4 ± 38.5 | 48.7 ± 61.7 | 35.8 ± 21.9 | 27.5 ± 22.3 | 39.5 ± 28.1 |
| AST (IU/L) | 55.7 ± 53.4 | 48.6 ± 65.0 | 61.8 ± 72.0 | 44.5 ± 24.6 | 61.5 ± 44.1 |
| γ-GT (IU/L) | 103.4 ± 148.6 | 76.8 ± 119.7 | 171.0 ± 188.7 | 65.1 ± 62.2 | 104.5 ± 148.4 |
| ALP (U/L) | 416.3 ± 361.7 | 306.6 ± 235.4 | 418.7 ± 363.5 | 405.3 ± 198.8 | 483.6 ± 443.4 |
| T-Bil (mg/dL) | 1.2 ± 1.4 | 0.8 ± 0.4 | 1.0 ± 0.7 | 1.8 ± 2.2 | 1.3 ± 1.5 |
| ChE (ΔPH) | 0.7 ± 0.3 | 1.0 ± 0.2 | 0.8 ± 0.2 | 0.5 ± 0.2 | 0.5 ± 0.2 |
| Na (mEq/L) | 138.5 ± 3.7 | 140.3 ± 2.2 | 139.0 ± 3.2 | 137.2 ± 5.1 | 138.3 ± 3.7 |
| PLT (×10$^4$ cells/μL) | 15.5 ± 8.4 | 18.8 ± 5.2 | 21.7 ± 8.6 | 12.7 ± 10.3 | 12.4 ± 7.3 |
| PT (%) | 81.0 ± 20.2 | 103.8 ± 10.5 | 89.6 ± 18.6 | 68.0 ± 17.9 | 78.8 ± 19.6 |
| FIB-4 index | 5.7 ± 8.6 | 3.8 ± 2.8 | 6.0 ± 4.6 | 4.5 ± 4.3 | 7.1 ± 12.1 |
| ALBI score | −2.4 ± 0.68 | −3.0 ± 0.3 | −2.7 ± 0.6 | −1.9 ± 0.6 | −2.1 ± 0.6 |
| DCP (mAU/mL) | 13,933 ± 48,142 | 24 ± 0 | 25,404.2 ± 73,946.8 | 20.3 ± 5.5 | 15,199.8 ± 44,683.1 |
| AFP (ng/mL) | 13,048 ± 96,646 | 4.3 ± 2.4 | 4,645.2 ± 20,973.3 | 6.7 ± 5.6 | 18,613.9 ± 110,886.3 |
| BMI | 23.7 ± 4.6 | 24.3 ± 3.4 | 22.2 ± 3.6 | 25.2 ± 7.8 | 23.3 ± 3.9 |
| T2DM (+/−) | 93/149 | 25/37 | 11/27 | 17/19 | 40/66 |
| L-FABP (ng/mL) | 11.8 ± 11.0 | 7.0 ± 5.3 | 13.1 ± 13.6 | 9.1 ± 7.0 | 15.0 ± 12.4 |

Note: Values are presented as mean ± SD.
Abbreviations: AIH-PBC, autoimmune hepatitis–primary biliary cholangitis; ALD, alcoholic liver disease; BMI, body mass index; and T2DM, type 2 diabetes mellitus.

serum L-FABP levels were significantly correlated with AST in all groups and ALT in three groups. Serum L-FABP levels were also significantly correlated with ALB, ALP, γ-GT, ChE, Na, and PT in one or more groups (Supporting Table S1). Due to serum L-FABP levels being significantly correlated with liver function, we investigated whether serum L-FABP levels are associated with ALBI score and the presence of HCC. We also compared serum L-FABP levels with serum ALB, a known clinical prognostic factor included in many scoring methodologies. Serum L-FABP levels were significantly increased in LC patients compared with CH patients (L-FABP: $P < 0.01$; ALB: $P < 0.0001$) (Fig. 1B), and were significantly correlated with ALBI score ($r = 0.457$; $P < 0.0001$) (Fig. 1C) as well as ALBI grade (G) (G1 versus G2: $P < 0.01$; G1 versus G3: $P < 0.0001$; G2 versus G3: $P < 0.01$) (Fig. 1D). As expected, serum ALB levels were significantly decreased, corresponding to ALBI grade ($P < 0.0001$) (Fig. 1D).

