ORIGINAL

Plasma Cystatin C Level is a Prognostic Marker of Morbidity and Mortality in Hospitalized Decompensated Cirrhotic Patients

Natsuda Aumpan[1], Tanabute Limprukkasem[1], Bubpha Pornthisarn[1], Ratha-korn Vilaichone[1,2], Soonthorn Chonprasertsuk[1], Patommatat Bhanthumkomol[1], Pichaya Tantiyavarong[3], and Sith Siramolpiwat[1,2]

[1]Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand
[2]Department of Medicine, Chulabhorn International College of Medicine (CICM), Thammasat University, Pathumthani, Thailand
[3]Division of Nephrology, Department of Internal Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

Abstract : Introduction : Cystatin C (CysC) is biomarker for early detection of acute kidney injury (AKI). However, there is limited evidence in decompensated cirrhotic patients without AKI at admission. This study aimed to assess CysC as a predictor of 90-day mortality. Methods : Decompensated cirrhotic patients without AKI were prospectively enrolled. CysC and creatinine were measured within 24 hours of admission and compared between patients with in-hospital complications (AKI, hepatorenal syndrome (HRS), acute-on-chronic liver failure (ACLF)) *vs.* those without, and survivors *vs.* non-survivors. The AUROC and cut-off point of CysC in predicting 90-day mortality were determined. Results : Of 137 decompensated cirrhotic patients, 46 without AKI at admission were included (58.7% male, age 60.8 ± 11.2 years, MELD 13.1 ± 5.1, ChildA/B/C 43.5%/39.1%/17.4%). The mean CysC level tended to be higher in patients with ACLF (1.52 ± 0.60 *vs.* 1.11 ± 0.28, p = 0.05), and significantly higher in non-survivors than survivors (1.61 ± 0.53 *vs.* 1.08 ± 0.28, p = 0.013). The 90-day mortality rate was 21.7%. After adjusting with age and bacterial infection on admission, CysC level ≥ 1.25 mg/L was significantly associated with 90-day mortality. The CysC cut-off level ≥ 1.25 mg/L provided 80% sensitivity and 75% specificity for predicting 90-day mortality. Conclusion : Plasma CysC within 24 hours could be used as a predictor for 90-day mortality and development of ACLF in decompensated cirrhotic patients. J. Med. Invest. 68 : 302-308, August, 2021

Keywords : *Cystatin C, Cirrhosis, Acute kidney injury, Acute-on-chronic liver failure*

INTRODUCTION

Acute kidney injury (AKI) is a common complication associated with high morbidity and mortality in decompensated cirrhotic patients (1, 2). Approximately 20% of hospitalized patients with cirrhosis experience deterioration of renal function predominantly caused by prerenal azotemia and acute tubular necrosis (3). Early diagnosis of AKI in cirrhotic patients is essential to prevent its progression and reduce mortality. Serum creatinine, an indicator of renal function, is widely used for the diagnosis of AKI in clinical practice. However, several limitations should be taken into consideration as serum creatinine might overestimate renal function, especially in cirrhotic patients (4, 5). Falsely decreased creatinine in cirrhosis can be resulted from 4 main reasons : diminished creatinine production due to sarcopenia (6) ; dilution effect from increased volume of distribution (7) ; increased renal tubular creatinine excretion (8) ; bilirubin interference in creatinine estimation (9). Therefore, these drawbacks have inspired researchers to find new biomarkers to provide more accurate assessment of renal function in cirrhotic patients.

Cystatin C (CysC) is non-glycosylated, low-molecular-weight protein produced by all nucleated cells at a constant rate. Since 1994, CysC has been introduced as a surrogate marker for determining glomerular filtration rate (10). CysC is freely filtered by glomerulus, completely reabsorbed, and metabolized by proximal tubular cells. In contrast to creatinine, CysC is not affected by demographic factors and muscle mass (11). These properties make CysC a good candidate for estimating renal function in cirrhotic patients. Previous studies suggested that CysC might be a predictor of early renal dysfunction in cirrhotic patients (12-16). Moreover, the CysC level of more than 1.5 mg/L could predict 90-day mortality in decompensated cirrhotic patients with sensitivity and specificity of 73.3% and 52.5%, respectively (12). The changes in CysC level also demonstrated stronger association with dialysis or mortality than serum creatinine in cirrhotic patients with AKI (16).

Until now, there have been limited data regarding prognostic ability of plasma CysC in hospitalized decompensated cirrhotic patients. This study aimed to determine the efficacy of plasma CysC as a predictor of morbidity and mortality in acutely decompensated cirrhotic patients who had no AKI on the day of admission.

