ORIGINAL ARTICLE OPEN ACCESS

Urine TIMP2.IGFBP7 Reflects Kidney Injury After Moderate Volume Paracentesis in Patients With Ascites: A Randomized Control Study

Anuchit Suksamai[1] | Sanpolpai Khaoprasert[1] | Amnart Chaiprasert[2] | Sakkarin Chirapongsathorn[1]

[1]Division of Gastroenterology and Hepatology, Department of Medicine, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand | [2]Division of Nephrology, Department of Medicine, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand

Correspondence: Sakkarin Chirapongsathorn (sakkarin.chi@pcm.ac.th)

Received: 19 September 2024 | **Revised:** 1 April 2025 | **Accepted:** 12 April 2025

Keywords: AKI | decompensated cirrhosis | novel biomarker | urine IGFBP7 | urine TIMP2

ABSTRACT

Background: Urinary biomarkers may predict acute kidney injury (AKI) in cirrhosis with ascites in a moderate volume paracentesis setting.

Objective: The study aimed to assess the risk and consequence of AKI and its progression in patients with decompensated cirrhosis undergoing paracentesis using a urine test measuring tissue inhibitor of metalloproteinases-2 (TIMP2) and insulin-like growth factor-binding protein 7 (IGFBP7).

Methods: A randomized, controlled trial was performed. All outpatients with decompensated cirrhosis with ascites and diuretic complications were enrolled and randomized into 3 and 5 L paracentesis groups. Serial urine samples were analyzed for TIMP2.IGFBP7 concentration before and after paracentesis.

Results: A total of 90 patients with decompensated cirrhosis were consecutively enrolled during the study period. After screening, 29 patients were enrolled in the 3-L paracentesis group, and 25 patients were enrolled in the 5-L paracentesis group. The mean of the MELD score was 8 ± 1.2. Urine TIMP2.IGFBP7 > 2, rising urine TIMP2, and rising urine TIMP2/urine Cr were shown in patients within the 5-L group for 48% ($p = 0.015$), 32% ($p = 0.049$), and 76% ($p = 0.010$) respectively, indicating a higher incidence of renal tubular injury markers in this group. Urine TIMP2.IGFBP7/1000 > 2 was statistically significant to predict a hemodynamic event ($p = 0.002$).

Conclusion: In cirrhotic patients with ascites undergoing paracentesis, a 5-L paracentesis volume was associated with a higher incidence of renal tubular injury markers.

Trail Registration: The national clinical registration number was TCTR20191116003.

1 | Introduction

Acute kidney injury (AKI) complicates the course of cirrhosis in over 20% of hospitalized patients with chronic liver disease, significantly impacting the prognosis [1]. Decompensated cirrhosis significantly increases the risk of renal failure due to splanchnic vasodilation and reduced systemic vascular resistance. Multiple risk factors contribute to this increased risk, including diuretic therapy for ascites, abdominal paracentesis, gastrointestinal bleeding, infections, or the development of hepatorenal syndrome, a severe complication with a high mortality rate [1–3]. Abdominal paracentesis, a common procedure in cirrhosis with tense ascites, can lead to AKI and hyponatremia, which can occur following both low volume and large volume paracentesis [4]. The mechanisms by which paracentesis without albumin infusion causes renal failure and hyponatremia in patients with tense ascites involve the

rapid reduction in intra-abdominal pressure. This can lead to rapid ascites reaccumulation, decreased effective blood volume, and subsequent impaired renal function [4, 5]. In patients with liver cirrhosis, malnutrition and muscle wasting can significantly amplify the misinterpretation of renal function based solely on serum creatinine levels. Additionally, ascites and peripheral edema can further reduce these levels by expanding the distribution of creatinine in the body [6, 7]. These raise concerns regarding the reliability of creatinine-based models, like the model of end-stage liver disease (MELD) score for accurately assessing mortality risk in all patients with cirrhosis [8]. The discovery of new biomarkers has recently been used in the field of acute kidney injury because of their simple access to urine analysis [9]. There have been some recent studies that have revealed the use of urinary biomarkers such as cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), kidney injury molecule 1 (KIM-1), and liver type fatty acid-binding protein (L-FABP) for the diagnosis of AKI in cirrhosis [10–12].