## SERUM L-FABP LEVELS SIGNIFICANTLY INCREASE IN HCC

We further investigated whether serum L-FABP levels were affected by HCC. Serum L-FABP levels were significantly elevated, and serum ALB levels were significantly decreased, in the presence of HCC compared with patients without HCC (both L-FABP and ALB: $P < 0.0001$) (Fig. 2A). Notably, serum L-FABP levels were 2-fold higher in the presence of HCC compared with the absence of HCC within the CH and LC cohort (both $P < 0.01$), and serum ALB levels were lower in the presence of HCC compared with the absence of HCC within the CH cohort ($P < 0.05$), but were similar regardless of HCC status in the LC cohort (Supporting Fig. S1). Moreover, serum L-FABP levels were significantly correlated with several blood tumor markers, namely, DCP ($r = 0.459$; $P < 0.0001$) (Fig. 2B) and AFP ($r = 0.221$; $P = 0.009$) (Fig. 2C). Serum
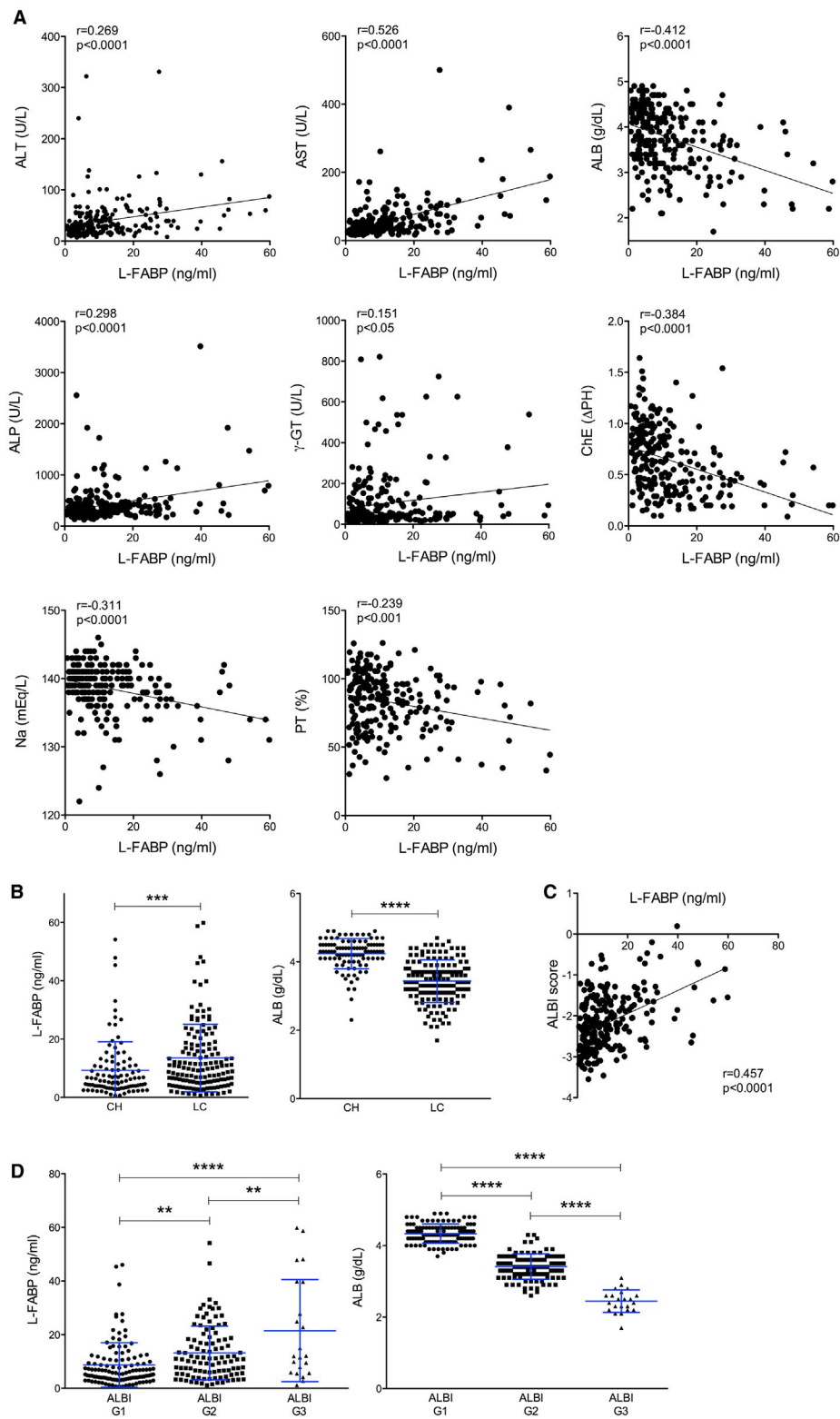
**FIG. 1.** Serum L-FABP levels are correlated with liver functions in human chronic liver disease. (A) Correlation between serum L-FABP levels and ALT, AST, ALB, ALP, γ-GT, ChE, Na, and PT. (B) Serum L-FABP and ALB levels in CH and LC groups. (C) Correlation between serum L-FABP levels and ALBI score. (D) Serum L-FABP and ALB levels in ALBI grade. Values are presented as mean ± SD. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$.
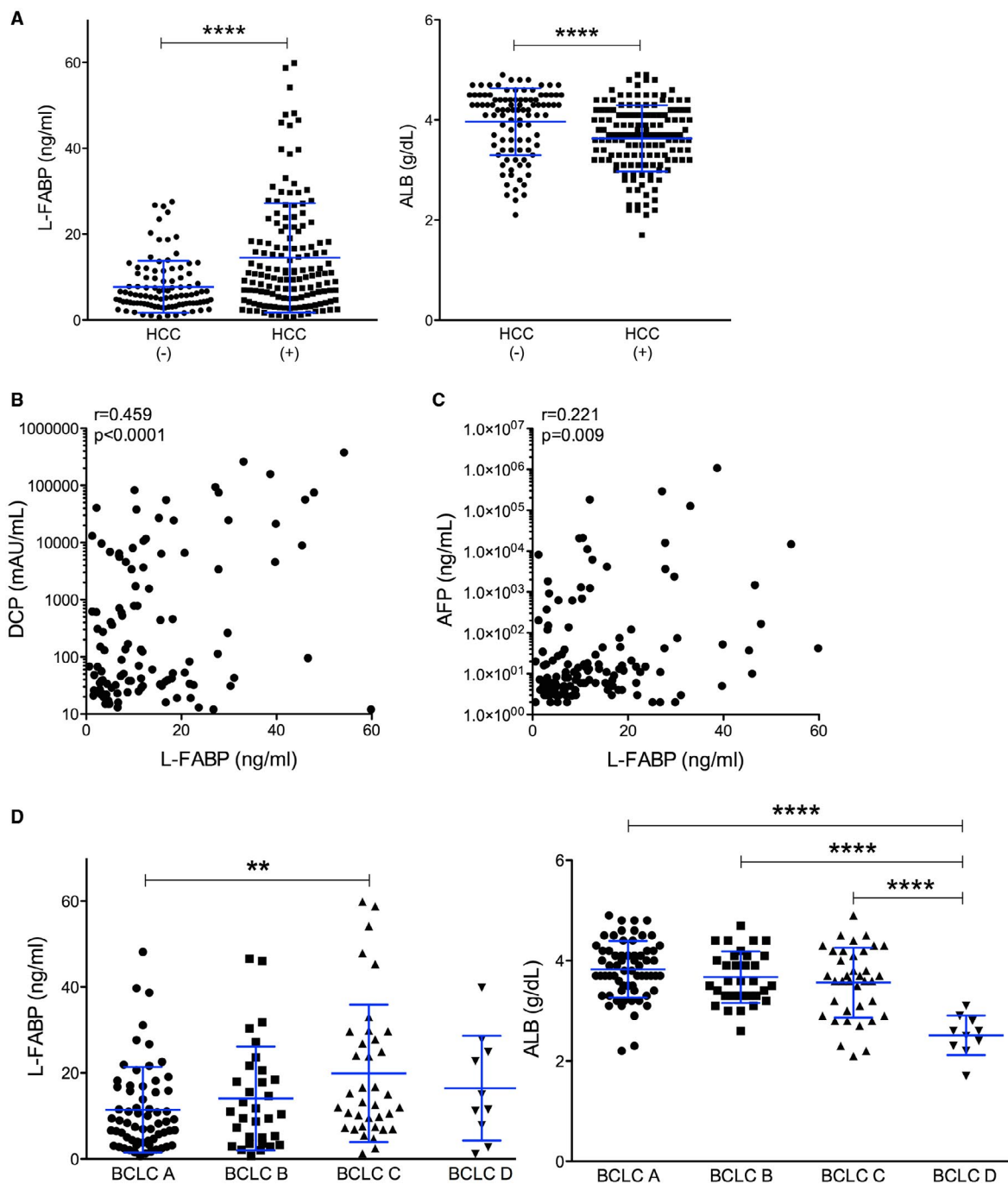
**FIG. 2.** Serum L-FABP levels are associated with HCC markers. (A) Serum L-FABP and ALB levels in the absence and presence of HCC in all cohorts. (B,C) Correlation between serum L-FABP levels and DCP (B) and AFP (C). (D) Serum L-FABP and ALB levels in BCLC stage. Values are presented as mean ± SD. ****$P < 0.0001$, **$P < 0.01$.

