Urine Liver-Type Fatty Acid Binding Protein; Biomarker for Diagnosing Acute Kidney Injury and Predicting Mortality in Cirrhotic Patients

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ABSTRACT

Objective: To determine impact of urine liver-type fatty acid binding protein (uL-FABP) and urine neutrophil gelatinase-associated lipocalin (uNGAL), which were biomarkers linked to acute kidney injury (AKI), in AKI diagnosis and prediction of 28-day mortality among hospitalized cirrhotic patients.

Materials and Methods: We prospectively enrolled hospitalized cirrhotic patients at a tertiary care university hospital between June 2018 and November 2019. The uL-FABP, uNGAL, and plasma NGAL (pNGAL) were collected within 48 hours of admission. Cutoff values of biomarkers for diagnosing AKI derived from receiver operating characteristic (ROC) curve. Logistic regression analysis was used to identify independent factors for 28-day mortality.

Results: We enrolled 109 cirrhotic patients in derivative cohort, 41.3% had AKI. Median uL-FABP, uNGAL, and pNGAL levels in AKI group were higher than non-AKI group: 8.1 vs. 2.8 ng/mL (p=0.002), 40.5 vs. 10.1 ng/mL (p<0.001), and 195.7 vs 81.4 ng/mL (p=0.001), respectively. Areas under the ROC curve of uL-FABP, uNGAL, and pNGAL for AKI diagnosis were 0.68, 0.73 and 0.68, respectively. Also, all biomarkers were significantly higher in mortality group. Multivariate analysis showed that the only independent predictor for 28-day mortality was uL-FABP 4.68 ng/mL (odd ratio 4.15, p=0.02).

Conclusion: UL-FABP, uNGAL, and pNGAL are associated with AKI in hospitalized cirrhotic patients. Moreover, uL-FABP 4.68 ng/mL was a significant independent predictor for 28-day mortality.

Keywords: Acute kidney injury; cirrhosis; liver-type fatty acid binding protein; mortality; neutrophil gelatinase-associated lipocalin; biomarker (Siriraj Med J 2024; 76: 198-208)

INTRODUCTION

Acute kidney injury (AKI) is a common complication in cirrhotic patients. Twenty to fifty percent of hospitalized cirrhotic patients had AKI, which was related to higher mortality and increased length of stay.[1,2] However, the diagnosis of AKI in cirrhotic patients has some limitations, including false low serum creatinine due to low muscle mass and an increase in serum bilirubin, which causes delayed diagnosis.[3]

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Prompt diagnosis of AKI and appropriate treatment in hospitalized cirrhotic patients are essential to reduce short-term mortality.[4] Several urinary biomarkers have been studied for their possible role in early diagnosis and predicting the risk of AKI progression. Among them, neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), and liver-type fatty acid binding protein (L-FABP) are currently demonstrated to guide the early diagnosis of AKI and differentiate types of AKI.[5] Urinary biomarkers for the early diagnosis of AKI are applied in several clinical settings other than cirrhosis, for example, post-cardiac surgery, pre-liver transplantation, and mixed intensive care units.[6-11] Recent research in the cirrhosis population has shown that these biomarkers can be utilized to diagnose AKI[12] and differentiate acute tubular necrosis (ATN) from non-ATN in patients with cirrhosis.[12,13]

Two biomarkers for early detection of AKI are L-FABP and NGAL. L-FABP prevents renal ischemic injury by binding to reactive oxygen species (ROS) and excretes them from proximal tubules into urine.[14] NGAL is produced in an ischemic state or after exposure to the renal toxin in tubular cells in thick ascending limbs or collecting ducts.[15] These biomarkers exhibited an increase in level prior to the elevation of serum creatinine, as early as four hours after the onset of AKI.[16] Therefore, this characteristic was more appropriate for the early detection of AKI in comparison to serum creatinine.

Several studies in cirrhotic patients demonstrated the role of urine nGAL (uNGAL) for diagnosis of new-onset AKI in hospitalized cirrhotic patients[17], increased levels of uNGAL and urine L-FABP (uL-FABP) in AKI progression[18], and in mortality group.[19] However, none of these studies evaluated the role of uL-FABP in the early diagnosis of AKI and mortality in cirrhotic patients.

The purpose of this study was to identify the accuracy to determine the relationship between biomarkers, including uL-FABP, uNGAL, and plasma NGAL (pNGAL), for the diagnosis of AKI and association with 28-day mortality in hospitalized cirrhotic patients.

MATERIALS AND METHODS

Patient population

We prospectively enrolled 139 consecutive hospitalized cirrhotic patients with a risk of AKI at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Participants who were admitted between June 2018 and November 2019 were enrolled. Patients were divided into 2 cohorts: 109 patients in derivative cohort, and 30 patients in validation cohort gathered in subsequent 6 months to validate the performance of biomarkers for the diagnosis of AKI and the prediction of mortality. Inclusion criteria included a known diagnosis of cirrhosis, presence of risk of AKI, which included gastrointestinal bleeding, bacterial infection, diarrhea, vomiting, poor intake, large-volume paracentesis, excessive diuretics usage, taking nephrotoxic drugs, decompensated cirrhosis, and age ≥18 years. The exclusion criteria were prior organ transplantation, end-stage renal disease with renal replacement therapy at the time of enrollment, acute interstitial nephritis, acute glomerulonephritis, post-renal AKI, current use of immunosuppressive agents other than treatment of severe alcoholic hepatitis, severe extrahepatic disease, and pregnancy. The protocol was approved by the Institutional Review Board and the Ethics Committee of the Faculty of Medicine, Chulalongkorn University (IRB number 196/61), and was registered at https://www.thaiclinicaltrials.org/show/TCTR20211121002. The registration identification number is TCTR20211121002. All patients, or their legal guardian, gave written informed consent in accordance with the Declaration of Helsinki prior to study enrollment. The manuscript was prepared and revised according to the STARD 2015 checklist.

Study design

Baseline characteristics, clinical data, and laboratory data were obtained within the first 48 hours of admission. The second urine samples and other laboratory data were collected within 48 hours of AKI diagnosis if the patients developed new-onset AKI in admission. Both cirrhotic or other complications were recorded and managed standardly by primary physicians. Patients were follow-up for a minimum of 28 days, and the 28-day mortality rate was recorded.