Novel biomarkers offer improved assessment of kidney function and AKI in cirrhotic patients, though with varying degrees of accuracy and specificity. Cystatin C, superior to creatinine, enhances GFR accuracy when combined with creatinine, especially in reduced GFR and low muscle mass, and predicts mortality better than the MELD score. However, its combined equations are less accurate in cirrhosis than in the general population [13–16]. Urinary NGAL shows promise for early AKI detection and distinguishing acute tubular necrosis (ATN) from HRS [17–19]. Urinary IL-18 and KIM-1 are elevated in ATN and may aid in AKI subtyping, but significant overlap limits their individual diagnostic accuracy [11, 20–23]. Urinary L-FABP reflects renal tubular damage and may serve as an AKI marker via Toll-like receptor type 4 (TLR-4) activation, which is also elevated in cirrhotic AKI [24–26].

Tissue inhibitors of metalloproteinases-2 (TIMP2) and insulin-like growth factor-binding protein 7 (IGFBP7) are expressed and secreted by primary kidney epithelial cells, with IGFBP7 primarily from proximal tubules and TIMP2 more strongly from distal tubules [27]. During the early phase of cellular damage induced by inflammation, ischemia, oxidative stress, or receiving renal toxicity agents, TIMP2 and IGFBP7 are associated with G1 cell cycle arrest in renal tubular cells. This G1 arrest effectively prevents their progression to the G_2 and M phases of the cell cycle [27–29]. This arrest is a protective mechanism aimed at preventing further cellular damage and allowing for repair. The increased synthesis of TIMP2 and IGFBP7 leads to their release into the tubular lumen. These proteins are then excreted in the urine, resulting in elevated urinary levels. The detection of these biomarkers in the urine indicates that the kidney has experienced cellular stress [30].

According to previous studies, TIMP2 and IGFBP7 have only been used in critically ill patients who have been admitted with respiratory illness, cardiovascular disease, neurological disease, surgical problems, septic shock, and trauma and have been used as biomarkers to predict early AKI after 12 h [30]. The urinary TIMP2 and IGFBP7 were measured in ng/mL and multiplied with each other for increased power of detection. Rising urinary TIMP2 and IGFBP7 levels represent that there was more tubular secretion of biomarkers as a result of renal injury. The relative risk (95% CI) of developed AKI for subjects testing at urinary [TIMP2]·[IGFBP7] values of ≤ 0.3 versus > 0.3–2 were 4.7 (1.5–16), and 12 (4.2–40) for urinary [TIMP2]·[IGFBP7] values of < 0.3 versus > 2. For the 0.3 cutoff, sensitivity was 89%, and specificity was 50%. For 2.0, sensitivity was 42%, and specificity was 95%. This study also found that the risk for major adverse kidney events (death, dialysis or persistent renal dysfunction) within 30 days elevated sharply for [TIMP2]·[IGFBP7] above 0.3 and doubled when values were > 2.0 [30–32]. However, there is no data on these two biomarkers in patients with cirrhosis.

In cirrhotic patients undergoing paracentesis, particularly large-volume paracentesis without adequate albumin replacement, the rapid reduction in intra-abdominal pressure can lead to hemodynamic instability. This instability can result in renal hypoperfusion, causing ischemic injury to the tubular cells. The injured tubular cells then respond by upregulating and secreting TIMP2 and IGFBP7, leading to their increased urinary excretion. The ascites reaccumulation and decreased effective blood volume that occur after paracentesis further exacerbate the hemodynamics that cause tubular damage.