L-FABP levels were gradually increased corresponding to BCLC stage (BCLC A versus BCLC C: $P < 0.01$), and serum ALB levels were significantly decreased in BCLC D (BCLC A, B, or C versus BCLC D: $P < 0.0001$) (Fig. 2D). These results indicate that serum L-FABP levels were continuously elevated in CH, LC and HCC, and can be used in the diagnosis of chronic liver diseases, including those coupled with HCC.

## SERUM L-FABP LEVELS ARE A PROGNOSTIC FACTOR FOR SURVIVAL

We examined the rate of survival within our study group and found that 71 patients, out of 242, died (called deceased in the following sentences) within the follow-up period of 1,054 days (median of 677 days). Within 180 days of our follow-up period, we could no longer account for 14 patients due to hospital transfer. According to hospital records, all causes of death were considered liver-related. Serum L-FABP levels were significantly increased and ALB levels were significantly decreased in the deceased group compared with the survival group (L-FABP and ALB: $P < 0.0001$) (Fig. 3A). ROC analyses yielded area under the curve (AUC) values of 0.765 (95% confidence interval [CI]: 0.6989-0.8303; $P < 0.0001$) for serum L-FABP levels (Fig. 3B), whereas an AUC value of 0.709 (95% CI: 0.6363-0.7812; $P < 0.0001$) was observed for ALB levels (Fig. 3B). Finally, we calculated the cut-off value of serum L-FABP levels to be 6.8 ng/mL, and ALB levels to be 3.2 g/dL. Patients with serum L-FABP > 6.8 ng/mL showed significantly increased ALT, AST, $\gamma$-GT, ALP, FIB-4 index, ALBI score, DCP, AFP, and an elevated presence of HCC, whereas ALB, ChE, Na, and PLT were significantly decreased in patients with serum L-FABP ≤ 6.8 ng/mL (Table 2). The survival rate was significantly decreased in patients with serum L-FABP > 6.8 ng/mL compared with patients with L-FABP ≤ 6.8 ng/mL ($P < 0.0001$) (Fig. 3C) and similar observations in patients with ALB ≤ 3.2 g/dL ($P < 0.0001$) (Fig. 3D). Furthermore, being male ($P < 0.01$) (Fig. 3E) and over 65 years old ($P < 0.01$) (Fig. 3F) appeared to negatively impact overall survival rate. This phenomenon was the same in the cohort presenting with HCC (L-FABP: $P < 0.0001$ and ALB: $P = 0.002$). Furthermore, serum L-FABP levels were more accurate than ALBI score in HCC patients when determining prognosis (L-FABP: AUC 0.731 and ALBI score: AUC 0.649, $P = 0.002$) (Supporting Fig. S2). We further analyzed the rate of survival within our four targeted groups: CH-non-HCC, CH-HCC, LC-non-HCC, and LC-HCC (Supporting Fig. S3A-D). Based on ROC analysis, serum L-FABP was a better prognostic marker than ALB when we the compared the CH-non-HCC, CH-HCC, and LC-HCC groups (Supporting Fig. S3A,B,D). In model 1 we used univariate Cox regression analysis, which included the ALBI score without ALB, and found that HCC (hazard ratio [HR] 8.0; $P < 0.001$), serum L-FABP (HR 7.6; $P < 0.001$), LC (HR 3.2; $P < 0.001$), ALBI score (HR 3.2; $P < 0.001$), gender (HR 2.1; $P = 0.007$), and age (HR 1.0; $P < 0.001$) were independent predictors of survival (Table 3 and Supporting Table S2). Moreover, we used multivariate Cox regression analyses to assess serum L-FABP (HR 4.0; $P < 0.001$), HCC (HR 3.7; $P = 0.001$), ALBI score (HR 2.7; $P < 0.001$), and age (HR 1.0; $P = 0.049$) as independent predictors of survival, but excluded FIB-4 index values, LC, and gender in our calculations (Table 3). In model 2 we used univariate and multivariate Cox regression analyses, including ALB without ALBI score, and found HCC (HR 4.0; $P < 0.001$), serum L-FABP (HR 3.8; $P = 0.001$), ALB (HR 3.4; $P < 0.001$), and age (HR 1.0; $P = 0.029$) to be independent predictors of survival (Supporting Table S2). These results reveal serum L-FABP levels to be a prognostic factor in chronic liver disease, such as CH and LC, including patients presenting with concomitant HCC.