Sample collection and biomarker measurement

Urine and blood samples were collected within the first 48 hours of admission and centrifuged at 3,000 revolutions per minute (rpm) at 25°C for 10 minutes before being stored at -80°C until assayed. UL-FABP was measured by latex turbidimetric immunoassay using a Norudia® L-FABP (Sekisui Medical CO., Ltd., Tokyo, Japan), with a lower detection limit of 1.5 ng/mL. A UL-FABP level below this value was reported as 0.75 ng/mL. Urine and plasma NGAL were tested by enzyme-linked immunosorbent assay (ELISA) (R&D, Minneapolis, MN, USA). Both results were reported in ng/mL. All biomarker testing was performed by two scientists (JD., ST.) in the critical care laboratory center of nephrology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand.

Definitions of variables

The diagnosis of cirrhosis was based on clinical, imaging, laboratory, or histology assessments. AKI was defined by Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Acute Kidney Injury 2012 as an increase in serum creatinine by ≥0.3 mg/dL within 48 hours or an increase in serum creatinine to ≥1.5 times from baseline, which is known or presumed to have occurred within the prior 7 days. We did not use urine volume depletion < 0.5 mL/kg/h for 6 hours as one of the criteria due to inaccuracy of urine output monitoring. AKI in cirrhotic patients is categorized into 3 types. The first is prerenal azotemia, including hepatorenal syndrome (HRS), defined by revised consensus recommendations of the International Club of Ascites 2015[3]; the second is intrinsic renal AKI, including ATN, acute interstitial nephritis (AIN), and glomerulonephritis; and the last post-renal obstruction.[20] Acute on chronic liver failure (ACLF) was defined and graded according to European Association for the Study of the Liver (EASL) criteria.[21] In this study, HRS was separated from prerenal azotemia. Scoring systems including a model for end-stage liver disease (MELD), chronic liver failure-sequential organ failure assessment (CLIF-SOFA), SOFA, and Child-Turcotte-Pugh (CTP) score were calculated at the time of enrollment.

Treatment outcomes

The primary outcome was the performance of uL-FABP, uNGAL, and pNGAL for the diagnosis of AKI compared to creatinine which is a standard of care in hospitalized cirrhotic patients. The secondary outcome was factors in predicting 28-day mortality in hospitalized cirrhotic patients.

Statistical analysis

A sample size of 99 patients was needed to identify AKI using a uNGAL cutoff value 56 ng/mL published in the previous study with 77% sensitivity in diagnosis of AKI, 29% prevalence of AKI in cirrhotic patients[17], for 80% power, and a two-sided α of 0.05. Categorical variables were analyzed by Chi-square or Fisher's exact test, and continuous variables were analyzed by Student's t-test or Mann-Whitney test. Normally distributed variables are reported as the means with standard deviations, and nonnormally distributed variables are reported as medians with interquartile ranges (IQRs). The area under the receiver operating characteristic curve (AUC) was calculated to assess the performance of biomarkers for the diagnosis and discrimination of AKI and the prediction of mortality. Univariate and multivariate logistic regression models were used to evaluate the association between these biomarkers and mortality. All statistical analyses were performed using the SPSS statistical analysis package (version 23.0.0; SPSS Inc., Chicago, Illinois, USA), and a p-value of < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

One hundred and fifty-eight hospitalized cirrhotic patients with a risk of AKI were included. Of these, 19 patients (12%) were excluded due to end-stage renal disease (10 patients, 6.3%), delayed sample collection (3, 1.9%), anuria (3, 1.9%), and incomplete data (3, 1.9%). A total of 139 patients were finally enrolled in the study. We consecutively assigned participants in the whole dataset into a derivation cohort for 109 patients (80%) and a validation cohort for 30 patients (20%) (Fig 1).

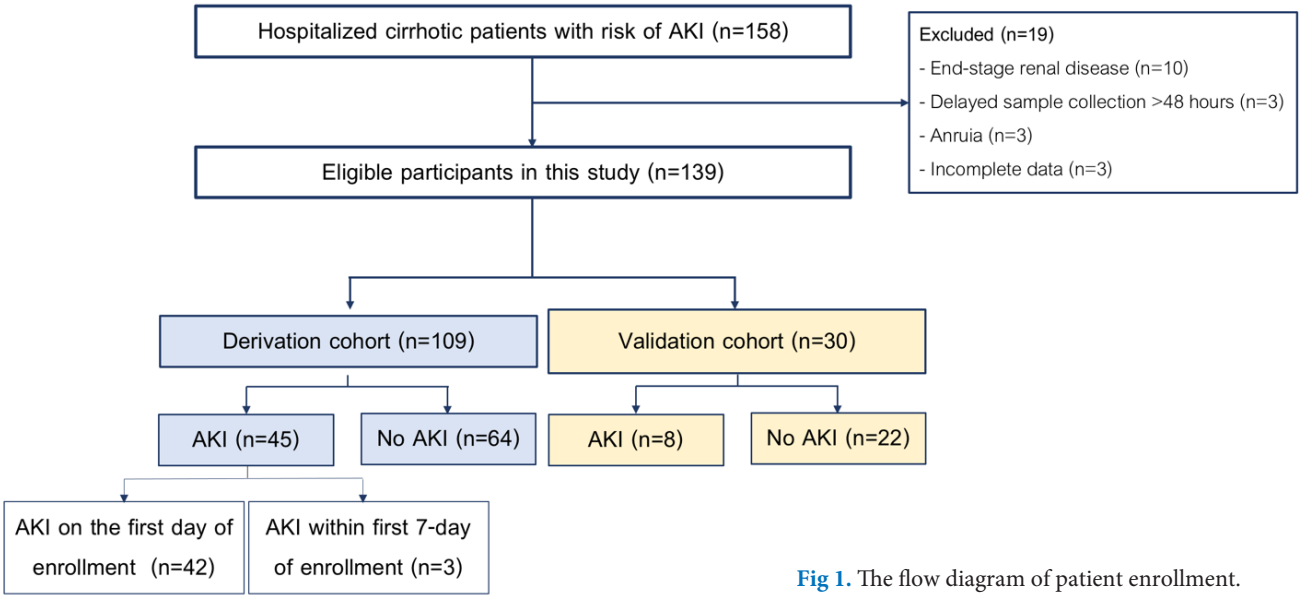

Fig 1. The flow diagram of patient enrollment.