Large volume paracentesis is an effective treatment for refractory ascites. There is one study that suggested that a single 5-L paracentesis in patients with cirrhosis and tense, diuretic-resistant ascites without albumin infusion is safe and causes no disturbances in systemic and renal hemodynamics 48 h after the procedure [33]. However, there have been a small number of patients studied and no data on early detection of acute kidney injury (AKI). This study aimed to determine whether urinary TIMP2 and IGFBP7 can predict AKI, a reduction in glomerular filtration rate (GFR), or alterations in mean arterial blood pressure (MAP) following moderate-volume paracentesis, comparing a 3 versus a 5-L volume of paracentesis.

2 | Methods

2.1 | Trial Design

This is a randomized, prospective controlled trial.

2.2 | Randomization and Blinding

The eligible patients were randomized with the use of a computer-generated block of four randomizations and stratified into two groups, which received abdominal paracentesis for 3 or 5 L of ascites. The trial statistician, independent from the study team, generated the random order in which participants would be assigned to groups. The research assistant, following the random order, assigned the participants to two abdominal paracentesis groups without knowing the order themselves. The main investigator, medical laboratory technologist, and patients were blinded to the randomization list.

2.3 | Participants, Eligibility Criteria, and Settings

This trial was conducted from December 4, 2018, to December 3, 2021. Although institutional review board (IRB) approval was obtained on December 4, 2018, trial registration occurred after the

study initiation due to constraints in the test kit procurement process. Consequently, screening of the first patient was commenced on November 16, 2019. The authors confirm that all ongoing and related trials for this intervention are registered. Eligible individuals were outpatients with cirrhosis aged between 18 and 80 years who were diagnosed with criteria of diuretic-resistance or diuretic-intractable ascites according to a combination of clinical, biochemical, and imaging studies such as ultrasonography, computed tomography, and magnetic resonance imaging, as well as the presence of varices, ascites, or liver biopsy.

All consecutive patients were screened and approached for enrollment by investigators at the Gastroenterology and Hepatology outpatient clinic, Phramongkutklao Hospital, Bangkok, Thailand. Patients had to provide written, informed consent before any study procedures occurred. Exclusion criteria were patients with end-stage renal disease receiving renal replacement therapy, patients with acute kidney injury during enrollment, patients with unstable hemodynamics during previous abdominal paracentesis, patients with cardiac arrhythmia or heart failure, patients with pacemaker devices owing to the inappropriate measurement of heart rate and blood pressure, shock during enrollment, defined as a mean arterial pressure (MAP) of less than 65 mmHg and signs of poor tissue perfusion, patients who had received radio-contrast media or received nonsteroidal anti-inflammatory drugs within the previous 2 weeks, urinary tract infection, and pregnancy. Patients with deviation from the intended liters of ascites release as randomized, for instance, randomized to 5 L and only 3 L came out, were also excluded. Patients who developed infections or sepsis during the study period were then excluded. We ensured no diuretic dose adjustments were made during the two-week follow-up and reviewed patient medication records to confirm the absence of any nephrotoxic drug therapy during this period. All participants with confirmed ascites had a location of needle puncture for abdominal paracentesis determined by bedside ultrasonography to estimate the amount of ascites that could be safely released per group. All enrolled cirrhotic patients with ascites did not receive an albumin infusion or other volume expander during abdominal paracentesis, because no more than 5 L of ascites were removed. Information on medical history, current use of medications, and diuretic dosage, along with the cause of cirrhosis, was recorded for each patient. Body weight, height, and calculated body mass index (BMI) as body weight (kg) divided by height (m) squared (kg/m^2) were measured. Laboratory evaluations included hemoglobin, platelet count, prothrombin time, international normalized ratio (PT/INR), albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, blood urea nitrogen, and creatinine, which were collected from all participants. Child-Pugh score (CTP), the model for end-stage liver disease (MELD and MELD-Na) scores, and glomerular filtration rate (GFR) according to the chronic kidney disease epidemiology collaboration (CKD-EPI) were performed to assess the severity of liver and kidney impairment.