## SERUM L-FABP LEVELS ARE A PROGNOSTIC FACTOR IN PATIENTS WHO MAINTAINED LIVER FUNCTION (ALB > 3.5)

ALB level is a well-established prognostic factor in liver disease and is incorporated into various scoring systems, such as ALBI grade and CPS. However, ALB levels are not affected by HCC, suggesting that the current scoring systems may not diagnose HCC patients in a cohort with high ALB levels. Therefore, we investigated whether serum L-FABP levels are useful as a prognostic factor in a subgroup of patients who maintain liver function with ALB > 3.5 g/dL, which is indexed as class A within CPS. In our chosen subgroup, serum L-FABP levels were significantly increased in the deceased group compared with the survival group ($P < 0.0001$) (Fig. 4A), and ALB levels were significantly decreased in the deceased group ($P < 0.01$) (Fig. 4A). Notably, ROC analysis yielded AUC values of 0.751 (95% CI: 0.6593-0.8416; $P < 0.0001$) for serum L-FABP and an AUC value of 0.643 (95% CI: 0.5420-0.7436; $P = 0.011$) for serum
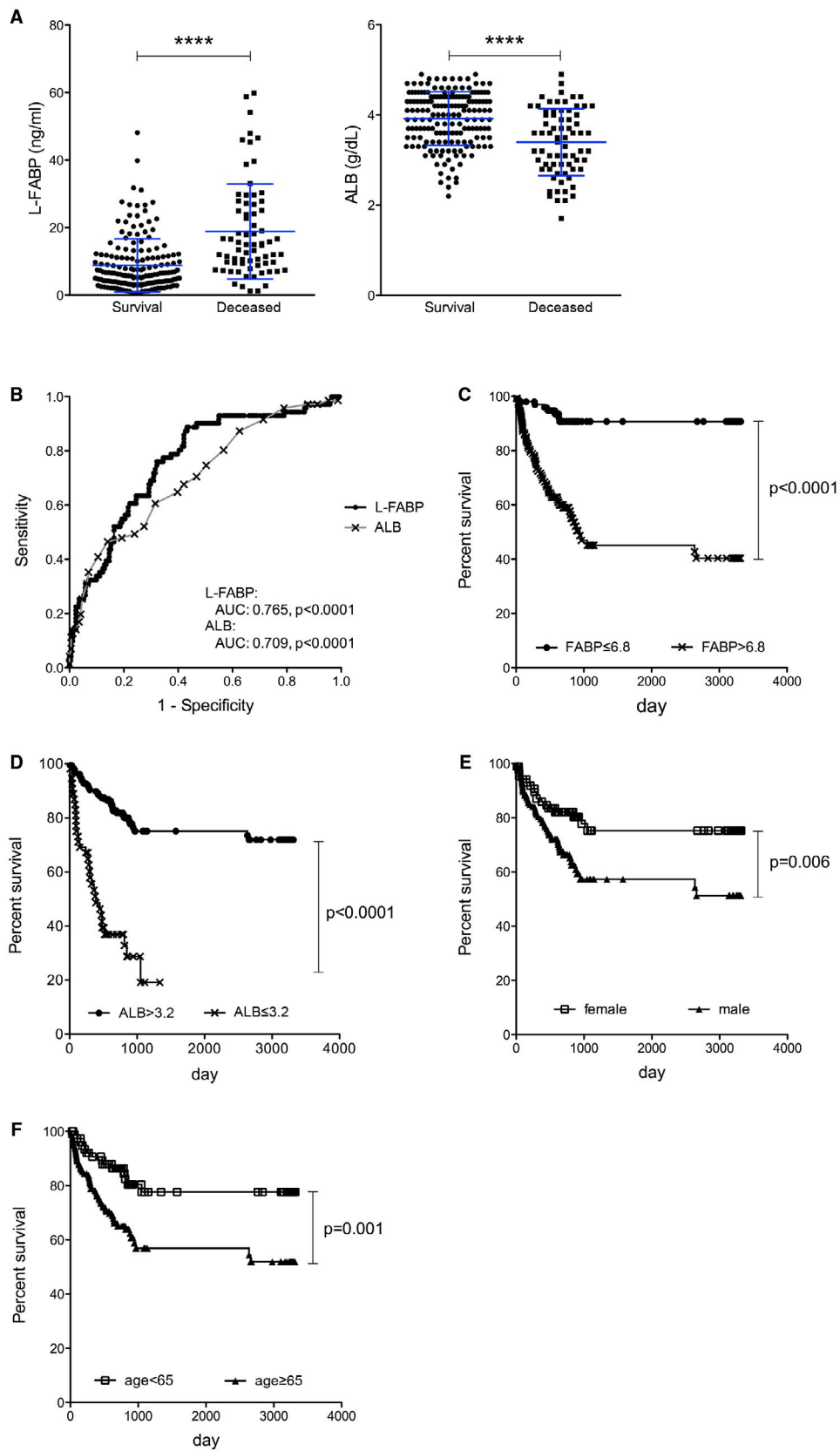
**FIG. 3.** Survival was improved in human chronic liver diseases influenced by several factors: serum L-FABP levels, ALB levels, age, or gender. (A) Serum L-FABP and ALB levels in survival and deceased groups in all cohorts. (B) ROC curve of serum L-FABP levels and ALB levels. (C-F) Survival curve in human chronic liver diseases with serum L-FABP levels (C), ALB levels (D), age (E), or gender (F). Values are presented as mean ± SD. ****$P$ < 0.0001.