The derivation cohort included a total of 109 patients; 85 (78%) were male, and 51 (46.8%) had CTP class C. The mean age was 59.0±12.3 years, and the median MELD score was 21.0 (IQR 16-27). The most common causes of cirrhosis were alcoholic liver disease (34 patients, 31.2%), followed by chronic hepatitis B (30, 27.5%), chronic hepatitis C (27, 24.8%), and metabolic dysfunction-associated steatohepatitis (7, 6.4%). Fifty-one patients had hepatocellular carcinoma (46.8%). The most common risks of AKI included gastrointestinal bleeding (44, 40.4%), followed by bacterial infection (42, 38.5%), and liver decompensation without identified precipitating causes (11, 10%) (Supplementary Table 1).

The baseline demographic, clinical, and laboratory data of cirrhotic patients with and without AKI were shown in Table 1. A total of forty-five patients had AKI,

TABLE 1. Patient characteristics and baseline laboratory parameters of derivation cohort (n=109).

Variables	Total (n=109)	No AKI (n=64)	AKI (n=45)	p-value
Age (years), mean ±S.D.	59.0±12.3	58.3±12.6	60.1±11.9	0.440
Male sex, n (%)	85 (78%)	50 (78.1%)	35 (77.8%)	0.970
Cause of cirrhosis, n (%)				0.38
HBV/HCV	57 (52.3%)	37 (57.8%)	20 (44.4%)	
Alcohol	34 (31.2%)	18 (28.1%)	16 (35.6%)	
MASH	7 (6.4%)	4 (6.3%)	3 (6.7%)	
Cryptogenic	7 (6.4%)	3 (4.7%)	4 (8.9%)	
Other	4 (3.7%)	2 (3.1%)	2 (4.4%)	
Cancer, n (%)	55 (50.5%)	30 (46.9%)	25 (55.6%)	0.370
HCC	51 (92.7%)	28 (93.3%)	23 (92%)	1.000
Others	3 (5.5%)	1 (3.3%)	2 (8%)	
Laboratory baseline (median, IQR)				
WBC (x10^3/µL)	8.42 (6.65-12.63)	7.83 (6.10-9.89)	9.98 (7.13-14.45)	0.005
% Neutrophil	78 (70.35-84.55)	77.15 (70.17-82.55)	82 (70.4-86.8)	0.045
Neutrophil/lymphocyte ratio	6.2 (3.5-10.0)	5.3 (3.5-8.0)	8.2 (4.1-11.7)	0.011
Platelet (x10^3/µL)	117 (74-178)	106 (68-156)	140 (78-229)	0.041
INR	1.55 (1.37-1.79)	1.5 (1.36-1.66)	1.72 (1.43-2.06)	0.006
Creatinine (mg/dL)	1.0 (0.73-1.44)	0.79 (0.65-1.0)	1.54 (1.24-1.99)	<0.001
Sodium (mmol/L)	133 (128.5-135)	134 (131-137)	131 (127-133)	0.001
TB (mg/dL)	2.73 (1.75-6.43)	2.53 (1.35-4.57)	3.84 (2.13-13.43)	0.003
Albumin (g/dL)	2.6 (2.25-3.15)	2.65 (2.3-3.2)	2.6 (2.1-3.1)	0.240
Lactate (mmol/L)	3.2 (1.6-5.25)	2.05 (1.36-3.4)	4 (2.5-8.7)	0.003
MELD score	21 (16-27)	17 (13.25-22)	28 (22-31)	<0.001
MELD-Na score	22 (17-22)	22 (17-22)	21 (17-28)	0.748
CTP score				0.010
A	17 (15.6%)	15 (23.4%)	2 (4.4%)	
B	41 (37.6%)	25 (39.1%)	16 (35.6%)	
C	51 (46.8%)	24 (37.5%)	27 (60%)	
Plasma NGAL (ng/mL)	125.4 (54.7-251.4)	81.4 (42.2-185.2)	195.7 (80.9-408.4)	0.001
Urine NGAL (ng/mL)	15.1 (6.1-74.7)	10.1 (2.7-26.3)	40.5 (10.4-186.9)	<0.001
Urine L-FABP (ng/mL)	4.2 (2.0-14.3)	2.8 (1.7-8.4)	8.1 (2.6-28.4)	0.002

Abbreviations: AKI; acute kidney injury. CTP; Child-Turcotte-Pugh. HBV; hepatitis B virus. HCC; hepatocellular carcinoma. HCV; Hepatitis C virus. INR; international normalized ratio. IQR; interquartile range. L-FABP; liver-type fatty acid-binding protein. MELD; model for end-stage liver disease. MASH; metabolic dysfunction-associated steatohepatitis. MELD-Na; model for end-stage liver disease-sodium. NGAL; neutrophil gelatinase-associated lipocalin. S.D.; standard deviation. TB; total bilirubin. WBC; white blood cell count.

forty-two (93.3%) had AKI on the first day of enrollment and three (6.7%) patients developed AKI within the first 7 days of enrollment. The most common causes of AKI were prerenal (39 patients, 86.7%), ATN (3, 6.7%), HRS (2, 4.4%), and unclassified (1, 2.2%). The significant laboratories associated with AKI were higher level of white blood cell counts (WBC) (9.98 vs 7.83 x103/μL; p=0.005); percentage of neutrophil (82 vs 77; p=0.045); neutrophil to lymphocyte ratio (NLR) (8.2 vs 5.3; p=0.011); higher INR level (1.72 vs 1.50 mmol/L; p=0.006); higher ALP (178 vs 108; p=0.032); higher both total bilirubin (TB) (3.84 vs 2.53; p=0.003) and direct bilirubin (2.56 vs 1.24; p<0.001), and higher level of venous lactate (4.0 vs 2.0; p=0.003). In addition, the factors associated with AKI in cirrhotic patients were high MELD score (26.9 vs 17.9; p<0.001), and presence of advanced stage CTP C (60.0% vs 4.4%; p=0.010).

