Neutrophil Gelatinase-Associated Lipocalin Level Is a Prognostic Factor for Survival in Rat and Human Chronic Liver Diseases

Kyoko Yoshikawa, Motoh Iwasa, Akiko Eguchi, Shinichi Kojima, Naohiko Yoshizawa, Mina Tempaku, Ryosuke Sugimoto, Norihiko Yamamoto, Kazushi Sugimoto, Yoshinao Kobayashi, Hiroshi Hasegawa, and Yoshiyuki Takei

Chronic liver disease patients often have complications, such as hepatocellular carcinoma (HCC) and acute bacterial infection. Model for end-stage liver disease and Child-Pugh scores are useful prognostic factors for chronic liver diseases but not for all chronic conditions, such as HCC. Our investigative aim targeted the prognostic abilities of neutrophil gelatinase-associated lipocalin (NGAL) in rat and human chronic liver diseases. Blood NGAL levels were measured by enzyme-linked immunosorbent assay in rats with cirrhosis and 96 patients with chronic liver disease and HCC. We examined the correlation between blood NGAL levels and liver functions as well as survival. In our rat model, liver NGAL expression was assessed by immunostaining, real-time quantitative polymerase chain reaction, and immunoblot. In rats with cirrhosis, blood NGAL levels were continuously and significantly elevated in the deceased group and were significantly correlated with liver functions. Liver NGAL, toll-like receptor 4, and interleukin-6 levels were increased in the deceased group compared to the survival group. Blood NGAL levels were significantly correlated with liver NGAL levels, indicating blood NGAL was derived from the liver. In patients with chronic liver disease, blood NGAL levels were associated with liver function and renal function. Blood NGAL levels were significantly increased in patients with chronic liver disease with HCC compared to without HCC. For the survival group, 38 out of 96 patients were dead in the average follow-up period of 9.9 months. The patients with blood NGAL ≤119 ng/mL had significantly longer rates of survival compared to patients with blood NGAL >119 ng/mL. *Conclusion*: Blood NGAL predicts the survival rate in rat and human chronic liver diseases. Our findings suggest blood NGAL may be prognostic of survival in chronic liver diseases complicated by HCC. (*Hepatology Communications* 2017;1:946-956)

Introduction

Neutrophil gelatinase-associated lipocalin (NGAL) is a binding partner with matrix metalloproteinase-9 in neutrophils[1] and is established as a clinical biomarker for acute renal injury[2] based on NGAL function, a kidney differentiation inducer,[3] and a kidney protector.[4] NGAL expression is also up-regulated in various tissues, including the liver, induced by a variety of stimulators, such as bacterial infection. Furthermore, several clinical studies have shown that NGAL levels are a prognostic

Abbreviations: AKI, acute kidney injury; Alb, albumin; ALT, alanine aminotransferase; Bil, total bilirubin; BUN, blood urea nitrogen; Cr, creatinine; CRP, C-reactive protein; eGFR, estimated glomerular filtration rates; HCC, hepatocellular carcinoma; IL, interleukin; LPS, lipopolysaccharide; MELD, model for end-stage liver disease; mRNA, messenger RNA; NGAL, neutrophil gelatinase-associated lipocalin; PCR, polymerase chain reaction; ROC, receiver operating curve; TLR4, toll-like receptor 4.

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factor in acute-on-chronic liver-failure patients[5] and that NGAL is associated with survival rate in patients with cirrhosis who present with bacterial infection[6] or hepatocellular carcinoma (HCC).[7] The role and clinical significance of NGAL is not fully understood in the liver even though the mechanism of NGAL induction has been investigated in fatty liver, hepatitis C, and liver regeneration.[8-10]

Patients with chronic liver disease often have complications, such as kidney damage, esophageal varices hemorrhage, bacterial infection, and HCC, resulting in a poor rate of survival. For patients with chronic liver disease with complications, a single factor, such as serum hyaluronic acid, is not sufficient to predict survival.[11] In contrast, Child-Pugh or Model for End-Stage Liver Disease (MELD) scores, generated with multiple factors and variables, are used in the assessment and prognosis of cirrhosis[12] or decompensated cirrhosis,[13] respectively. However, the predicted rate of survival is also influenced by the stage of HCC, which is not included in both scores but often occurs in chronic liver diseases. Therefore, we need to further develop highly precise, predictive, prognosis factors for chronic liver diseases.

In this study, we investigated the association of blood NGAL level and liver NGAL expression with survival rate in rats with cirrhosis administered CCl_4 for 21 weeks. We also validated the association of blood NGAL levels with liver function and survival rate in human chronic liver diseases, including patients with HCC.

Materials and Methods

ANIMALS

Our animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hokudo Co., Ltd (Sapporo, Japan). The cirrhosis rat model has been described in detail.[14] Briefly, Wister rats aged 6 weeks (n = 21) were orally administered CCl_4 at 1.0 mL/kg twice a week for 5 weeks to establish the rat model of cirrhosis. To maintain cirrhosis, these rats then received CCl_4 at 0.5 mL/kg orally twice a week for 16 weeks (total 21 weeks). We divided the rats into two groups: survival and deceased. The survival group contained rats that survived for 21 weeks post-CCl_4 administration, whereas the deceased group contained rats that died within 21 weeks of CCl_4 administration. The control designation was early rats with cirrhosis 5 weeks post-CCl_4 administration.