List of abbreviations :
CysC, Cystatin C ; AKI, acute kidney injury ; ACLF, acute-on-chronic liver failure ; HRS, hepatorenal syndrome ; MELD ; model for end-stage liver disease ; HIV, human immunodeficiency virus ; GFR, Glomerular filtration rate ; CKD-EPI, chronic kidney disease epidemiology collaboration ; ROC, receiver operating characteristic ; AUROC, area under the ROC curve ; SBP, spontaneous bacterial peritonitis ; NASH, non-alcoholic steatohepatitis ; PHT, portal hypertension ; WBC, white blood cell ; AST, aspartate transaminase ; ALT, alanine transaminase; ALP, alkaline phosphatase ; INR, international normalized ratio

MATERIALS AND METHODS

Study design and population

This prospective observational study was conducted at Thammasat University Hospital between October 1, 2017 and November 30, 2019. The inclusion criteria were hospitalized patients diagnosed with acute decompensated cirrhosis aged between 18 and 80 years old. The exclusion criteria were the presence of AKI on the day of admission, chronic kidney disease (CKD), pregnancy, hypothyroidism or hyperthyroidism, history of steroid use, previous liver or kidney transplantation, hepatocellular carcinoma, HIV infection, receiving immunosuppressive drugs for any disease except severe alcoholic hepatitis, or refusal to participate in the study.

This study received ethical approval by the Human Research Ethics Committee of Thammasat University, Thailand and was conducted according to the good clinical practice guideline, as well as the Declaration of Helsinki. Written informed consent was obtained from all participants. The patient's relative could provide written informed consent instead of the patient himself in case of being diagnosed with hepatic encephalopathy. The informed consent was then requested from the patient after the resolution of hepatic encephalopathy for continuation in the study.

Study protocol

All patients were included in this study within 24 hours of admission. Demographic data, etiologies of cirrhosis, underlying medical conditions, history of present illness, physical examination, and treatment outcomes were recorded. Laboratory results including a complete blood count, a comprehensive metabolic panel, coagulation tests, hemoculture, ascitic fluid analysis and culture, urinalysis and urine culture were recorded. The Child-Pugh score, the model for end-stage liver disease (MELD) score, and MELD-Na score were calculated to assess severity of liver impairment. Blood samples for determining plasma CysC and serum creatinine were collected from each patient at the same time within 24 hours of admission. At least 1-ml plasma sample was obtained by centrifugation and then stored at -20°C until CysC analysis was performed by using nephelometry (13). All patients received standard treatment for acute decompensated cirrhosis. The 90-day mortality rate was prospectively evaluated.

The primary aim was to determine the efficacy of plasma CysC as a predictor of 90-day mortality in acutely decompensated cirrhotic patients without AKI on the day of admission. The secondary aim was to evaluate the efficacy of plasma CysC as a predictor of in-hospital AKI, hepatorenal syndrome (HRS) and acute-on-chronic liver failure (ACLF) in acutely decompensated cirrhotic patients.

Definitions

Acute decompensation was defined as an acute development of one or more of the following complications : ascites, hepatic encephalopathy, gastrointestinal bleeding, or bacterial infection in a patient with cirrhosis (17).

Acute-on-chronic liver failure (ACLF) was defined according to the diagnostic criteria established in the CANONIC study (17).

Acute kidney injury (AKI) was defined as an increase in serum creatinine of ≥ 0.3 mg/dL within 48 hours ; or a percentage increase in serum creatinine of $\geq 50\%$ from baseline which is known or presumed to have occurred within the previous 7 days (9).

Hepatorenal syndrome (HRS) was defined as the diagnosis of cirrhosis and ascites in concurrent with the diagnosis of AKI. In addition, the diagnostic criteria included no response of AKI after 2 consecutive days of diuretic withdrawal and plasma volume expansion with 1 gram of albumin per kilogram of body weight, absence of shock, no current or recent use of nephrotoxic drugs, and no macroscopic signs of structural kidney injury (9).

Glomerular filtration rate (GFR) was calculated by using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation based on serum creatinine, or CysC (18).

Chronic kidney disease (CKD) was defined as either structural or functional renal abnormalities. The presence of either one of markers for kidney damage (e.g., albuminuria, urine sediment abnormalities) or decreased GFR <60 ml/min/1.73 m² for >3 months is considered as CKD (19).

Statistical analysis

All statistical analyses were performed by using STATA software version 12.0 (Stata Corp, Texas, USA). The demographic data and clinical characteristics were analyzed by Fisher's exact test, or Chi-square test where appropriate. Laboratory results were compared between 90-day survivors and non-survivors using Student's t-test. Plasma CysC level was presented as mean and standard deviation and evaluated for association with the development of in-hospital complications (AKI, HRS, ACLF), and 90-day mortality. The receiver operating characteristic (ROC) curve analysis of CysC for predicting 90-day mortality was performed, and the optimal cut-off point which yielded the highest sensitivity and specificity was selected. The area under the ROC curve (AUROC) of CysC and MELD score for predicting 90-day mortality was compared. Statistical significance was defined as p-value of less than 0.05.

Sample size for two-sample comparison of means was calculated in order to achieve 90% power using a two-sided test with an alpha level of 0.05. The means of plasma CysC used in the calculation were from the previous study in which patients who survived had a mean plasma CysC of 1.5 ± 0.23 mg/L, whereas patients who died within 90 days had a mean of 1.9 ± 0.33 mg/L (12). The estimated total sample size was at least 42.