The hospital events and complications

The hospital events and complications during hospitalization were shown in Supplementary Table 1. Of 109 patients in derivation cohort, 27 patients (24.8%) expired, 15 patients (13.8%) developed bacterial infection, and 8 patients (7.3%) had organ failure. The rate of hospital-acquired bacterial infection (22.2% vs 7.8%; p=0.803) and new-onset organ failure (13.3% vs 3.1%; p=0.063) during admission were not different between patients with and without AKI. The bacterial infection mostly occurred on the average of day 7 from admission (range 2-27 days). The rate of overall infection was significantly higher in the AKI group than in the non-AKI group (53.3% vs 28.1%; p=0.004). Major sources of infection were spontaneous bacterial peritonitis (SBP) (17 patients, 39.5%), followed by septicemia (10, 23.8%).

Biomarkers and AKI diagnosis

All of 3 biomarkers, pNGAL, uNGAL, and uL-FABP, in patients with AKI were significantly higher than those in patients without AKI as follow: 195.7 vs 81.4 ng/mL (p=0.001), 40.5 vs 10.1 ng/mL (p<0.001), and 8.1 vs 2.8 ng/mL (p=0.002), respectively (Table 1).

The AUC analysis showed that all biomarkers could be used to diagnose AKI in hospitalized cirrhotic patients with comparable accuracy. The AUCs of pNGAL was 0.68 (95% CI 0.57-0.78, p=0.002), uNGAL was 0.73 (95% CI 0.63-0.82, p<0.001), uL-FABP was 0.68 (95% CI 0.57-0.78, p=0.002) compared to creatinine as a standard of care was 0.90 (95% CI 0.83-0.96, p<0.001) for AKI diagnosis (Fig 2A).

The optimal cutoff of each biomarker was determined according to receiver operating characteristic (ROC) curve analysis. The cutoff of uL-FABP was 4.68 ng/mL, providing 68.2% sensitivity and 65.6% specificity; uNGAL was 13.3 ng/mL, with 72.7% sensitivity and 62.5% specificity; and pNGAL was 127.35 ng/mL, with 63.6% sensitivity and 61.3% specificity (Table 2). The combination of multiple biomarkers improved the specificity for the diagnosis of AKI, but the sensitivity was reduced. UL-FABP combined with uNGAL had 56.8% sensitivity and 78.1% specificity, uL-FABP combined with pNGAL had 48.8% sensitivity and 80.6% specificity, and uNGAL combined with pNGAL had 51.2% sensitivity and 74.2% specificity. The combination of all biomarkers had 41.9% sensitivity and 83.9% specificity.

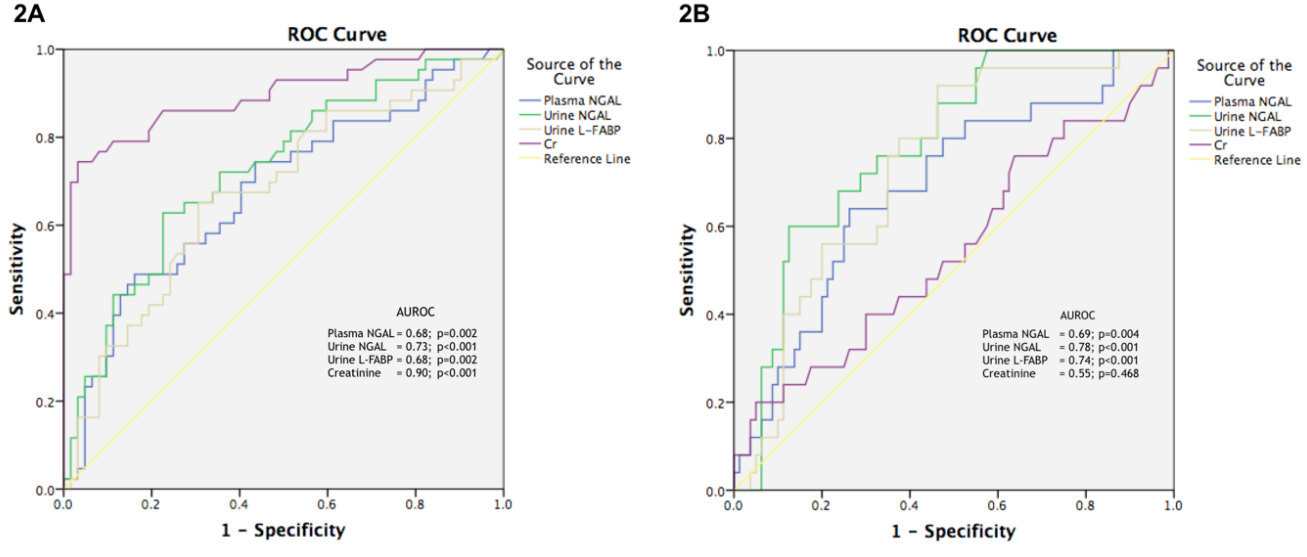

Fig 2A. Performance of pNGAL, uNGAL, uL-FABP, and creatinine for AKI diagnosis in hospitalized cirrhotic patients (n=109). **Fig 2B.** Performance of pNGAL, uNGAL, uL-FABP, and creatinine for predicting 28-day mortality in hospitalized cirrhotic patients (n=109).

TABLE 2. The performance of biomarkers for AKI diagnosis in hospitalized cirrhotic patients in a derivation cohort.