All subjects were properly instructed and consented to participate in this trial by signing the informed consent regulation provided by the Institutional Review Board of the Royal Thai Army Medical Department Committee (IRB number R164h/61) and registered at www.clinicaltrials.in.th. The registration number was TCTR20191116003. The Institutional Review Board of the Royal Thai Army Medical Department Committee uses the World Medical Association: DECLARATION OF HELSINKI, GUIDELINES FOR GOOD CLINICAL PRACTICE: ICH Harmonized Tripartite Guideline, Council for International Organizations of Medical Sciences (CIOMS), CODE of FEDERAL REGULATIONS: Title 45 Public Welfare; Part 46 Protection of Human Subjects, and the Belmont Report to regulate the ethics concerns in publications. Informed consent was obtained from all subjects, and all methods were conducted according to the relevant guidelines and regulations. All patients provided complete informed consent to participate in the study.

2.4 | Sample Collection, Method of Measurement and Interventions

Urine samples, collected pre- and post-abdominal paracentesis, were centrifuged, and the supernatant was stored at −20°C for TIMP2 and IGFBP7 measurement using sandwich enzyme-linked immunosorbent assays (ELISA). Samples underwent no freeze–thaw cycles, and protease inhibitors were omitted. A blinded medical technologist performed the assays. TIMP2 was measured using Quantikine ELISA by Abcam, Cambridge, U.S.A. with a 2-fold urine dilution and a 2-h incubation. IGFBP7 was measured using SimpleStep ELISA by Bio-Techne, R&D Systems Inc., Minneapolis, U.S.A. with a 1:800 urine dilution and a 1-h incubation. Both ELISAs involved standard curve generation and optical density readings at 450 nm, following the manufacturer's instructions.

The urinary TIMP2 and IGFBP7 were measured in ng/mL and multiplied by each other for increased power of detection. Urinary creatinine was measured to calculate ratios with TIMP2 and IGFBP7. These ratios were used to normalize biomarker levels for variations in urine concentration, which can be influenced by factors such as hydration status and urine output, thereby providing a more accurate reflection of renal tubular secretion. Rising urinary TIMP2 and IGFBP7 levels represent that there was more tubular secretion of biomarkers compared between before and after abdominal paracentesis. The result of urine TIMP2 × IGFBP7/1000 > 2 means that patients have a high risk of acute kidney injury event according to a Sapphire study from Kashani et al. [30] and supported by other studies [31, 32].

Urine samples were collected before and immediately after completing abdominal paracentesis to evaluate the primary endpoint. Blood pressure and heart rate were recorded every 30 min until the end of the procedure. Eligible patients were then followed up every 2 weeks until 12 weeks, and total abdominal paracentesis times, admission, and death due to cirrhotic complications were recorded to assess the secondary endpoints. Serum creatinine at 2 weeks of follow-up was collected to define the acute kidney injury. Acute kidney injury was defined according to KDIGO guidelines, which include a serum creatinine increase of more than 50% within 7 days or an absolute increase of more than 0.3 mg/dL within 48 h. Given the low risk of acute, severe kidney injury following outpatient abdominal paracentesis, and considering the gradual nature of ascites reaccumulation, a two-week follow-up period was deemed to be sufficient to assess for potential further kidney injury [34]. Serum creatinine levels at 12 weeks of follow-up were evaluated, and GFRs were estimated by the CKD-EPI equation ($mL/min/1.73m^2$) to define the rapid