RESULTS

Patient characteristics

Of 137 decompensated cirrhotic patients admitted during the study period, 91 patients (27 with AKI, 59 with chronic kidney disease, and 5 with hepatocellular carcinoma) were excluded from the study. A total of 46 patients were prospectively enrolled (27 men and 19 women) with a mean age of 60.8 ± 11.2 (range 40-80) years. All patients were diagnosed with acute decompensated cirrhosis without AKI on the day of admission. The average serum creatinine within the previous 3 months before the study entry of all patients was 0.8 ± 0.2 mg/dl, meanwhile the previous urinary examination (available in 32/46 patients) showed no significant abnormalities (e.g., albuminuria, urine sediments). The most common causes of cirrhosis were alcohol (50%), chronic hepatitis B infection (17.4%) and non-alcoholic steatohepatitis (17.4%). There were 20 (43.5%), 18 (39.1%), and 8 (17.4%) patients classified as Child-Pugh class A, B, and C, respectively. The mean MELD and MELD-Na score were 13.1 ± 5.1 and 15.1 ± 5.4, respectively. The most common comorbidities were diabetes (39.1%), hypertension (34.8%), and dyslipidemia (17.4%), whereas 18 (39.1%) patients had no comorbidity. Majority of patients had bacterial infection (37%), and portal hypertension-related gastrointestinal bleeding (34.8%) as presenting symptoms of decompensation. Baseline characteristics of all patients were demonstrated in the Table 1.

Laboratory results at baseline including complete blood count, renal and liver function test were demonstrated in the Table 2.

The mean serum creatinine was 0.85 ± 0.17 mg/dL, and mean plasma CysC was 1.20 ± 0.41 mg/L. The means of GFR estimated by serum creatinine and plasma CysC were 86.3 and 68.3 ml/min, respectively. There was no significant difference of the mean CysC level according to Child-Pugh classes (1.09 ± 0.27 mg/L for Child-Pugh A *vs.* 1.22 ± 0.33 mg/L for Child-Pugh B *vs.* 1.41 ± 0.72 mg/L for Child-Pugh C, p = 0.164).

The 90-day mortality

There were 10 patients (21.7%) who died within 90 days. Six deaths were due to sepsis with multiorgan failure, two from massive gastrointestinal hemorrhage, and another two from ACLF with progressive liver deterioration. All 5 patients who developed in-hospital AKI died within 90 days, 4 of whom died during admission, while the other one survived and subsequently died 41 days after being discharged from hospital due to sepsis with multiorgan failure. Baseline characteristics along with laboratory results were compared between 90-day survivors and non-survivors as demonstrated in the Table 1 and 2. The mean age of non-survivors was significantly higher than survivors (70.1 ± 8.4 *vs.* 58.2 ± 10.5, p = 0.002). Alcoholic cirrhosis was more prevalent in survivors, whereas cirrhosis due to unidentified causes was more common in non-survivors. Non-survivors had significantly higher MELD-Na score than survivors (18.9 ± 6.6 *vs.* 14.1 ± 4.6, p = 0.010). Meanwhile the MELD score was higher in non-survivor (15.2 ± 6.5 *vs.* 12.5 ± 4.6), however no statistical significance was reached (p = 0.136). The diagnosis of bacterial infection (70% *vs.* 27.8%, p = 0.025), and spontaneous bacterial peritonitis (SBP) (50% *vs.* 8.3%, p = 0.007) on current admission were more prevalent in non-survivors than survivors. In addition, the mean CysC level was 1.39 ± 0.42 mg/L in the group with bacterial infection compared to 1.08 ± 0.36 mg/L in the group without (p = 0.012). The average length of hospital stay was 8 ± 7 days. Non-survivors had slightly longer average length of hospital stay than survivors (9.9 ± 9.5 days *vs.* 7.9 ± 6.7 days, p = 0.459).

Table 1. Baseline and clinical characteristics of included patients and comparison between 90-day survivors and non-survivors