LIVER AND BLOOD PREPARATION

Blood was collected at 9, 13, 17, and 21 weeks after established cirrhosis and kept at −80 °C. All rats that survived were killed at the termination of treatment under anesthesia. Whole rat blood was collected and disgorged into tubes with or without anticoagulant. Liver tissue was fixed in formalin for 24 hours and embedded in paraffin, and the remaining liver tissue was quickly frozen in liquid nitrogen and stored at −80 °C. Serum was used for measurement of albumin (Alb), total bilirubin (Bil), alanine aminotransferase (ALT), and creatinine (Cr), which was performed at Hokudo Co., Ltd. Plasma was used for NGAL enzyme-linked immunosorbent assay (R&D systems, Minneapolis, MN) and cystatin C enzyme-linked immunosorbent assay (R&D systems), according to the manufacturer's instructions.

LIVER IMMUNOSTAINING

Liver sections were prepared and stained for NGAL (GeneTex, Irvine, CA) or myeloperoxidase (myeloperoxidase antibody 1; Thermo Fisher Scientific) by immunohistochemistry using paraffin-embedded samples according to the manufacturer's instructions. All

ARTICLE INFORMATION:

From the Department of Gastroenterology and Hepatology, Graduate School of Medicine, Mie University, Mie, Japan.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Akiko Eguchi, Ph.D.
Department of Gastroenterology and Hepatology
Graduate School of Medicine, Mie University
2-174 Edobashi
Tsu Mie, 514-8507, Japan
E-mail: akieguchi@clin.medic.mie-u.ac.jp
Tel: + 81-59-231-5017

photographs were taken by KEYENCE (BZ-X710) (KEYENCE, Japan).

IMMUNOBLOT ANALYSIS

For immunoblot analysis, 20 μg of whole-liver lysate was resolved by TGX precast gels (BioRad, Hercules, CA) and transferred to polyvinylidene fluoride membranes (BioRad). Blotted membranes were incubated with anti-NGAL antibody (Abcam, Cambridge, United Kingdom) followed by peroxidase-conjugated secondary antibody (GE Healthcare Life Sciences, Pittsburgh, PA). Protein bands were visualized using enhanced chemiluminescence reagents (Thermo Fisher Scientific) and digitized using a charge-coupled device camera (LAS4000 mini; Fuji film, Japan). Expression intensity was quantified by Multi Gauge (Fuji). Protein load was verified using β-actin (GeneTex) antibody.

REAL-TIME POLYMERASE CHAIN REACTION

Total RNA was isolated from liver tissue using Trizol (Thermo Fisher Scientific) followed by DNase treatment (Thermo Fisher Scientific), according to the manufacturer's instructions. Complementary DNA was synthesized from 500 ng of total RNA using the complementary DNA synthesis kit (Takara, Shiga, Japan). Real-time polymerase chain reaction (PCR) quantification was performed with forward and reverse primers and KAPA SYBR FAST quantitative PCR master mix (KAPA Biosystems, Woburn, MA) in a 7300 Real-Time PCR Detection System (Thermo Fisher Scientific). The PCR primers were used to amplify each gene as listed in Supporting Table S1. Mean values were normalized to β2 microglobulin for messenger RNA (mRNA).

HUMAN SAMPLES

The study protocol was approved by the ethics committee of Mie University, Mie, Japan. From December 2013 to August 2016, patients (N = 96) were recruited by the stage of their chronic liver disease, which was clinically diagnosed by blood tests, ultrasound or computed tomography images, FibroScan (Echosens, French) results, and esophageal varix by endoscopy, as general protocol. Liver biopsy was performed when the diagnosis was difficult using the general protocol. HCC was diagnosed based on histologic findings or typical imaging characteristics and classified according to Barcelona Clinic Liver Cancer staging classification.[15] Patients who had other malignancies within the past 3 years, severe hepatic failure (serum Bil level > 5 mg/dL), 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 and were excluded. Blood samples were collected for measurement of serum NGAL, Alb, Bil, aspartate transaminase, ALT, gamma glutamyl transferase, blood urea nitrogen (BUN), Cr, estimated glomerular filtration rates (eGFR),[16] Na, C-reactive protein (CRP), branched-chain amino acids and tyrosine molar ratio, platelet count, prothrombin time (%), alpha-fetoprotein, and protein induced by vitamin K absence-II. The fibrosis index based on 4 factors was calculated.[17]

STATISTICAL ANALYSES

All data were expressed as mean ± SEM unless otherwise noted. Data were analyzed using unpaired *t* tests or Mann-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 curve (ROC) analysis for survival. The predicted value of NGAL and Alb levels or NGAL and Na levels was calculated by multiple regression analysis, and the predicted value was applied to calculate the cutoff value using ROC analysis. 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 Graph Pad (Graph Pad Software Inc., CA) for comparison of continuous variables. Differences were considered to be significant at $P < 0.05$.