Bio-marker	AUC (95%CI)	p-value	Cut-off (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR
pNGAL	0.68 (0.57-0.78)	0.002	127.35	63.6	61.3	53.8	70.4	1.64	0.59
uNGAL	0.73 (0.63-0.82)	<0.001	13.3	72.7	62.5	57.1	76.9	1.94	0.44
uL-FABP	0.68 (0.57-0.78)	0.002	4.68	68.2	65.6	57.7	75.0	1.98	0.48
pNGAL with CF	0.88 (0.82-0.95)	<0.001	127.35	13.6	100	100	62	0	0.86
uNGAL with CF	0.90 (0.84-0.95)	<0.001	13.3	13.6	100	100	62.7	0	0.86
uL-FABP with CF	0.89 (0.83-0.95)	<0.001	4.68	13.6	100	100	62.7	0	0.86

Clinical factors included bacterial infection, BUN >20, CLIF-OF>10, and MELD score > 20

Abbreviations: AUC; area under the ROC curve. CF; clinical factors. L-FABP; liver-type fatty acid-binding protein. NGAL; neutrophil gelatinase-associated lipocalin. NPV; negative predictive value. PPV; positive predictive value. 95%CI; 95% confidence interval. +LR; positive likelihood ratio. -LR; negative likelihood ratio.

Univariate and multivariate analysis for the AKI diagnosis is shown in Supplementary Table 2. The predictors for the diagnosis of AKI were uNGAL ≥ 13.3 ng/mL (OR 5.75, 95% CI 1.53-21.66, p=0.01), MELD score > 20 (OR 5.03, 95% CI 1.33-19.01, p=0.02), bacterial infection (OR 3.63, 95% CI 1.09-12.09, p=0.04), CLIF-OF score (OR 1.60, 95% CI 1.08-2.36, p=0.02), and BUN (OR 1.07, 95% CI 1.03-1.12, p=0.002). We hypothesized that adding these clinical predictors might improve the accuracy of the studied biomarkers for AKI diagnosis. Clinical factors including presence of bacterial infection, BUN > 20, CLIF-OF > 10, and MELD score > 20 were incorporated into the biomarker in order to assess its sensitivity and specificity. As all clinical factors mentioned were incorporated along with the biomarkers at the optimal cutoff point, the test's specificity and positive predictive value demonstrated an increase in the results, as shown in Table 2. Additionally, the AUC for diagnosing AKI in hospitalized cirrhotic patients increased to 0.89 (95% CI 0.83-0.95) for uL-FABP, 0.90 (95% CI 0.84-0.96) for uNGAL, and 0.88 (95% CI 0.82-0.95) for pNGAL, as shown in Table 2.

According to small number of patients with ATN (3 patients) and HRS (2 patients), there was no significant difference in the levels of each biomarker among patients with prerenal azotemia, ATN, and HRS (p=0.18 for uL-FABP, p= 0.81 for uNGAL, p=0.08 for pNGAL) (Supplementary Table 3).

Biomarkers and prediction of 28-day mortality

Of 109 patients in derivation cohort; the 28-day overall mortality was 24.8%. Patients who died within 28 days after admission had a higher proportion of AKI (70.4% vs 31.7%; p<0.001), presence of cancer (77.8% vs 41.5%; p=0.001), and new-onset organ failure after admission (18.5% vs 3.7%; p=0.02) than those who survived (Supplementary Table 4). Serum sodium was not significantly different between these two groups (133 vs 129, p=0.06). The MELD and CLIF-OF scores were greater in the mortality group; 29 vs 20 (p<0.001) and 9 vs 6 (p<0.001) respectively. The concentrations of the biomarkers uL-FABP, uNGAL, and pNGAL were greater in deceased patients compared to those in survivors; 14 vs 2.72 ng/mL (p<0.001), 104.7 vs 10.3 ng/mL (p<0.001), and 209.3 vs 91.3 ng/mL (p=0.01), respectively.

The AUCs of pNGAL was 0.69 (95% CI 0.57-0.81, p=0.004), uNGAL was 0.78 (95% CI 0.69-0.88, p<0.001), uL-FABP was 0.74 (95% CI 0.64-0.84, p<0.001), and creatinine was 0.55 (95%CI 0.41-0.68, p=0.47) for predicting 28-day mortality (Fig 2B). The performance of all studied

biomarkers for predicting mortality is shown in Table 3. Among them, uL-FABP had the highest sensitivity and specificity to predict 28-day mortality.

By using multivariate analysis, the only independent predictor for 28-day mortality was high uL-FABP ≥ 4.68 ng/mL (OR 4.15, 95%CI 1.21-14.29) (Table 4). There were no clinical factors to predict 28-day mortality; therefore, we combined multiple biomarkers to predict 28-day mortality. The specificity for the predicting mortality was increased, but the sensitivity was reduced. UL-FABP combined with uNGAL had 66.7% sensitivity and 74.1% specificity, uL-FABP combined with pNGAL had 63% sensitivity and 77.8% specificity, and uNGAL combined with pNGAL had 59.3% sensitivity and 68.3% specificity. The combination of all biomarkers had 55.6% sensitivity and 81.5% specificity.

Validation cohort

To validate the role of the performance of biomarkers for the diagnosis and discrimination of AKI and the prediction of mortality, we analyzed an independent cohort of 30 cirrhotic patients consecutively recruited within a subsequent 6-month period. Baseline characteristics of patients in the derivation and validation cohorts were summarized in Supplementary Table 5. All differences between the two cohorts were not statistically significant, with the exception of gender, where males comprised the majority of the derivation cohort and females comprised the majority of the validation cohort. Of 30 patients, 13 (43.3%) were male with mean age 62.8±13.0 years. There were 8 patients (26.7%) who had AKI. The baseline demographic, clinical, and laboratory data of cirrhotic patients with and without AKI of validation cohort were shown in Supplementary Table 6. The performance of biomarkers for AKI diagnosis and prediction of 28-day mortality in the validation cohort were shown in Supplementary Table 7 & 8, respectively.

The AUCs of pNGAL was 0.82 (95% CI 0.66-0.98; p=0.01), uNGAL was 0.76 (95% CI 0.54-0.97; p=0.046), uL-FABP was 0.48 (95% CI 0.22-0.73; p=0.85) compared to creatinine as a standard of care was 0.97 (95%CI 0.92-1.00; p<0.001) for AKI diagnosis (Fig 3A). Moreover, the AUCs of pNGAL was 0.71 (95% CI 0.41-1.00; p=0.13), uNGAL was 0.79 (95% CI 0.55-1.00; p=0.04), uL-FABP was 0.49 (95% CI 0.21-0.77; p=0.93), and creatinine was 0.77 (95% CI 0.58-0.95; p=0.05) for predicting 28-day mortality (Fig 3B).