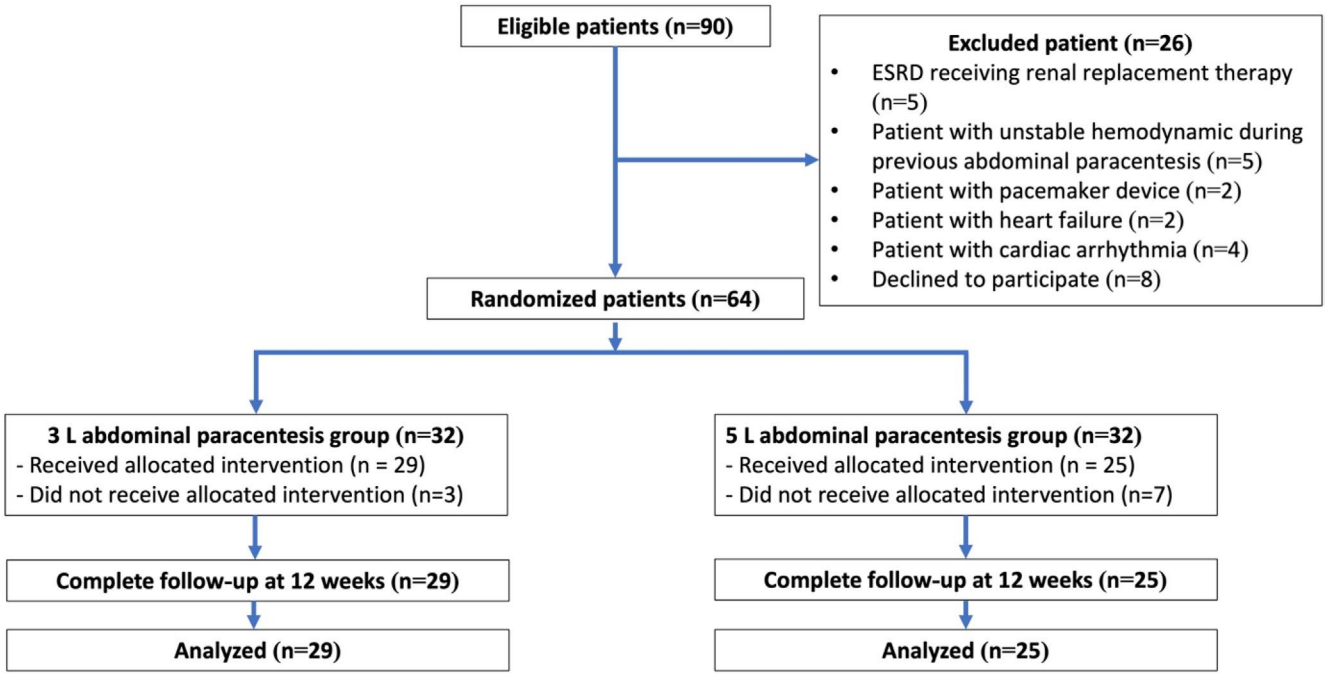


FIGURE 1 | Flowchart of the study.

progression of kidney function within 3 months. A flowchart of the study is shown in Figure 1.

2.5 | Outcome Measurement

2.5.1 | Primary Outcome

The primary endpoint was the development of any stage of AKI according to the KDIGO guidelines within 2 weeks of follow-up. The reference values for serum creatinine were obtained at the time of enrollment.

2.5.2 | Secondary Outcomes

A hemodynamic event was defined by decreased systolic blood pressure ≥ 10 mmHg, decreased mean arterial blood pressure ≥ 10 mmHg, or a risen heart rate ≥ 20 beats per minute observed within 120 min after abdominal paracentesis [35, 36].

Rapid decline of GFR was defined as a sustained decline in eGFR of more than 5 mL/min/1.73 m^2/year [37, 38].

Rates of hospitalization, mortality, and total abdominal paracentesis times within 12 weeks were collected.

2.6 | Statistical Analysis

The main endpoint of the study selected to calculate the sample size was the comparison of urine TIMP2 and IGFBP7 between the 3 and 5 L abdominal paracentesis groups. Sample size calculation was determined from the study of Kashani et al. [30] To detect a 20% difference in the proportion of patients with positive urine TIMP2 and IGFBP7 levels between the 3 and 5-L paracentesis groups, a sample size of 24 patients per group was calculated, assuming a two-sided alpha error of 5% and a power of 90% (beta error of 10%).