Results

BLOOD NGAL LEVELS REFLECT LIVER FUNCTION AND DETERMINE SURVIVAL IN RATS WITH CIRRHOSIS

To investigate whether blood NGAL levels are associated with survival in chronic liver diseases, we used a

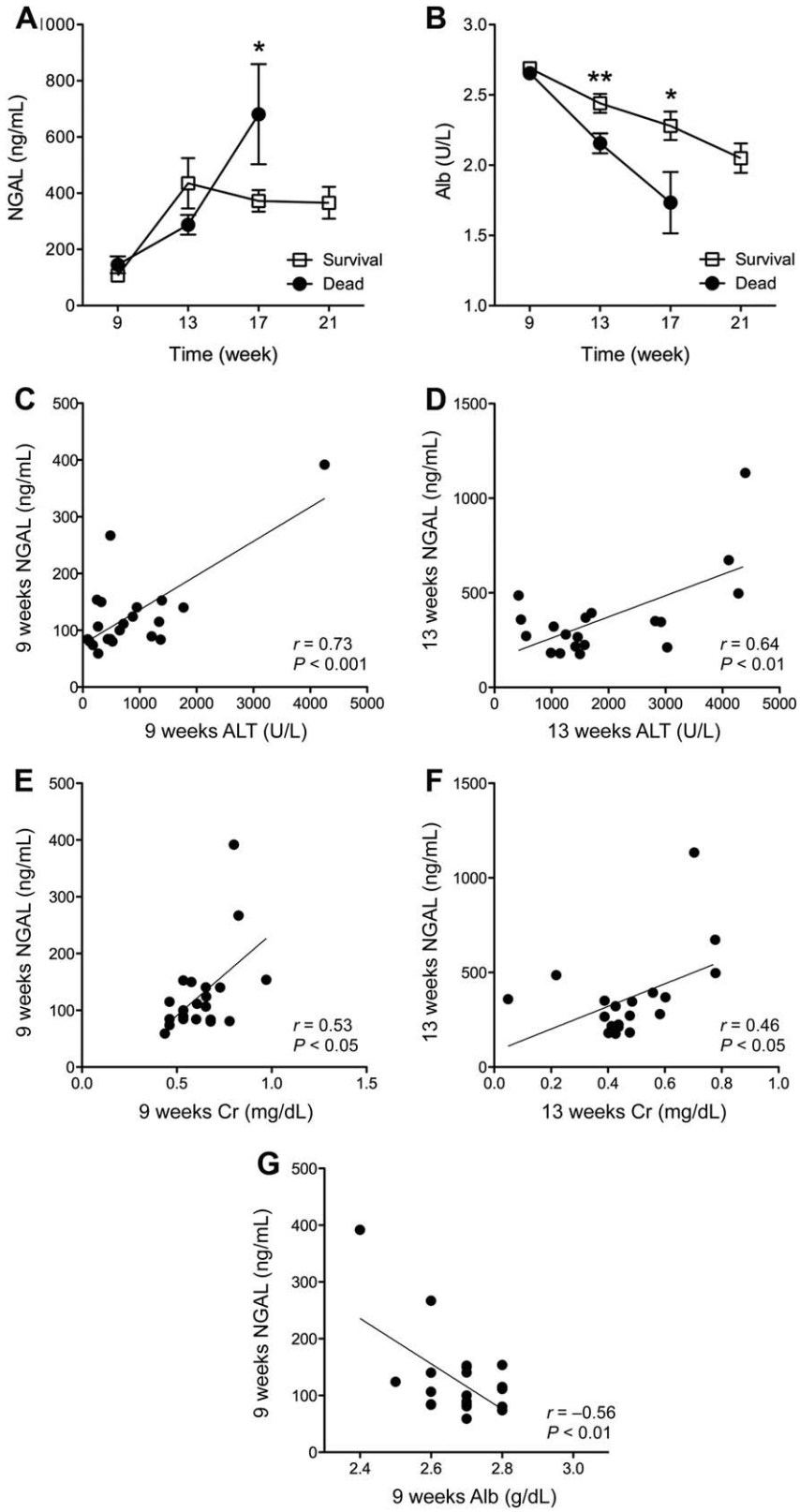

FIG. 1. Blood NGAL levels were correlated with liver and kidney function in rats with cirrhosis. (A,B) Time course of (A) blood NGAL or (B) Alb levels in deceased and survival groups of rats with cirrhosis. $^{**}P < 0.01$, $^{*}P < 0.05$. (C,D) Correlation between blood NGAL levels and ALT at (C) 9 weeks or (D) 13 weeks in rats with cirrhosis administered CCl_4. (E,F) Correlation between blood NGAL levels and Cr at (E) 9 weeks or (F) 13 weeks in rats with cirrhosis. (G) Correlation between blood NGAL levels and Alb at 9 weeks in rats with cirrhosis. Values are mean ± SEM.