Factors	Total (N = 46)	Survivors (N = 36)	Non-survivors (N = 10)	P-value*
Male (%)	27 (58.7%)	23 (63.9%)	4 (40%)	0.277
Mean age	60.8 ± 11.2	58.2 ± 10.5	70.1 ± 8.4	0.002
Body mass index (kg/m²)	23.7 ± 4.2	23.6 ± 4.0	23.9 ± 5.1	0.855
Comorbidities				
Diabetes mellitus	18 (39.1%)	14 (38.9%)	4 (40%)	1.000
Hypertension	16 (34.8%)	10 (27.8%)	6 (60%)	0.074
Dyslipidemia	8 (17.4%)	4 (11.1%)	4 (40%)	0.055
Cardiovascular disease	4 (8.7%)	2 (5.6%)	2 (20%)	0.201
None	18 (39.1%)	15 (41.7%)	3 (30%)	0.717
Underlying liver diseases				
Cirrhosis etiology				
Alcohol	23 (50.0%)	22 (61.1%)	1 (10%)	0.004
Chronic hepatitis B	8 (17.4%)	7 (19.4%)	1 (10%)	0.664
Chronic hepatitis C	6 (13.0%)	3 (8.3%)	3 (30%)	0.107
NASH	8 (17.4%)	5 (13.9%)	3 (30%)	0.344
Cryptogenic	4 (8.7%)	1 (2.8%)	3 (30%)	0.028
Child-Pugh class				0.616
A	20 (43.5%)	17 (47.2%)	3 (30%)	
B	18 (39.1%)	13 (36.1%)	5 (50%)	
C	8 (17.4%)	6 (16.7%)	2 (20%)	
MELD (Mean ± SD)	13.1 ± 5.1	12.5 ± 4.6	15.2 ± 6.5	0.136
MELD-Na (Mean ± SD)	15.1 ± 5.4	14.1 ± 4.6	18.9 ± 6.6	0.010
Previous decompensation	28 (60.9%)	22 (61.1%)	6 (60%)	1.000
Acute decompensation present on current admission				
Bacterial infection	17 (37%)	10 (27.8%)	7 (70%)	0.025
SBP	8 (17.4%)	3 (8.3%)	5 (50%)	0.007
PHT-related gastrointestinal bleeding	16 (34.8%)	15 (41.7%)	1 (10%)	0.130
Hepatic encephalopathy	6 (13.0%)	5 (13.9%)	1 (10%)	1.000
Ascites	2 (4.3%)	2 (5.6%)	0 (0%)	1.000

*The p-value of < 0.05 represents significant difference between survivors and non-survivors.
Abbreviations : NASH = non-alcoholic steatohepatitis, MELD = model for end-stage liver disease, SBP = spontaneous bacterial peritonitis PHT = portal hypertension

Table 2.   Laboratory findings of included patients and comparison between 90-day survivors and non-survivors

Factors	Total (N = 46)	Survivors (N = 36)	Non-survivors (N = 10)	P-value*
Complete blood count				
Hemoglobin (g/dL)	9.4 ± 2.2	9.6 ± 2.3	8.6 ± 2.5	0.205
WBC count (x10^9/L)	8.4 ± 4.3	8.8 ± 4.6	7.2 ± 2.6	0.297
Platelet count (x10^9/L)	109 ± 47	115 ± 48	89 ± 43	0.136
Renal function test				
Serum creatinine (mg/dL)	0.85 ± 0.17	0.82 ± 0.16	0.97 ± 0.14	0.010
Plasma cystatin C (mg/L)	1.20 ± 0.41	1.08 ± 0.28	1.61 ± 0.53	0.013
Glomerular filtration rate				
CKD-EPI Cr (ml/min)	86.3 ± 18.3	91.3 ± 16.1	68.3 ± 14.2	< 0.001
CKD-EPI CysC (ml/min)	68.3 ± 26.2	75.1 ± 24.0	43.8 ± 18.4	< 0.001
Liver function test				
Total protein (g/dl)	6.8 ± 1.0	6.8 ± 1.0	6.7 ± 1.1	0.779
Serum albumin (g/dl)	2.4 ± 0.6	2.5 ± 0.6	2.1 ± 0.5	0.114
Total bilirubin (mg/dl)	3.2 ± 3.7	2.9 ± 3.4	4.3 ± 4.5	0.307
Direct bilirubin (mg/dl)	1.9 ± 2.7	1.6 ± 2.5	2.8 ± 3.5	0.209
AST (U/L)	86 ± 89	93 ± 98	61 ± 41	0.324
ALT (U/L)	49 ± 81	53 ± 91	34 ± 11	0.520
ALP (U/L)	157 ± 105	152 ± 72	173 ± 187	0.580
Coagulation test				
INR	1.52 ± 0.42	1.42 ± 0.31	1.90 ± 0.55	0.024
Other				
Uric acid (mg/dl)	6.8 ± 2.3	6.7 ± 2.6	6.9 ± 2.4	0.68

*The p-value of < 0.05 represents significant difference between survivors and non-survivors.
Abbreviations : CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration, Cr = creatinine, CysC = cystatin C, WBC = white blood cell, AST = aspartate transaminase, ALT = alanine transaminase, ALP = alkaline phosphatase, INR = international normalized ratio

All renal function parameters on the first day of admission in non-survivors were worse than survivors. Serum creatinine (0.97 ± 0.14 *vs.* 0.82 ± 0.16, p = 0.01) and plasma CysC (1.61 ± 0.53 *vs.* 1.08 ± 0.28, p = 0.013) in non-survivors were significantly higher than survivors, while creatinine- and CysC-based glomerular filtration rate (GFR) were lower. International normalized ratio (INR) was also higher in patients who died (1.90 ± 0.55 *vs.* 1.42 ± 0.31, p = 0.024). Other laboratory results including complete blood count and liver function test were not significantly different between 2 groups.