### TABLE 2. PATIENT CHARACTERIZATION SEPARATED BY SERUM L-FABP LEVELS

| Variables | L-FABP ≤ 6.8 (n = 105) | L-FABP > 6.8 (n = 137) | P Value |
|---|---|---|---|
| Gender (male/female) | 58/47 | 95/42 | $P$ = 0.024 |
| Age (years) | 65.9 ± 11.3 | 68.3 ± 10.7 | $P$ = 0.079 |
| Etiology (HBV/HCV/NASH/ALD/AIH-PBC/others) | 7/40/18/14/11/12 | 16/74/8/19/4/19 | |
| HCC (+/−) | 45/60 | 99/38 | $P$ < 0.0001 |
| ALB (g/dL) | 4.0 ± 0.58 | 3.6 ± 0.70 | $P$ < 0.0001 |
| ALT (IU/L) | 31.6 ± 39.6 | 45.6 ± 37.0 | $P$ < 0.0001 |
| AST (IU/L) | 37.7 ± 24.5 | 69.5 ± 64.5 | $P$ < 0.0001 |
| γ-GT (IU/L) | 75.2 ± 108.5 | 128.0 ± 172.9 | $P$ = 0.014 |
| ALP (U/L) | 358.9 ± 299.0 | 460.3 ± 398.7 | $P$ = 0.003 |
| T-Bil (mg/dL) | 1.2 ± 1.5 | 1.2 ± 1.2 | $P$ = 0.160 |
| ChE (ΔPH) | 0.8 ± 0.3 | 0.6 ± 0.3 | $P$ < 0.0001 |
| Na (mEq/L) | 139.5 ± 3.0 | 137.8 ± 3.9 | $P$ = 0.001 |
| PLT (×10$^4$ cells/μL) | 16.2 ± 6.5 | 15.1 ± 9.6 | $P$ = 0.014 |
| PT (%) | 82.4 ± 21.9 | 80.3 ± 19.2 | $P$ = 0.392 |
| FIB-4 index | 4.2 ± 3.5 | 6.9 ± 10.9 | $P$ = 0.003 |
| ALBI score | −2.5 ± 0.58 | −2.0 ± 0.69 | $P$ < 0.0001 |
| DCP (mAU/mL) | 2005 ± 7144 | 19,898 ± 57,957 | $P$ = 0.0004 |
| AFP (ng/mL) | 242.1 ± 1153 | 21,034 ± 12,2739 | $P$ = 0.002 |

Note: Manny-Whitney U test. Values are presented as mean ± SD.
Abbreviations: AIH-PBC, autoimmune hepatitis–primary biliary cholangitis; and ALD, alcoholic liver disease.

### TABLE 3. UNIVARIATE AND MULTIVARIATE COX REGRESSION ANALYSIS FOR PREDICTORS OF SURVIVAL IN ALL PATIENTS (MODEL 1)

| Variables | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | P Value | HR | 95% CI | P Value |
| Gender Male | 2.107 | 1.231-3.605 | 0.007 | | | |
| Age | 1.049 | 1.023-1.076 | <0.001 | 1.026 | 1.000-1.053 | 0.049 |
| LC | 3.152 | 1.822-5.452 | <0.001 | | | |
| HCC | 8.014 | 3.834-16.754 | <0.001 | 3.679 | 1.698-7.968 | 0.001 |
| ALBI score | 3.207 | 2.321-4.432 | <0.001 | 2.654 | 1.809-3.893 | <0.001 |
| FIB-4 >3.25 | 1.385 | 0.862-2.227 | 0.178 | | | |
| L-FABP >6.8 | 7.564 | 3.621-15.804 | <0.001 | 3.993 | 1.868-8.534 | <0.001 |