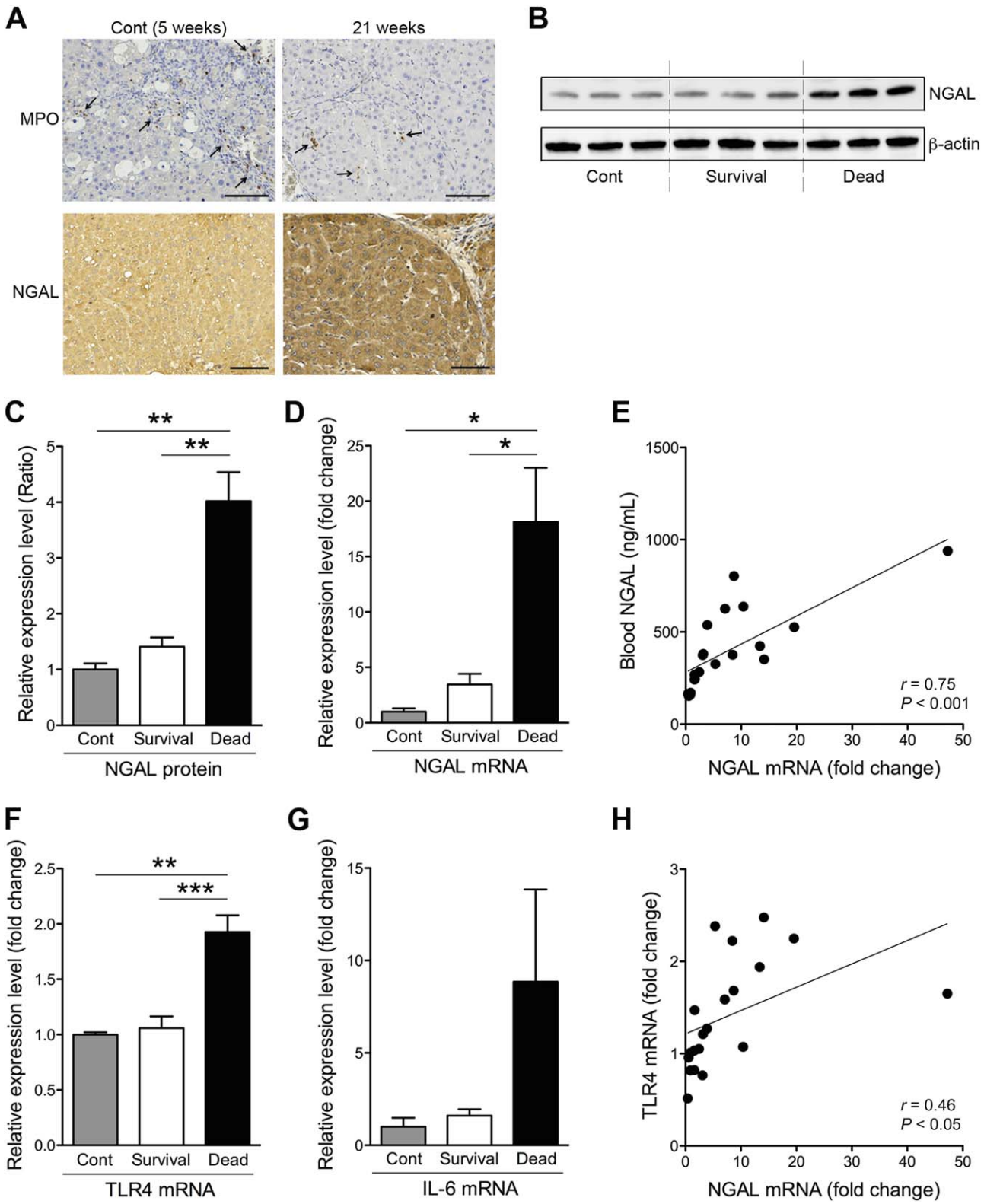

FIG. 2. Liver NGAL, TLR4, and IL-6 levels were elevated in deceased group of rats with cirrhosis, and liver NGAL levels were correlated with blood NGAL levels. (A) Immunohistochemical staining of liver sections from rats with cirrhosis specific for NGAL or MPO (neutrophils) at 5 weeks (control) or 21 weeks of CCl_4 administration. Scale bar, 100 μm. Arrows point to MPO-positive cells/area. (B) Protein expression of NGAL and β-actin in whole liver, using immunoblotting. (C) Bar graph shows quantification of NGAL normalized with β-actin. (D,F,G) Gene expression of (D) NGAL, (F) TLR4, or (G) IL-6 genes as measured by qPCR. All gene expression levels were normalized to housekeeping control *β2 microglobulin* and shown relative to the expression levels of control rats. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. (E,H) Correlation between liver NGAL mRNA levels and (E) TLR4 mRNA levels or (H) blood NGAL levels. Values are mean ± SEM. Abbreviations: Cont, control; MPO, myeloperoxidase; qPCR, quantitative PCR.