Development of AKI, HRS, and ACLF

Five patients (10.9%) developed AKI during admission. The causes of AKI were prerenal azotemia (60%) and hepatorenal syndrome (40%). The mean of plasma CysC tended to be higher in patients who developed AKI (1.82 ± 0.71 *vs.* 1.12 ± 0.29 mg/L, p = 0.093), whereas there was no significant difference of baseline serum creatinine between 2 groups (0.94 ± 0.15 *vs.* 0.84 ± 0.17 mg/dL, p = 0.214). Of the 5 patients with in-hospital AKI, all had normal serum creatinine, whereas 4 patients (80%) had elevated plasma CysC on the first day of admission. Two patients who developed HRS during admission had higher mean of plasma CysC than those without, however no statistical significance was reached (2.03 ± 1.00 *vs.* 1.16 ± 0.34 mg/L, p = 0.434). On the contrary, there was no significant difference of baseline serum creatinine between patients who developed HRS and those who did not (0.87 ± 0.21 *vs.* 0.85 ± 0.17 mg/dL, p = 0.892).

ACLF were diagnosed in 10 patients (21.7%), 4 of whom died during admission. Half of all ACLF patients had renal failure as one of organ failure. The mean of plasma CysC tended to be higher in patients who developed ACLF (1.52 ± 0.60 *vs.* 1.11 ± 0.28 mg/L, p = 0.05), whereas there was no significant difference in baseline serum creatinine between 2 groups (0.93 ± 0.16 *vs.* 0.83 ± 0.17 mg/dL, p = 0.102).

Predictors of AKI, HRS, ACLF, and 90-day mortality

The ROC curve analysis showed that the AUROC of plasma CysC in predicting 90-day mortality was 0.85 (95% CI 0.71-0.97), which was marginally superior to the MELD score (AUROC 0.64, 95% CI 0.43-0.84, p = 0.04). The best cut-off value of plasma CysC for predicting 90-day mortality was ≥ 1.25 mg/L, which yielded sensitivity and specificity of 80% and 75%, respectively. Patients with CysC of ≥ 1.25 mg/L demonstrated significant association with 90-day mortality (OR 12 ; 95%CI 2.14-67.24, p = 0.005).

In terms of in-hospital AKI, the AUROC curve of plasma CysC in predicting in-hospital AKI was 0.79 (95% CI 0.39-1.0). The cut-off value of plasma CysC ≥ 1.25 mg/L for predicting in-hospital AKI provided sensitivity and specificity of 80% and 68%, respectively. The AUROC curve of plasma CysC in predicting in-hospital ACLF was 0.75 (95% CI 0.56-0.93). The cut-off value of plasma CysC ≥ 1.25 mg/L for predicting in-hospital ACLF yielded sensitivity and specificity of 70% and 72%, respectively. Patients with CysC of ≥ 1.25 mg/L demonstrated

a significant association with the development of ACLF (OR 6.07 ; 95%CI 1.31-28.20, p = 0.021). Plasma CysC as a predictor for development of in-hospital AKI, HRS, ACLF, and 90-day mortality was demonstrated in the Table 3.

Table 4 shows univariate logistic regression analysis of factors associated with 90-day mortality. Baseline demographic data including severity of liver cirrhosis, type of decompensation, and initial laboratory parameters were included in the analysis. By univariate logistic regression analysis, age, MELD-Na score, bacterial infection on admission, SBP on admission, and plasma CysC level ≥ 1.25 mg/L were significantly associated with 90-day mortality. Taking into account that the number of event in this study was low, we therefore decided to include only 3 factors in the multivariate regression model : age, bacterial infection, and plasma CysC level ≥ 1.25 mg/L. The MELD-Na score was excluded because it may collinear with plasma Cys C level given that both factors were based on the patients' kidney function. Similarly, SBP was excluded because of its collinearity with bacterial infection. As shown, after adjusting with age and bacterial infection, plasma CysC level ≥ 1.25 mg/L was independently associated with 90-day mortality with an adjusted OR of 6.70 (95%CI 1.05-42.73), however the statistical significant was borderline (p = 0.044).

DISCUSSION

This is a prospective observational study investigating the prognostic ability of plasma CysC in predicting in-hospital complications and 90-day mortality in acutely decompensated cirrhotic patients without AKI at admission. The data suggested that plasma CysC level within 24 hours after admission was significantly higher in 90-day non-survivors, and it could be used as a reliable marker for prediction of in-hospital ACLF, and 90-day

mortality in these patients. In addition, by multivariate analysis, plasma CysC ≥ 1.25 mg/L was an independent predictor of 90-day mortality.

Acute kidney injury frequently occurs as a complication leading to grave prognosis in decompensated cirrhosis (1, 2). These patients are generally susceptible to intravascular volume depletion resulted from many conditions such as gastrointestinal bleeding, diarrhea, or sepsis-induced microvascular leakage (3). These precipitating events cause a decrease in effective arterial blood volume which can activate neurohormonal system, subsequently induce renal vasoconstriction, and contribute to AKI (3). Apart from precipitating factors, the severity of cirrhosis itself can provoke AKI through the mechanism of reduced renal blood flow due to portal hypertension in HRS (20). Our study demonstrated a lower number of patients who developed in-hospital AKI compared with other study (10.9% *vs.* 53%) (21). This could be explained by the fact that our study mainly comprised Child-Pugh A cirrhosis with relatively low MELD score resulting in a lower rate of complications and mortality (22). However, another study revealed a comparable number of hospitalized decompensated cirrhotic patients who later developed renal dysfunction (14%) during admission using different criteria for renal dysfunction (serum creatinine ≥ 1.5 mg/dL) (12). It should be noted that the demographic factors including age and male preponderance of our cohort were almost similar to the previous study (12).