ALB (Fig. 4B). We calculated the cutoff value of serum L-FABP levels to be 6.8 ng/mL, and ALB levels to be 4.2 g/dL. The survival rate was significantly decreased in patients with serum L-FABP > 6.8 ng/mL compared with patients with L-FABP ≤ 6.8 ng/mL ($P$ < 0.0001) (Fig. 4C) as well as patients presenting with ALB ≤ 4.2 g/dL ($P$ = 0.002) (Fig. 4D). Furthermore, being male ($P$ = 0.006) (Fig. 4E) and over 65 years old ($P$ = 0.005) (Fig. 4F) were predictive of a poor survival rate. Using univariate Cox regression analysis, HCC (HR 21.8;
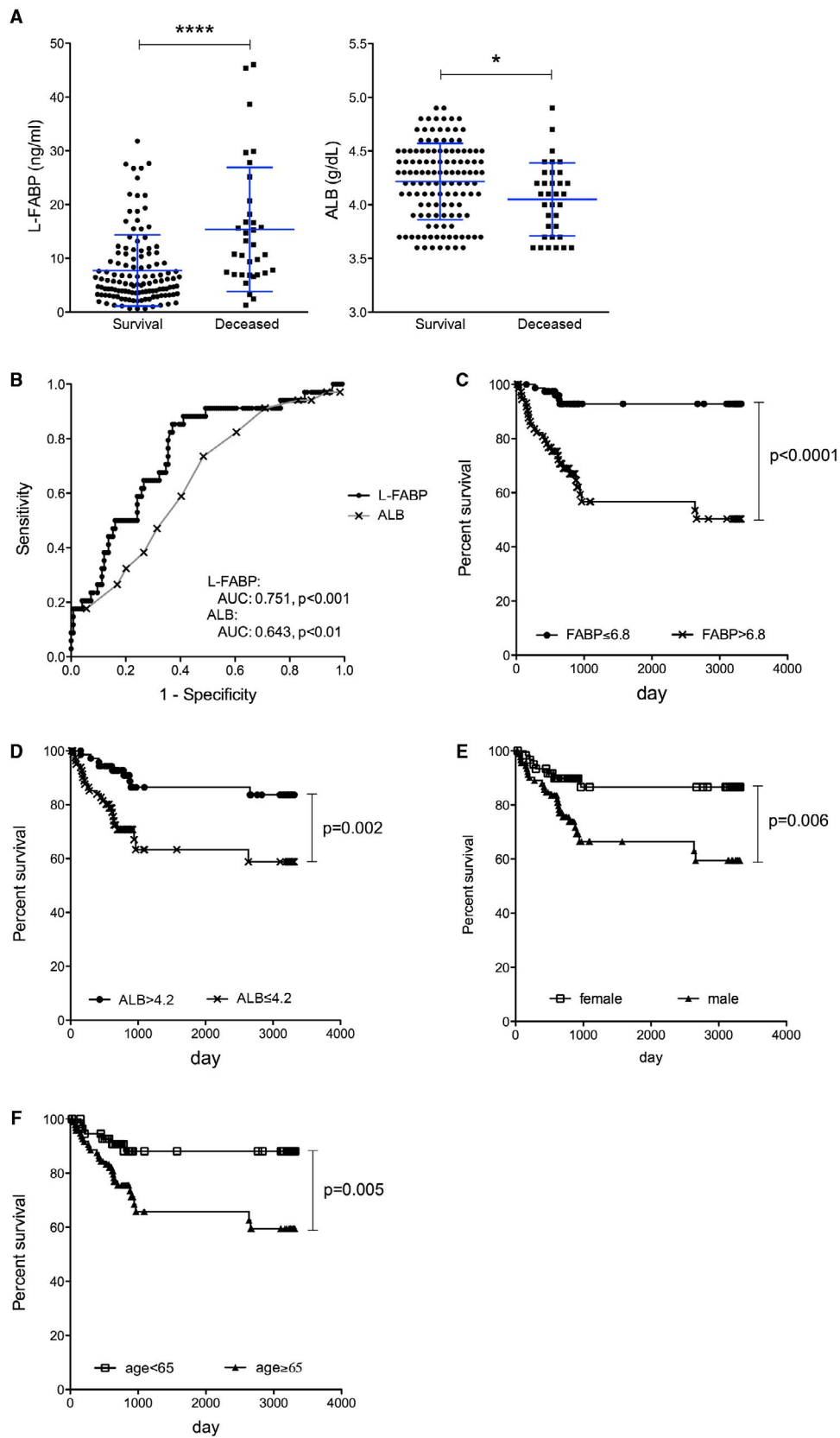
**FIG. 4.** Survival was improved in human chronic liver diseases influenced by several factors: serum L-FABP levels, ALB levels, age, or gender in the subcohort who maintained liver function (ALB > 3.5). (A) Serum L-FABP and ALB levels in survival and deceased groups in the cohort who maintained liver function (ALB > 3.5). (B) ROC curve of serum L-FABP levels and ALB levels. (C-F) Survival curve in human chronic liver diseases with serum L-FABP levels (C), ALB levels (D), age (E), or gender (F). Values are presented as mean ± SD. ****$P < 0.0001$, *$P < 0.05$.

### TABLE 4. UNIVARIATE AND MULTIVARIATE COX REGRESSION ANALYSIS FOR PREDICTORS OF SURVIVAL IN THE COHORT WHO MAINTAINED LIVER FUNCTION

| Variables | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | $P$ Value | HR | 95% CI | $P$ Value |
| Gender<br>  Male | 2.633 | 1.192-5.819 | 0.017 | | | |
| Age | 1.070 | 1.031-1.110 | <0.001 | | | |
| LC | 2.552 | 1.285-5.070 | 0.007 | | | |
| HCC | 21.830 | 6.032-78.998 | <0.001 | 13.884 | 3.861-49.928 | <0.001 |
| ALBI score | 3.738 | 1.513-9.234 | 0.004 | | | |
| FIB-4<br>  >3.25 | 1.194 | 0.614-2.320 | 0.602 | | | |
| L-FABP<br>  >6.8 | 7.643 | 2.964-19.708 | <0.001 | 4.430 | 1.696-11.572 | 0.002 |

$P < 0.001$), serum L-FABP (HR 7.6; $P < 0.001$), ALBI score (HR 3.7; $P = 0.004$), gender (HR 2.6; $P = 0.017$), LC (HR 2.6; $P = 0.007$), and age (HR 1.1; $P < 0.001$) were independent predictors of survival (Table 4). Using multivariate Cox regression analyses in our subgroup of patients, HCC (HR 13.9; $P < 0.001$) and serum L-FABP (HR 4.4; $P = 0.002$) were independent predictors of survival; however, FIB-4 index value, ALBI score, LC, age, and gender were not selected (Table 4). These results reveal that serum L-FABP level is a prognostic factor in chronic liver diseases from CH to LC, including those with concomitant HCC, and patients who maintained liver function.