cirrhosis rat model administered with CCl_4 for 21 weeks (5 weeks to establish cirrhosis and an additional 16 weeks to determine survival percentage), which we have reported.[14] Our macroscopic findings showed that the end stage of all rats with cirrhosis, survival (n = 10) and deceased (n = 11), had liver failure with atrophy of the liver and enlargement of the spleen plus ascites (n = 8) and pleural fluid (n = 9), but the frequency of ascites and pleural fluid was not changed between survival and deceased groups. Blood NGAL levels were consistently up-regulated in a time-dependent manner and were significantly increased in the deceased group when compared to the survival group ($P < 0.05$; Fig. 1A), whereas NGAL levels were slightly decreased at 17 weeks in the survival group (Fig. 1A). Alb levels were decreased in a time-dependent manner in both deceased and survival groups but were significantly reduced in the deceased group compared to the survival group ($P < 0.01$ at 13 weeks; $P < 0.05$ at 17 weeks; Fig. 1B). Cystatin C levels were not changed between the survival and deceased groups (Supporting Fig. S1). We further investigated the correlation between blood NGAL levels and liver/renal functions, such as ALT, Cr, and Alb, at 9 weeks and 13 weeks. Blood NGAL levels were significantly correlated with ALT at 9 weeks ($P < 0.001$; Fig. 1C) and 13 weeks ($P < 0.01$; Fig. 1D) and with Cr at 9 weeks ($P < 0.05$; Fig. 1E) and 13 weeks ($P < 0.05$; Fig.1F). Blood NGAL levels were negatively correlated with Alb at 9 weeks ($P < 0.01$; Fig. 1G).

LIVER NGAL EXPRESSION IS ASSOCIATED WITH SURVIVAL AND CORRELATED WITH BLOOD NGAL LEVELS

Several reports have shown that liver NGAL expression is elevated during acute liver injury.[8,18] Therefore, we examined whether NGAL expression is up-regulated in liver cirrhosis and associated with survival. As a potential source of NGAL expression, neutrophil infiltration was limited in the control group (5 weeks of CCl_4 administration) and the survival group (given an additional 16 weeks of CCl_4 administration) of rats with cirrhosis as indicated by immunohistochemistry with myeloperoxidase antibody (Fig. 2A). In contrast, NGAL was expressed in the hepatocytes of the control rats and was increased in the survival rats, which we assessed by immunohistochemistry with NGAL antibody (Fig. 2A). These results indicated that hepatocytes are the major source of NGAL expression. Liver NGAL expression from whole liver lysates was significantly up-regulated in the deceased group when compared to the survival group ($P < 0.05$) as assessed by immunoblotting (Fig. 2B,C). Liver NGAL mRNA levels were also significantly increased in the deceased group when compared to the control ($P < 0.05$) or survival ($P < 0.05$) group (Fig. 2D). Notably, blood NGAL levels were significantly correlated with liver NGAL mRNA levels ($P < 0.001$; Fig. 2E), suggesting blood NGAL was derived from the cirrhotic liver. NGAL expression is regulated by the toll-like receptor 4 (TLR4) to interleukin (IL)-6 signaling pathway, which led us to further investigate liver TLR4 and IL-6 mRNA levels.[18] Liver TLR4 mRNA levels were significantly elevated in the deceased group when compared to the control ($P < 0.01$) or survival ($P < 0.001$) group (Fig. 2F), and liver IL-6 mRNA levels showed a modest but not significant increase in the deceased group (Fig. 2G). Liver NGAL mRNA levels were significantly correlated with liver TLR4 mRNA levels ($r = 0.46$, $P < 0.05$; Fig. 2H).

BLOOD NGAL LEVELS ARE A PROGNOSTIC FACTOR FOR SURVIVAL IN HUMAN CHRONIC LIVER DISEASE

We demonstrated that blood NGAL levels were associated with liver function and survival in rats with cirrhosis. Therefore, we further investigated whether blood NGAL levels can be used as a prognostic factor in human chronic liver diseases. We recruited 96 patients (63 male and 33 female; median age 69 ± 1 years) consisting of 74 patients with cirrhosis and 22 patients with chronic liver disease. The cohort of study patients was admitted to our study due to a variety of causative agents: 11 with hepatitis B virus, 35 with hepatitis C virus, 5 with nonalcoholic steatohepatitis, 19 with alcoholism, 7 with primary biliary cholangitis/autoimmune hepatitis, and 19 unknown. Of the 96 patients, 54 had HCC in addition to chronic liver disease. Blood NGAL levels were significantly correlated with Alb ($r = -0.36$, $P < 0.001$; Fig. 3A), BUN ($r = 0.47$, $P < 0.001$; Fig. 3B), Cr ($r = 0.30$, $P < 0.01$; Fig. 3C), Na ($r = -0.31$, $P < 0.01$; Fig. 3D), and estimated glomerular filtration rate (eGFR; $r = -0.35$, $P < 0.001$; Fig. 3E), suggesting a predictive ability inherent in blood NGAL levels for liver damage and renal function.[19] There were no significant correlations between NGAL levels and Bil, transaminases,