Cystatin C has been recognized as a promising biomarker to evaluate renal function for several years (10). Studies have reported that it could overcome limitations of serum creatinine and facilitate early detection of AKI in cirrhotic patients (12-16). So far, few studies have evaluated the role of plasma CysC in hospitalized cirrhosis with acute decompensation (12, 15). The recent prospective study in hospitalized cirrhotic patients using CANONIC database has found that higher plasma CysC level was associated with the development of renal dysfunction, ACLF

Table 3. Plasma cystatin C as a predictor for development of in-hospital AKI, HRS, ACLF, and 90-day mortality

Outcome	Cystatin <1.25 mg/L (N = 29)	Cystatin ≥1.25 mg/L (N = 17)	Odds ratio (95% CI)	P-value
AKI	1 (3.4%)	4 (23.5%)	8.62 (0.87-84.9)	0.065
HRS	0	2 (11.8%)	-	0.131
ACLF	3 (10.3%)	7 (41.2%)	6.07 (1.31-28.20)	0.021
Death within 90 days	2 (6.9%)	8 (47.1%)	12 (2.14-67.24)	0.005

Abbreviations : AKI = acute kidney injury, HRS = hepatorenal syndrome, ACLF = acute-on chronic liver failur

Table 4. Univariate and multivariate analysis of factors associated with 90-day mortality

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age	1.11 (1.03-1.20)	0.006	1.07 (0.98-1.17)	0.133
Child-Pugh class A	1	-		
Child-Pugh class B	2.18 (0.44-10.83)	0.341		
Child-Pugh class C	1.89 (0.25-14.19)	0.537		
MELD-Na	1.18 (1.02-1.37)	0.022		
Bacterial infection	6.07 (1.31-28.2)	0.021	1.99 (0.29-13. 41)	0.477
SBP on admission	11 (1.98-61)	0.006		
Plasma cystatin C ≥ 1.25 mg/L	12 (2.14-67.24)	0.005	6.70 (1.05-42.73)	0.044

Abbreviations : MELD = model for end-stage liver disease, SBP = spontaneous bacterial peritonitis

and 90-day mortality (12). However, it should be noted that more than one-third of the included patients in the previous study had impaired renal function during the inclusion period. This fact potentially limits the clinical application of plasma CysC as an early marker of kidney injury in decompensated cirrhosis if one already had impaired renal function at baseline. Therefore, our study aimed to explore the predictive ability of plasma CysC in decompensated cirrhosis who had no AKI at admission. In the present study, we found that the mean plasma CysC was higher in patients who died within 90 days compared with those who were alive. Moreover, patients who developed in-hospital AKI, HRS and ACLF tended to have higher CysC level than patients without these conditions. The mean CysC values of patients who had in-hospital AKI and HRS (1.82 and 2.03 mg/L, respectively) were quite comparable to what have been reported in the earlier study (1.6 and 2.1 mg/L, respectively) (12). In addition, we demonstrated that the cut-off value of plasma CysC level ≥ 1.25 mg/L yielded the best sensitivity for prediction of 90-day mortality. The cut-off point was slightly lower than previously reported, which could be possibly attributed to variability of patients included and ethnic diversity(12).

Most of patients (80%) who developed in-hospital AKI in this study had elevated plasma CysC but normal serum creatinine level on the first day of admission. This suggested that elevated baseline plasma CysC level could predict AKI earlier than a rise in serum creatinine in hospitalized decompensated cirrhotic patients. In addition to renal outcomes, this study disclosed an association between higher CysC level and the development of ACLF. This information is clinically relevant as ACLF patients carry an exceptionally high morbidity and mortality rates (17). Therefore, an early identification of ACLF by a simple and reliable biomarker should be encouraged. Supporting this idea, in the present study, all ACLF patients died within 90 days indicating an ominous prognosis especially when renal failure was one of end organ damage.

Mortality rate has long been a primary focus in cirrhosis research field. Patients with compensated cirrhosis has remarkably longer median survival time than the decompensated ones (> 12 *vs.* 2 years, respectively) (23). The transition from compensated to decompensated cirrhosis can be accelerated by complications at any time during the disease course. Our study revealed a slightly lower 90-day mortality rate than prior studies, which might be caused by a higher number of patients with early cirrhosis included in the present study (12, 24). Regarding causes of death, sepsis with multiorgan failure was the most frequent cause, which was similar to what has been previously reported (23). In the present study, we have demonstrated that, in the multivariate regression analysis, after adjusting with age and bacterial infection on admission, plasma CysC level was significantly associated with 90-day mortality. This finding emphasizes a potential advantage of plasma CysC level as a predictor for short-term mortality. However, taking into consideration that the statistical significance level was marginal, more data are needed to clarify this issue.