DISCUSSION

In patients with cirrhosis, AKI is a serious problem that can increase mortality, but the diagnosis is often delayed due to false low serum creatinine levels.[1-3] There is still an urgent need for new biomarkers to diagnose AKI development and poor outcome in hospitalized cirrhotic patients. The three main findings of the study are as follows: 1) baseline uL-FABP, uNGAL, and pNGAL are related to AKI and 28-day mortality in hospitalized patients with cirrhosis, 2) uNGAL demonstrated fair discriminating ability in diagnosing AKI, in contrast to pNGAL and uL-FABP. However, combining clinical factors with these biomarkers was able to improve their accuracy for AKI diagnosis. The discriminating ability to predict 28-day mortality was shown to be fair only for uNGAL and uL-FABP, but not for pNGAL. 3) Baseline

TABLE 3. The performance of biomarkers for prediction of 28-day mortality in hospitalized cirrhotic patients in a derivation cohort.

Biomarker	AUC (95%CI)	p-value	Cut-off (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR
pNGAL	0.69 (0.57-0.81)	0.004	127.35	68.0	56.8	32.7	85.2	1.58	0.56
uNGAL	0.78 (0.69-0.87)	<0.001	13.3	77.8	56.8	37.5	88.5	1.81	0.39
uL-FABP	0.74 (0.64-0.84)	<0.001	4.68	81.5	63.0	42.3	91.1	2.19	0.29

Abbreviations: AUC; area under the ROC curve. L-FABP; liver-type fatty acid-binding protein. LR; likelihood ratio. NGAL; neutrophil gelatinase-associated lipocalin. NPV; negative predictive value. PPV; positive predictive value. 95%CI; 95% confidence interval. +LR; positive likelihood ratio. -LR; negative likelihood ratio.

TABLE 4. Univariate and multivariate analysis for prediction of 28-day mortality in hospitalized cirrhotic patients.

Factors	Univariate analysis OR (95%CI)	p-value	Multivariate analysis OR (95%CI)	p-value
Age	1.02 (0.98-1.06)	0.30		
Bacterial infection	1.61 (0.67-3.87)	0.29		
AKI	5.11 (1.98-13.20)	0.001	2.20 (0.71-6.77)	0.17
Biomarkers				
pNGAL ≥ 127.35 ng/mL	3.04 (1.19-7.72)	0.02	1.56 (0.48-5.03)	0.46
uNGAL ≥ 13.3 ng/mL	4.60 (1.68-12.61)	0.003	1.64 (0.46-5.81)	0.45
uL-FABP ≥ 4.68 ng/mL	7.48 (2.56-21.82)	<0.001	4.15 (1.21-14.29)	0.02
uNGAL≥ 13.3 ng/mL and uL-FABP ≥ 4.68 ng/mL	5.71 (2.23-14.66)	<0.001		
Laboratories baseline				
Neutrophil/lymphocyte ratio	1.02 (0.99-1.05)	0.11		
INR	3.47 (1.40-8.57)	0.01		
Creatinine	1.43 (0.89-2.31)	0.14		
Sodium	0.93 (0.86-1.01)	0.06		
Sodium < 130 mmol/L	3.57 (1.43-8.90)	0.01	2.60 (0.87-7.75)	0.09
ACLF grade				
1	2.58 (0.67-9.83)	0.17		
2	5.15 (1.30-20.37)	0.02		
3	12.88 (2.25-73.71)	0.004		
MELD > 20	3.33 (1.22-9.11)	0.02		
SOFA	1.27 (1.05-1.53)	0.01		
New-onset organ failure	5.98 (1.33-27.02)	0.02	4.30 (0.68-27.07)	0.12

Abbreviations: ACLF; acute-on-chronic liver failure. AKI; acute kidney injury. CTP; Child-Turcotte-Pugh. INR; international normalized ratio. IQR; interquartile range. L-FABP; liver-type fatty acid-binding protein. MELD; model for end-stage liver disease. NGAL; neutrophil gelatinase-associated lipocalin. SOFA; Sequential Organ Failure Assessment.

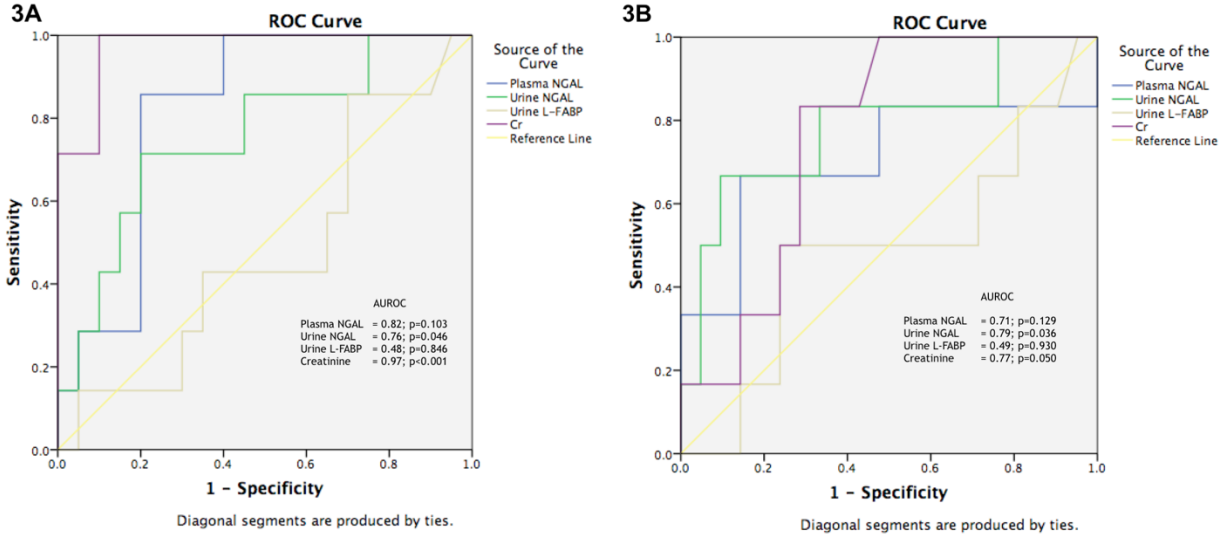

Fig 3A. Performance of pNGAL, uNGAL, uL-FABP, and creatinine for AKI diagnosis in hospitalized cirrhotic patients in the validation cohort (n=30). **Fig 3B.** Performance of pNGAL, uNGAL, uL-FABP, and creatinine for predicting 28-day mortality in hospitalized cirrhotic patients in the validation cohort (n=30).

uL-FABP was an independent predictor of 28-day mortality in hospitalized patients with cirrhosis and may be useful to guide clinicians for close monitoring and early management.