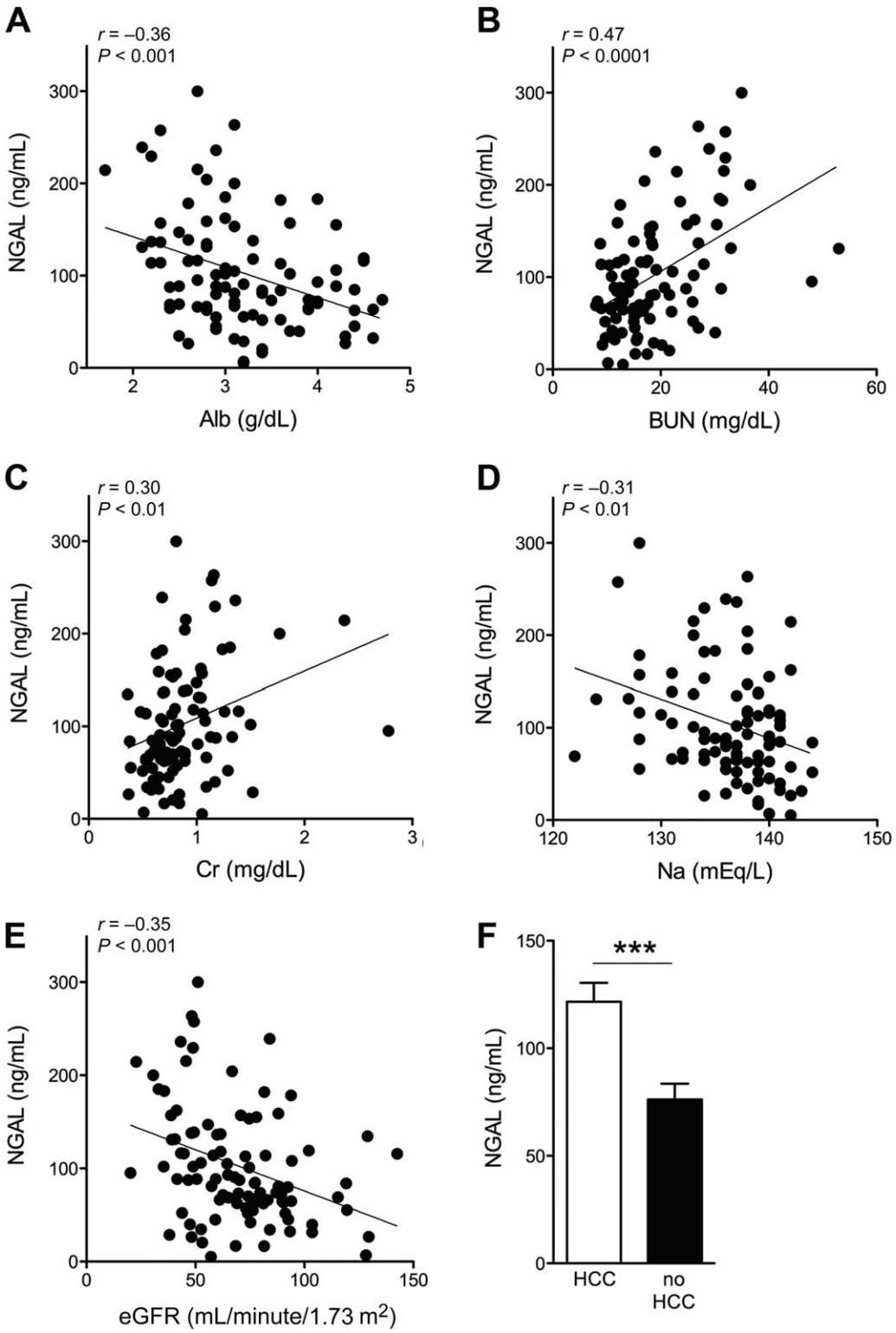

FIG. 3. Blood NGAL levels in human chronic liver disease were correlated with liver and kidney functions and were elevated in patients with chronic liver disease complicated with HCC. (A-E) Correlation of blood NGAL levels with (A) Alb, (B) BUN, (C) Cr, (D) Na, or (E) eGFR. (F) Bar graph shows blood NGAL levels in human chronic liver diseases complicated with or without HCC. $^{***}P < 0.001$. Values are mean ± SEM.

TABLE 1. PATIENT CHARACTERIZATION SEPARATED BY BLOOD NGAL LEVELS, 119 ng/mL

	NGAL ≤ 119	NGAL > 119
Patient number	68	28
Sex (M/F)	48/20	15/13
Age (years)	67.1 ± 1.4	72.8 ± 1.6*
HBV/HCV/NASH/AL/AIH-PBC/others	8/22/4/15/6/13	3/13/1/4/1/6
HCC (+/−)	32/36	22/6†
BCLC (0/A/B/C/D)	4/8/12/6/5	2/1/4/9/6
AFP (ng/mL)	5,814 ± 3154	1,355 ± 701
PIVKA-II (mAU/mL)	135,624 ± 121,354	15,776 ± 5,165
Alb (g/dL)	3.4 ± 0.1	2.8 ± 0.1‡
PT (%)	80.9 ± 2.6	76.9 ± 4.3
AST (IU/L)	66 ± 8	86 ± 15
ALT (IU/L)	44 ± 7	43 ± 6
GGT (IU/L)	142 ± 20	111 ± 23
T-Bil (mg/dL)	1.7 ± 0.2	1.7 ± 0.4
BTR (μmol/L)	4.5 ± 0.28	3.97 ± 0.41
PLT ($\times 10^4$ cells/μL)	14.5 ± 1	17.2 ± 2.6
BUN (mg/dL)	16.5 ± 0.8	25.1 ± 1.8‡
Cr (mg/dL)	0.83 ± 0.04	0.99 ± 0.07
eGFR (mL/minute/1.73 m^2)	74.6 ± 2.9	57.5 ± 4.4†
Na (mEq/L)	137 ± 1	134 ± 1†
CRP (mg/dL)	1.67 ± 0.36	2.63 ± 0.54