Another point worth mentioning in the present study was that bacterial infection and SBP were significantly associated with 90-day mortality in the univariate analysis. It is well known that bacterial infection is very common among hospitalized cirrhotic patients, and is associated with an increase risk of mortality (25). From the pathophysiological standpoint, bacterial infection is distinct from other decompensations given that it can cause an exaggerated systemic inflammation that is responsible for organ failure in patients with cirrhosis (26). Of note, a recent global scale study has demonstrated that 48% of ACLF patients were documented with bacterial infection, of which the majority were SBP, pneumonia, and infections caused by multidrug-resis-

tant organisms (27).

There were some limitations in this study. First, our study had a relatively small sample size, however, the number of participants exceeded the minimum number determined by sample size calculation. Second, AKI was diagnosed by International Club of Ascites definitions based on serum creatinine, which could underestimate renal dysfunction in cirrhotic patients. However, serum creatinine is the most widely used biomarker of kidney function in clinical practice. Third, our study lacked validation cohort. Therefore, our results including the optimal cut-off point of plasma CysC need to be redetermined in the future study. Finally, the result of the ROC analysis showing that plasma CysC was superior to the MELD score for 90-day mortality prediction should be interpreted with caution because the sample size and the event rate in the present may be too small for the ROC analysis, as well as the significance level was considerably borderline.

In conclusion, plasma cystatin C within 24 hours after admission can be used as a renal biomarker for prognostic prediction of 90-day mortality, and the development of in-hospital ACLF in hospitalized decompensated cirrhotic patients who have no AKI at admission. This information could facilitate early identification and raise awareness of ACLF in these patients.

FUNDING

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTERESTS

Natsuda Aumpan, Tanabute Limprukkasem, Bubpha Pornthisarn, Ratha-korn Vilaichone, Soonthorn Chonprasertsuk, Patommatat Bhanthumkomol, and Pichaya Tantiyavarong have no conflicts of interest to declare. Sith Siramolpiwat has served a speaker for Ferring Pharmaceuticals Thailand.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Belcher JM, Garcia-Tsao G, Sanyal AJ, Bhogal H, Lim JK, Ansari N, Coca SG, Parikh CR, Consortium T-A : Association of AKI with mortality and complications in hospitalized patients with cirrhosis. Hepatology 57(2) : 753-62, 2013
2. Fagundes C, Barreto R, Guevara M, Garcia E, Sola E, Rodriguez E, Graupera I, Ariza X, Pereira G, Alfaro I, Cardenas A, Fernandez J, Poch E, Gines P : A modified acute kidney injury classification for diagnosis and risk stratification of impairment of kidney function in cirrhosis. J Hepatol 59(3) : 474-81, 2013
3. Garcia-Tsao G, Parikh CR, Viola A : Acute kidney injury in cirrhosis. Hepatology 48(6) : 2064-77, 2008

4. Davenport A : Difficulties in assessing renal function in patients with cirrhosis : potential impact on patient treatment. Intensive Care Med 37(6) : 930-2, 2011

5. Mindikoglu AL, Opekun AR, Mitch WE, Magder LS, Christenson RH, Dowling TC, Weir MR, Seliger SL, Howell CD, Raufman JP, Rana A, Goss JA, Khaderi SA, Vierling JM : Cystatin C Is a Gender-Neutral Glomerular Filtration Rate Biomarker in Patients with Cirrhosis. Dig Dis Sci 63(3) : 665-75, 2018

6. Francoz C, Sola E : Assessment of renal function in cirrhosis : Sarcopenia, gender and ethnicity matter. J Hepatol 70(5) : 828-30, 2019

7. Davenport A, Cholongitas E, Xirouchakis E, Burroughs AK : Pitfalls in assessing renal function in patients with cirrhosis--potential inequity for access to treatment of hepatorenal failure and liver transplantation. Nephrol Dial Transplant 26(9) : 2735-42, 2011

8. Caregaro L, Menon F, Angeli P, Amodio P, Merkel C, Bortoluzzi A, Alberino F, Gatta A : Limitations of serum creatinine level and creatinine clearance as filtration markers in cirrhosis. Arch Intern Med 154(2) : 201-5, 1994

9. Angeli P, Gines P, Wong F, Bernardi M, Boyer TD, Gerbes A, Moreau R, Jalan R, Sarin SK, Piano S, Moore K, Lee SS, Durand F, Salerno F, Caraceni P, Kim WR, Arroyo V, Garcia-Tsao G : Diagnosis and management of acute kidney injury in patients with cirrhosis : revised consensus recommendations of the International Club of Ascites. Gut 64(4) : 531-7, 2015

10. Shlipak MG, Mattes MD, Peralta CA : Update on cystatin C : incorporation into clinical practice. Am J Kidney Dis 62(3) : 595-603, 2013

11. Levin A : Cystatin C, serum creatinine, and estimates of kidney function : searching for better measures of kidney function and cardiovascular risk. Ann Intern Med 142(7) : 586-8, 2005