There was a clear association between the levels of the biomarkers tested and the occurrence of AKI or 28-day mortality. The levels of uL-FABP, uNGAL, and pNGAL were considerably greater in cirrhotic patients with AKI or death compared to cirrhotic patients who did not experience AKI or death. This is consistent with a previous study by Treeprasertsuk S. et al[17] that showed the advantage of using uNGAL in predicting AKI and poor outcomes. However, a recent study by Jiang QQ et al. demonstrated that there were no significant differences in uL-FABP and uNGAL levels between decompensated cirrhosis patients with AKI and those without AKI.[22] This result differed from our research. The possible reason is the difference in sample selection criteria. We included both decompensated and compensated cirrhosis in the AKI and non-AKI groups, whereas Jiang QQ et al included ACLF and decompensated cirrhosis in their study.

In our study, the performance of uL-FABP for prediction of death was found to be comparable to that of uNGAL. However, from multivariate analysis, only baseline uL-FABP was able to independently predict 28-day mortality. This could be explained by the different pathophysiology of both urine biomarkers. UL-FABP was demonstrated to have a linear correlation with hypoperfusion and liver injury, whereas uNGAL correlated with systemic inflammation and sepsis.[23] This current study included both infected and noninfected patients, and the majority were in the noninfected group, for instance, gastrointestinal bleeding and liver decompensation (Supplementary Table 1), which hypothesized hypoperfusion and liver injury. Moreover, the majority of deceased patients was in the non-infectious group, this data provided further support why uL-FABP and not NGAL was the sole predictor of mortality in this study. The finding that hospitalized cirrhotic patients with baseline uL-FABP ≥ 4.68 ng/mL had a 4-5-fold higher mortality risk than those with uL-FABP < 4.68 ng/mL with 81.5% sensitivity and 63% specificity was consistent with the results of a previous study which established that uL-FABP independently predicted AKI progression and mortality during admission.[18] From this information, uL-FABP might be useful for identifying high-risk patients for fatal outcomes and encouraging prompt management to reduce morbidity and mortality. However, due to insufficient sample size, the results of the validation cohort were not replicable.

The clinical features and laboratory profiles of cirrhotic patients at baseline also influenced their outcomes. Multivariate analysis from our data showed that MELD score > 20, CLIF-OF score, presence of bacterial infection, and BUN were independent predictors of AKI development. Interestingly, the previous study demonstrated the utility of NLR in predicting bacterial infection and short-term mortality[24] although our outcome was not as predicted. NLR did not reach statistical significance for prediction of 28-day mortality. We postulated that NLR representing dysregulation of the immune system in cirrhosis and/or decompensation especially the suppression of T lymphocytes, hence the majority affecting this biomarker was an infection-related complication. Though, less than half of the patients in our cohort had infectious causes, NLR was not a well providing prognostic marker in our study.

Additionally, we further evaluated the performance of AKI diagnosis and predicting mortality when combining these clinical parameters with biomarkers. The combination of these clinical factors improved the AUC of biomarkers from 0.67 to 0.89 for uL-FABP, 0.72 to 0.90 for uNGAL, and 0.68 to 0.88 for pNGAL in AKI diagnosis. When combining the clinical factors with biomarkers, the highest AUC achievable was 0.90 with uNGAL for AKI diagnosis. The specificity and positive predictive value of the test also improved. The prior study evaluated the association between the number of urine biomarkers (L-FABP, NGAL, IL-18, and albumin) above the cutoff for AKI development and mortality, as well as relative risk for the outcome.[18] As the number of biomarkers exceeding the threshold increased, so did the relative risk for AKI development and mortality. Thus, we investigated whether combining two biomarkers would improve their diagnostic sensitivity and specificity for AKI diagnosis and predicting mortality. The results showed that using two out of three biomarkers resulted in decreased sensitivity and increased specificity, which improved the reliability of the test for AKI diagnosis and prediction of 28-day mortality.

The validation cohort was established with the purpose of confirming the effectiveness of these biomarkers in mortality prediction and AKI diagnosis. In the validation cohort, uL-FABP lacked the ability to differentiate AKI or predict 28-day mortality owing to its AUC being less than 0.5. One potential constraint was the relatively small sample size of the validation cohort, which contained a relatively low proportion of individuals with AKI (26.7% vs. 41.3%, p=0.14) in comparison to the derivative cohort.

Regarding the differentiation of subtypes of AKI, this study included a small number of patients with ATN and HRS, and the levels of each biomarker did not differ significantly between subtypes of AKI. Thus,

more study is essential to determine the significance of biomarkers in diagnosing subtypes of AKI.

Finally, our study had some limitations. First, this study was a single-center study with sufficient sample size; however, the number of cirrhotic patients presenting with ATN or HRS was insufficient to establish a definitive conclusion regarding their ability to distinguish between the two conditions. Second, serum creatinine for the diagnosis of AKI might be underestimated and inaccurate for the diagnosis due to low muscle mass and increased serum bilirubin in cirrhotic patients.[3] And lastly, the small number of validation cohort limited the study's replicability. A future study that includes a larger number of patients in the AKI group should be explored to assess the effectiveness of the biomarker in predicting outcomes within this specific population.

CONCLUSION

Our prospective cohort study showed that structural urinary biomarkers were significantly higher in cirrhotic patients with AKI and with 28-day mortality. UNGAL for AKI diagnosis and uL-FABP for predicting mortality was shown to be acceptable. Together with clinical factors, these biomarkers had a better discriminating performance for the diagnosis of AKI than biomarkers alone. Furthermore, baseline uL-FABP ≥ 4.68 ng/mL was a valid predictor of 28-day mortality in hospitalized cirrhotic patients.