*$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$; values are mean ± SEM.
Abbreviations: AFP, alpha-fetoprotein; AIH, autoimmune hepatitis; AL, alcoholic liver disease; AST, aspartate transaminase; BCLC, Barcelona Clinic Liver Cancer; BTR, branched-chain amino acids to tyrosine ratio; GGT, gamma glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PIVKA-II; protein induced by vitamin K absence-II; PLT, platelet count; PT, prothrombin time; T-Bil, total bilirubin.

CRP, platelet count, prothrombin time, or fibrosis-4 index. Because Child-Pugh and MELD scores do not take into account the complications of HCC, we further investigated whether blood NGAL levels were affected by HCC in chronic liver diseases. Blood NGAL levels were significantly elevated in the presence of HCC coupled with chronic liver disease when compared to patients without HCC ($P < 0.001$; Fig. 3F), whereas there was no difference in the level of NGAL levels between presence and absence of cirrhosis. There were no significant correlations of NGAL levels with blood tumor marker levels or Barcelona Clinic Liver Cancer staging classification (data not shown). Regarding survival, 38 out of 96 patients were deceased in the average follow-up period of 9.9 months (median of 9 months). Thus, all causes of death were considered liver related. Finally, we calculated the cutoff value of blood NGAL levels as 119 ng/mL (area, 0.645; sensitivity, 0.842; specificity, 0.473) from ROC analysis of survival curves (Supporting Fig. S1). Alb, BUN, eGFR, Na, incidence of HCC, and age were significantly increased in patients with NGAL >119 ng/mL compared to patients with NGAL ≤119 ng/mL, although there was no difference in these patients concerning other factors, such as incidence of cirrhosis and blood tumor marker levels, other liver function tests, or CRP (Table 1{TABLE1}). We also calculated the cutoff value of blood Alb as 3.2 g/dL (area, 0.709; sensitivity, 0.614; specificity, 0.789) and Na as 135 mEq/L (area, 0.658; sensitivity, 0.811; specificity, 0.486) levels from ROC analysis of survival curves (Supporting Fig. S2). When we compared NGAL levels with Alb or Na levels, the ROC was 0.720 or 0.701, respectively (Supporting Fig. S2). The survival rate was significantly decreased in patients with NGAL >119 ng/mL compared to patients with NGAL ≤119 ng/mL ($P < 0.001$; Fig. 4A). Furthermore, Alb ≤ 3.2 g/dL ($P < 0.001$; Fig. 4B), Na ≤ 135 mEq/L ($P < 0.001$; Fig. 4C), and the presence of HCC ($P < 0.001$; Fig. 4D) were indicative of a poor survival rate. There was no difference in survival rates with respect to other factors, such as age, sex, or renal function (data not shown).

Discussion

Our study reveals that blood NGAL levels and liver NGAL expression are significantly increased in the deceased group of rats with cirrhosis, suggesting an association of blood NGAL levels with survival rate. We also verified that blood NGAL levels can be used as a prognostic factor in patients with chronic liver disease. Notably, blood NGAL levels appear to predict the rate of survival, even in patients presenting with HCC in addition to their underlying chronic liver disease.

Several animal studies have demonstrated that NGAL expression is increased in a variety of organs, such as lung and liver, and is caused by a bacterial infection in rodent acute models.[8,20] In addition, clinical studies show NGAL is expressed in the liver of patients with acute-on-chronic liver disease.[5] We also showed that NGAL is expressed in the liver, especially hepatocytes, of rats with cirrhosis and demonstrate that liver NGAL expression, which correlated with blood NGAL levels, was significantly elevated in the deceased group of rats with cirrhosis. This study is the first to identify liver NGAL expression as a prognostic factor in cirrhosis as part of the spectrum of chronic liver disease.

We found a significant correlation between NGAL mRNA levels and TLR4 mRNA levels in the livers of