12. Markwardt D, Holdt L, Steib C, Benesic A, Bendtsen F, Bernardi M, Moreau R, Teupser D, Wendon J, Nevens F, Trebicka J, Garcia E, Pavesi M, Arroyo V, Gerbes AL : Plasma cystatin C is a predictor of renal dysfunction, acute-on-chronic liver failure, and mortality in patients with acutely decompensated liver cirrhosis. Hepatology 66(4) : 1232-41, 2017

13. Gerbes AL, Gulberg V, Bilzer M, Vogeser M : Evaluation of serum cystatin C concentration as a marker of renal function in patients with cirrhosis of the liver. Gut 50(1) : 106-10, 2002

14. Seo YS, Park SY, Kim MY, Kim SG, Park JY, Yim HJ, Jang BK, Park SH, Kim JH, Suk KT, Kim JD, Kim TY, Cho EY, Lee JS, Jung SW, Jang JY, An H, Tak WY, Baik SK, Hwang JS, Kim YS, Sohn JH, Um SH : Serum cystatin C level : An excellent predictor of mortality in patients with cirrhotic ascites. J Gastroenterol Hepatol 33(4) : 910-7, 2018

15. Wu J, Wu Q, Wu M, Mao W : Serum Cystatin C Predicts Mortality in HBV-Related Decompensated Cirrhosis. Biomed Res Int 2019 : 7272045, 2019

16. Belcher JM, Sanyal AJ, Garcia-Tsao G, Ansari N, Coca SG, Shlipak MG, Parikh CR : Early trends in cystatin C and outcomes in patients with cirrhosis and acute kidney injury. Int J Nephrol 2014 : 708585, 2014

17. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V, Consortium CSIotE-C : Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. Gastroenterology 144(7) : 1426-37, 37 e1-9, 2013

18. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS, Investigators C-E : Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 367(1) : 20-9, 2012

19. Stevens PE, Levin A : Evaluation and management of chronic kidney disease : synopsis of the kidney disease : improving global outcomes 2012 clinical practice guideline. Ann Intern Med 158(11) : 825-30, 2013

20. Angeli P, Garcia-Tsao G, Nadim MK, Parikh CR : News in pathophysiology, definition and classification of hepatorenal syndrome : A step beyond the International Club of Ascites (ICA) consensus document. J Hepatol 71(4) : 811-22, 2019

21. Slack AJ, McPhail MJ, Ostermann M, Bruce M, Sherwood R, Musto R, Dew T, Auzinger G, Bernal W, O'Grady J, Heneghan MA, Moore K, Wendon JA : Predicting the development of acute kidney injury in liver cirrhosis--an analysis of glomerular filtration rate, proteinuria and kidney injury biomarkers. Aliment Pharmacol Ther 37(10) : 989-97, 2013

22. Christensen E : Prognostic models including the Child-Pugh, MELD and Mayo risk scores--where are we and where should we go? J Hepatol 41(2) : 344-50, 2004

23. D'Amico G, Garcia-Tsao G, Pagliaro L : Natural history and prognostic indicators of survival in cirrhosis : a systematic review of 118 studies. J Hepatol 44(1) : 217-31, 2006

24. Allegretti AS, Parada XV, Ortiz GA, Long J, Krinsky S, Zhao S, Fuchs BC, Sojoodi M, Zhang D, Karumanchi A, Kalim S, Nigwekar SU, Thadhani RI, Parikh SM, Chung RT : Serum Angiopoietin-2 Predicts Mortality and Kidney Outcomes in Decompensated Cirrhosis. Hepatology 69(2) : 729-41, 2019

25. Piano S, Brocca A, Mareso S, and Angeli P : Infections complicating cirrhosis. Liver Int 38 Suppl 1 : 126-33, 2018

26. Claria J, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, Amoros A, Titos E, Alcaraz-Quiles J, Oettl K, Morales-Ruiz M, Angeli P, Domenicali M, Alessandria C, Gerbes A, Wendon J, Nevens F, Trebicka J, Laleman W, Saliba F, Welzel TM, Albillos A, Gustot T, Benten D, Durand F, Gines P, Bernardi M, Arroyo V : Systemic inflammation in decompensated cirrhosis : Characterization and role in acute-on-chronic liver failure. Hepatology 64(4) : 1249-64, 2016

27. Wong F, Piano S, Singh V, Bartoletti M, Maiwall R, Alessandria C, Fernandez J, Soares EC, Kim DJ, Kim SE, Marino M, Vorobioff J, Barea RCR, Merli M, Elkrief L, Vargas V, Krag A, Singh SP, Lesmana LA, Toledo C, Marciano S, Verhelst X, Intagliata N, Rabinowich L, Colombato L, Kim SG, Gerbes A, Durand F, Roblero JP, Bruns T, Yoon EL, Girala M, Pyrsopoulos NT, Kim TH, Yim SY, Juanola A, Gadano A, Angeli P : Clinical features and evolution of bacterial infection-related acute-on-chronic liver failure. J Hepatol 74(2) : 330-9, 2021