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Author contributions

Study concept and design (SW, NS, ST), acquisition of data (SW, TT, RC, PK, PT, ST), analysis and interpretation of data (TP, KT, CP), drafting of the manuscript (SW, TP), critical revision of the manuscript for important intellectual content (NS, TT, KT, ST), administrative, technical, and material support (NS, ST), and study supervision (NS, ST).

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Conflict of interest statement

All authors declare no conflict of interest, no plagiarism, no fabrication, and no falsification.

REFERENCES

1. Piano S, Rosi S, Maresio G, Fasolato S, Cavallin M, Romano A, et al. Evaluation of the Acute Kidney Injury Network criteria in hospitalized patients with cirrhosis and ascites. J Hepatol. 2013;59(3):482-9.
2. Bucsics T, Krones E. Renal dysfunction in cirrhosis: acute kidney injury and the hepatorenal syndrome. Gastroenterol Rep (Oxf). 2017;5(2):127-37.
3. Angeli P, Gines P, Wong F, Bernardi M, Boyer TD, Gerbes A, et al. Diagnosis and management of acute kidney injury in patients with cirrhosis: revised consensus recommendations of the International Club of Ascites. J Hepatol. 2015;62(4):968-74.
4. Francoz C, Nadim MK, Durand F. Kidney biomarkers in cirrhosis. J Hepatol. 2016;65(4):809-24.
5. Siew ED, Ware LB, Ikizler TA. Biological markers of acute kidney injury. J Am Soc Nephrol. 2011;22(5):810-20.
6. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol. 2008;3(3):665-73.
7. Arthur JM, Hill EG, Alge JL, Lewis EC, Neely BA, Janech MG, et al. Evaluation of 32 urine biomarkers to predict the progression of acute kidney injury after cardiac surgery. Kidney Int. 2014;85(2):431-8.
8. Aberg F, Lempinen M, Hollmen M, Nordin A, Makisalo H, Isoniemi H. Neutrophil gelatinase-associated lipocalin associated with irreversibility of pre-liver transplant kidney dysfunction. Clin Transplant. 2014;28(8):869-76.
9. Doi K, Negishi K, Ishizu T, Katagiri D, Fujita T, Matsubara T, et al. Evaluation of new acute kidney injury biomarkers in a mixed intensive care unit. Crit Care Med. 2011;39(11):2464-9.
10. Siew ED, Ikizler TA, Gebretsadik T, Shintani A, Wickersham N, Bossert F, et al. Elevated urinary IL-18 levels at the time of ICU admission predict adverse clinical outcomes. Clin J Am Soc Nephrol. 2010;5(8):1497-505.
11. Ferguson MA, Vaidya VS, Waikar SS, Collings FB, Sunderland KE, Gioules CJ, et al. Urinary liver-type fatty acid-binding protein predicts adverse outcomes in acute kidney injury. Kidney Int. 2010;77(8):708-14.
12. Belcher JM, Sanyal AJ, Peixoto AJ, Perazella MA, Lim J, Thiessen-Philbrook H, et al. Kidney biomarkers and differential diagnosis of patients with cirrhosis and acute kidney injury. Hepatology. 2014;60(2):622-32.
13. Fagundes C, Pepin MN, Guevara M, Barreto R, Casals G, Sola E, et al. Urinary neutrophil gelatinase-associated lipocalin as biomarker in the differential diagnosis of impairment of kidney function in cirrhosis. Journal of hepatology. 2012;57(2):267-73.
14. Xu Y, Xie Y, Shao X, Ni Z, Mou S. L-FABP: A novel biomarker of kidney disease. Clin Chim Acta. 2015;445:85-90.
15. Ariza X, Graupera I, Coll M, Sola E, Barreto R, Garcia E, et al. Neutrophil gelatinase-associated lipocalin is a biomarker of acute-on-chronic liver failure and prognosis in cirrhosis. J Hepatol. 2016;65(1):57-65.
16. Portilla D, Dent C, Sugaya T, Nagothu KK, Kundi I, Moore P, et al. Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. Kidney Int. 2008;73(4):465-72.
17. Treeprasertsuk S, Wongkarnjana A, Jaruvongvanich V, Sallapant S, Tiranathanagul K, Komolmit P, et al. Urine neutrophil

gelatinase-associated lipocalin: a diagnostic and prognostic marker for acute kidney injury (AKI) in hospitalized cirrhotic patients with AKI-prone conditions. BMC Gastroenterol. 2015; 15:140.

18. Belcher JM, Garcia-Tsao G, Sanyal AJ, Thiessen-Philbrook H, Peixoto AJ, Perazella MA, et al. Urinary biomarkers and progression of AKI in patients with cirrhosis. Clin J Am Soc Nephrol. 2014;9(11): 1857-67.
19. Eguchi A, Hasegawa H, Iwasa M, Tamai Y, Ohata K, Oikawa T, et al. 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. Hepatol Commun. 2019;3(6):825-37.
20. Russ KB, Stevens TM, Singal AK. Acute Kidney Injury in Patients with Cirrhosis. J Clin Transl Hepatol. 2015;3(3):195-204.
21. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. Gastroenterology. 2013;144(7):1426-37, 1437.e1-9.
22. Jiang QQ, Han MF, Ma K, Chen G, Wan XY, Kilonzo SB, et al. Acute kidney injury in acute-on-chronic liver failure is different from in decompensated cirrhosis. World J Gastroenterol. 2018;24(21):2300-10.
23. Asada T, Isshiki R, Hayase N, Sumida M, Inokuchi R, Noiri E, et al. Impact of clinical context on acute kidney injury biomarker performances: differences between neutrophil gelatinase-associated lipocalin and L-type fatty acid-binding protein. Sci Rep. 2016;6:33077.
24. Sun J, Guo H, Yu X, Chen J, Zhu H, Qi X, et al. Evaluation of prognostic value of neutrophil-to-lymphocyte ratio in patients with acute-on-chronic liver failure or severe liver injury from chronic HBV infection. Eur J Gastroenterol Hepatol. 2021;33 (1S Suppl 1):e670-e680.