Baseline clinical and biochemical data were compared using independent t-tests or chi-square tests, depending on the normality of data distribution. Categorical variables were compared using chi-square tests or Fisher's exact test, depending on the sample size within categories. Continuous variables were presented as mean ± standard deviation (SD) for normally distributed data, median with interquartile range (IQR) for non-normally distributed data, while categorical variables were presented as percentage and frequency over the total available. The rapid decline of GFR at 12 weeks of follow-up was compared between groups using the Fisher exact test. A receiver operating curve (ROC) analysis was employed to calculate the area under the curve (AUC) for TIMP2.IGFBP7 to find the best cut-off values identifying AKI and hemodynamic event, respectively. Significance was established at a p value of <0.05. The analysis was done using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY).

3 | Results

3.1 | Study Population

This study screened 90 outpatients with decompensated cirrhosis from December 4, 2018, to December 3, 2021. Twenty-six patients were excluded, leaving 64 patients who were enrolled and randomized. Figure 1 summarizes the flowchart of the study. Patients who deviated from the intended liters of ascites release were also excluded. This resulted in 29 patients in the 3-L group and 25 patients in the 5-L group, whose data were analyzed.

The baseline demographic characteristics of the study population were similar between the two groups, as shown in Table 1. The mean age of patients was 64 years, and most of the patients were male (87%). The most common cause of decompensated cirrhosis in the study was alcoholic cirrhosis (50%). Most patients had CTP class B cirrhosis (78%). The means of MELD and MELD-Na scores were 12.9 ± 4.8 and 16.2 ± 5.4, respectively. The median dose of spironolactone was 50 mg in both groups. There were no statistically significant differences in the underlying disease of patients or biochemical values between the two groups (Table 1).

3.2 | Primary Outcome

The rates of AKI events within 2 weeks after abdominal paracentesis were similar between the 3 L, $n = 3$ (10%) and 5 L, $n = 3$ (12%) groups; $p = 0.85$ (Table 2). Urinary TIMP2 and IGFBP7 showed no statistical differences between patients who experienced AKI and those who did not (Table 3).

Statistically, rising urine TIMP2 and rising urine TIMP2/urine Cr were all higher with a 5 L paracentesis $n = 5$ (17%) versus $n = 12$ (48%); $p = 0.01$; $n = 3$ (10%) versus $n = 8$ (32%); $p = 0.049$; and $n = 12$ (41%) versus $n = 19$ (76%); $p = 0.01$, respectively. However, there was no statistically significant difference between the 3 and 5 L groups in rising urine IGFBP7, urine TIMP2.IGFBP7, urine IGFBP7/urine Cr, and urine TIMP2.IGFBP7/urine Cr. (Table 2).

3.3 | Secondary Outcome

Hemodynamic event was assessed during abdominal paracentesis with no statistical difference between the 3 and 5 L groups, $n = 3$ (10%) versus $n = 5$ (20%); $p = 0.31$. There were no statistically significant differences in the rates of rapid GFR decline between the two groups, $n = 16$ (55%) versus $n = 14$ (56%); $p = 0.95$. Ascites release times were statistically different between the 3 L group = 4 (3, 6) and the 5 L group = 6 (4, 6); $p = 0.032$. There was no statistical difference between the two groups in admission and death within 3 months of the follow-up (Table 2). Five patients died within 90 days of follow-up from sepsis ($n = 2$), spontaneous bacterial peritonitis ($n = 2$), and primary bacteremia ($n = 1$).

Rising urine IGFBP7, rising urine TIMP2.IGFBP7, rising urine IGFBP7/urine Cr, rising urine TIMP2.IGFBP7/urine Cr, and urine TIMP2 × IGFBP7/1000 > 2 showed significantly higher hemodynamic events than those without increases, $n = 9$ (19%) versus $n = 7$ (78%); $p > 0.001$, $n = 6$ (13%) versus $n = 6$ (75%); $p > 0.001$, $n = 18$ (39%) versus $n = 7$ (87%); $p = 0.011$, $n = 14$ (30%) versus $n = 7$ (87%); $p = 0.002$, and $n = 11$ (23%) versus $n = 6$ (75%); $p = 0.004$, respectively (Table 4).