## Discussion

Our study demonstrates that serum L-FABP levels are significantly correlated with liver function, as well as ALBI score, and increased in the LC group presenting with HCC. We also verified that serum L-FABP levels can be used as a prognostic factor in chronic liver disease patients. Notably, AUC values of serum L-FABP levels were higher than that of serum ALB levels in 242 chronic liver disease patients, as well as in the subgroup that maintained liver function

(ALB > 3.5). Using multivariate Cox regression analyses, serum L-FABP was the first criterion to be used as a prognostic factor in 242 patients and the second criterion (HCC was the first) for prognostic capacity in the subgroup who maintained liver function.

Serum L-FABP levels are strongly correlated with liver function when analyzed with ALT, AST, ALB, ALP, ChE, and Na. Furthermore, serum L-FABP levels were increased in patients with HCC compared with patients without HCC. A variety of experiments using mouse models and *in vitro* cell work showed that L-FABP regulates fatty acid metabolism associated with peroxisome proliferator–activated receptor alpha (PPARα in β-oxidation[16] and is involved in hepatocellular damage as well as oxidative stress, thus contributing to the progression of liver disease through increased hepatic steatosis and the subsequent activation of hepatic stellate cells.[17-19] Performing immunohistochemistry on liver sections showed L-FABP protein to be expressed in human HCC tissue associated with vascular endothelial growth factor A expression using 90 HCC cases. Furthermore, L-FABP promotes tumor growth and metastasis in animal models[14] and can be used for hepatocellular adenoma (HCA) subtype classification due to the decrease of L-FABP proteins in HCA.[20] The biological functions of L-FABP in

the liver will reflect the increase of serum L-FABP levels associated with overall liver function in human chronic liver disease patients, including those presenting with HCC.

ALB levels are included in a variety of scoring systems used to diagnose overall liver condition and to predict patient survival. Therefore, we compared serum L-FABP levels to ALB levels within various subgroups: (1) CH and LC, (2) ALBI grade, (3) presence or absence of HCC in all patients, and (4) presence or absence of HCC in LC. Serum L-FABP levels were significantly increased in LC patients when compared with CH patients and significantly correlated with ALBI grade, but these differences were eliminated, or observed to be slightly decreased, when measuring ALB levels. Furthermore, serum L-FABP levels and ALB levels were significantly elevated in the presence of HCC compared with the absence of HCC in all patients. Notably, we found that serum L-FABP levels were significantly elevated in the presence of HCC compared with the absence of HCC within the LC cohort, but ALB levels were not changed regardless of HCC status, meaning that serum L-FABP has an advantage in diagnosing HCC within the LC cohort, whereas ALB levels have a limitation due to not being influenced by HCC status. This finding is significantly important because LC patients have a greater risk of HCC compared with CH patients.

In this study, we also showed that patient survival rate can be predicted based on serum L-FABP levels greater than or equal to 6.8 ng/mL in chronic liver diseases, from CH to LC and HCC. The CPS and ALBI grade are used as predictive measures for patient prognosis in those who have cirrhosis and chronic liver diseases following liver failure,[1,3] but these scores do not consistently reflect complications, such as HCC, in chronic liver diseases. Serum AFP and DCP levels are used for HCC stratification in the clinic; however, these are not appropriate for HCC surveillance.[21] Although angiopoietin 2 and vascular endothelial growth factor levels are reported as potential prognostic factors in HCC patients, they have yet to be established as specific biomarkers.[22,23] Our data indicate that serum L-FABP levels are significantly increased in the deceased group compared with the survival group in the cohort presenting with HCC ($P < 0.0001$), suggesting that serum L-FABP levels have the potential to be a prognostic factor and may be useful as a diagnostic factor during HCC

treatment. Having said that, further study is required using a larger cohort of HCC patients, as well as using multiple samples per patient in order to establish reproducibility.

In conclusion, serum L-FABP level is a prognostic factor that correlates with functional measures of the liver and ALBI score in human chronic liver disease patients, including CH, LC, and HCC. Moreover, serum L-FABP levels will be useful in predicting the rate of survival in patients who maintain liver function during the course of their disease.

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# Supporting Information

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