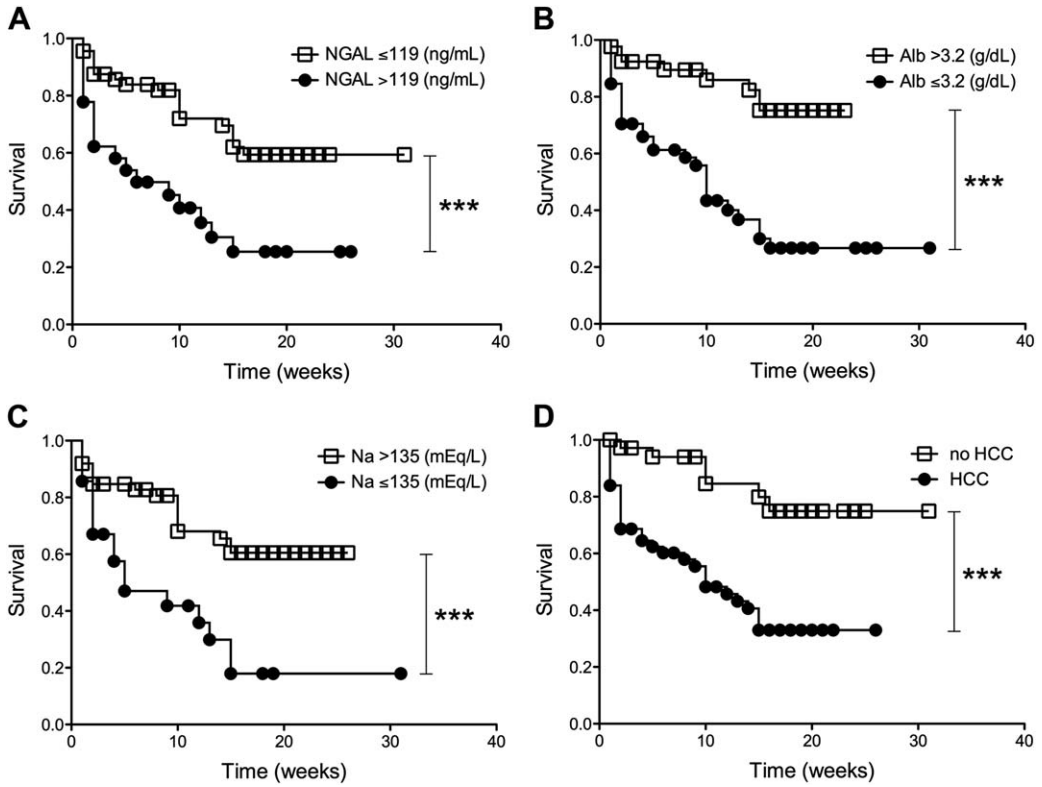

FIG. 4. Survival was improved in human chronic liver diseases guided by several factors: blood NGAL levels, blood Alb levels, blood Na levels, or complication with HCC. (A-D) Survival curve in human chronic liver diseases with (A) blood NGAL levels, (B) Alb levels, (C) Na levels, or (D) complicated with or without HCC. $^{***}P < 0.001$.

rats with cirrhosis. Several reports have shown that NGAL expression is mediated by lipopolysaccharide (LPS) in the lung and liver,[21] and NGAL is secreted from the cultured chondrocytes stimulated by LPS through TLR4 activation.[18] Up-regulation of NGAL expression is also induced by cytokines, such as IL-6, IL-1β, IL-10, IL-17, tumor necrosis factor-α, and tumor growth factor-β.[8,22] Because we did not observe a correlation between NGAL mRNA levels and levels of IL-6 mRNA in the cirrhotic rat liver, up-regulated liver NGAL expression may be mediated by infiltrated LPS in the cirrhotic liver following TLR4 activation. However, future studies are required to explore compounds[23] that lead to TLR4 expression and damage-associated molecular patterns that lead to TLR4 and IL-6 expression released from necrotic hepatocytes in the acute liver injury model.[24]

Patients with cirrhosis often have complications with acute kidney injury (AKI), resulting in a poor rate of survival.[25] The diagnosis of cirrhosis in patients complicated with AKI was difficult due to the diminished specificity of Cr, which is elevated in both acute tubular necrosis in AKI and hepatorenal syndrome. However, current reports show that NGAL is elevated in acute tubular necrosis but not elevated in hepatorenal syndrome, resulting in NGAL being useful as a biomarker for AKI diagnosis in addition to cirrhosis.[26] Our data also support the current findings that blood NGAL levels are significantly correlated with kidney function in rat and human chronic liver diseases.[2,4]

In this study, we also showed that patient survival rate can be predicted based on blood NGAL levels greater than or less than 119 ng/mL in chronic liver diseases, including those complicated by HCC. Child-Pugh and MELD scores are used as predictive measures of prognosis for patients who have cirrhosis and chronic liver diseases following liver failure,[12,13] but these scores do not consistently reflect complications, such as HCC, in chronic liver diseases. 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.[27,28] Previous clinical studies regarding NGAL function showed NGAL expression is increased in the early stages of bacterial infection[21,29] and strongly inhibits the proliferation of *Escherichia coli* and tubercle bacillus due to the ability of NGAL to chelate iron.[20,30] In addition, NGAL is expressed within hepatic tumors leading to an elevation of blood NGAL levels in patients with HCC.[7] Our data also indicate that the blood level of NGAL is increased in human chronic liver diseases complicated with HCC but is not associated with alpha-fetoprotein or protein induced by vitamin K absence-II, which is a biomarker of HCC. Based on this evidence and the results from our cirrhotic rat model, elevated human blood NGAL levels can determine cirrhosis despite multiple tangential complications, such as HCC, liver failure, infection, and AKI, which suggests a role for NGAL as a potentially new prognostic factor for survival and therapeutic outcomes in chronic liver diseases. Our findings warrant future larger clinical studies to further investigate the utility of monitoring blood NGAL as a prognostic factor of survival in chronic diseases of the liver.

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Author names in bold designate shared co-first authorship.

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