Mean arterial pressure was statistically significantly declined at 2 h of abdominal paracentesis compared with baseline within both groups, the 3 L group declined 3.6 (95% CI: 1.8–5.4), $p < 0.001$ and the 5 L group declined 5.2 (95% CI: 2.9–7.6), $p < 0.001$. Between the 2 groups there were no statistically significant declinations. (Figure 2).

TABLE 1 | Demographic data.

Variables	3 L group ($n = 29$)	5 L group ($n = 25$)	p
Age (years)	64 ± 10.2	65 ± 10.96	0.75
Male	24 (82%)	23 (92%)	0.47
Body weight (kg)	63 ± 13.2	62 ± 15.2	0.86
BMI (kg/m^2)	23.3 ± 4.6	22.4 ± 5.3	0.51
Etiology			
Alcohol	14 (48%)	13 (52%)	0.78
Hepatitis B virus	5 (18%)	5 (20%)	0.95
Hepatitis C virus	4 (14%)	2 (8%)	0.49
Metabolic associated steatohepatitis	4 (14%)	3 (12%)	0.84
Others	2 (6%)	2 (8%)	0.78
Child-Pugh score	8.9 ± 1.4	8.8 ± 1.05	0.96
CTP B	22 (76%)	20 (80%)	0.71
CTP C	7 (24%)	5 (20%)	0.71
MELD score	12.2 ± 4.4	13.5 ± 5.1	0.33
MELD-Na score	15.1 ± 5.5	17.4 ± 5.2	0.13
Diuretic dosage			
Spironolactone (mg)	50 (25, 50)	50 (25, 100)	0.22
Furosemide (mg)	20 (0, 20)	20 (0, 40)	0.40
Underlying disease			
Diabetes	15 (51%)	15 (60%)	0.54
Hypertension	9 (31%)	13 (52%)	0.11
Dyslipidemia	4 (13%)	6 (24%)	0.33
Coronary artery disease	1 (3%)	2 (8%)	0.46
Biochemical value			
Hemoglobin (g/dl)	10.2 ± 1.9	10.03 ± 1.5	0.68
Platelet (×10^9/L)	189 ± 78	213 ± 83	0.27
INR	1.22 ± 0.2	1.25 ± 0.2	0.72
Albumin (g/dL)	2.8 ± 0.6	2.8 ± 0.4	0.77
Bilirubin (mg/dL)	1.6 ± 2.04	2.3 ± 4.1	0.40
Sodium (mEq/L)	134 ± 4	133 ± 4	0.44
Creatinine (mg/dL)	1.2 ± 0.4	1.4 ± 0.7	0.20
CKD-EPI (ml/min/1.73m^2)	65 ± 28	62 ± 31	0.67

Note: Values presented as mean ± SD, or median (IQR) and n (%). p value corresponds to independent t test and chi-square test.

TABLE 2 | Urine biomarkers and outcomes between the two groups.

Variables	3 L group ($n=29$)	5 L group ($n=25$)	p
TIMP2.IGFBP7/1000 > 2	5 (17%)	12 (48%)	0.01
Rising urine TIMP2	3 (10%)	8 (32%)	0.049
Rising urine IGFBP7	8 (27%)	8 (32%)	0.72
Rising urine TIMP2.IGFBP7	5 (17%)	7 (28%)	0.34
Rising urine TIMP2/Urine Cr	12 (41%)	19 (76%)	0.01
Rising urine IGFBP7/Urine Cr	12 (41%)	13 (52%)	0.43
Rising urine TIMP2.IGFBP7/urine Cr	9 (31%)	12 (48%)	0.20
Acute kidney injury	3 (10%)	3 (12%)	0.85
Hemodynamic event	3 (10%)	5 (20%)	0.31
Change MAP (mmHg))	−3.6 ± 3.7	−5.2 ± 4.3	0.06
Ascites release times (times)	4 (3, 6)	6 (4, 6)	0.032
Rapid decline of GFR in 3 months	16 (55%)	14 (56%)	0.95
Admission within 3 months	7 (24%)	8 (32%)	0.52
Death within 3 months	3 (10%)	2 (8%)	0.76

Note: Value presented as *n* (%). *p* value corresponds to independent *t* test or Chi-Square test.

TABLE 3 | Prediction of AKI event by urinary biomarker.

Variables	No AKI ($n=48$)	AKI ($n=6$)	p
Rising urine TIMP2	10 (20%)	1 (17%)	0.811
Rising urine IGFBP7	15 (31%)	1 (17%)	0.461
Rising urine TIMP2. IGFBP7	12 (25%)	0 (0%)	0.165
Rising urine TIMP2/ Urine Cr	28 (58%)	3 (50%)	0.697
Rising urine IGFBP7/ Urine Cr	22 (46%)	3 (50%)	0.847
Rising urine TIMP2. IGFBP7/urine Cr	19 (40%)	2 (33%)	0.767
TIMP2.IGFBP7/1000 > 2	18 (38%)	4 (67%)	0.170

Note: Value presented as *n* (%). *p* value corresponds to Chi-Square test.

Urine TIMP2.IGFBP7 more than 3.435 (AUC 0.85, 95% CI 72–0.99; $p=0.002$), rising urine TIMP2.IGFBP7/urine Cr more than 0.0027 (AUC 0.84, 95% CI 0.65–1; $p=0.002$) and rising urine IGFBP7/urine Cr more than 0.0025 (AUC 0.80, 95% CI 0.06–0.95; $p=0.006$) were statistically significant to predict a hemodynamic event (Figure 3).

4 | Discussion

This study had four key findings. First, urine TIMP2.IGFBP7/1000 > 2 shows good performance in predicting the rapid decline of GFR and hemodynamic events, crucial for early intervention and improved prognosis in patients with decompensated cirrhosis. Second, urinary TIMP2 and IGFBP7 demonstrated greater sensitivity for early detection of tubular injury compared with serum creatinine, enabling proactive measures to prevent further kidney damage. Third, the high prevalence of rapid GFR decline within 90 days in both the 3 and 5 L abdominal paracentesis groups highlighted the potential for renal complications regardless of ascites volume removed. Furthermore, this study indicated that renal tubular injury could occur even when less than 5 L of ascites are released in decompensated cirrhosis.

In patients with cirrhosis, increased portal pressure triggers splanchnic vasodilation. This vasodilation is caused, at least in part, by the release of nitric oxide, which leads to decreased systemic vascular resistance (SVR) and a subsequent decrease in arterial blood pressure. To compensate for the drop in blood pressure and maintain adequate perfusion to vital organs like the kidneys, the heart increases its cardiac output [39]. However, splanchnic vasodilation does not directly decrease cardiac output. Instead, it leads to a reduction in effective arterial blood volume. This triggers the activation of the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system (SNS), and the release of arginine vasopressin (AVP) [40]. These hormones collectively promote renal sodium and water reabsorption, contributing to ascites development and progression. Abdominal paracentesis can have significant hemodynamic effects. It leads to a reduction in effective arterial blood volume, which in turn triggers renal vasoconstriction. If this vasoconstriction cannot be adequately compensated for by an increased cardiac output, renal blood flow may markedly decrease, predisposing patients to acute kidney injury [41]. Alterations in both systemic and intrarenal hemodynamics, accompanied by abnormal autoregulation of renal blood flow over time, lead to decreased GFR [42].

Early detection of Acute Kidney Injury (AKI) remains challenging due to limitations in current methods, such as the low

TABLE 4 | Prediction of hemodynamic event by urinary biomarker.

Variables	No hemodynamic event ($n=46$)	Hemodynamic event ($n=8$)	p
Rising urine TIMP2	8 (17%)	3 (37%)	0.19
Rising urine IGFBP7	9 (19%)	7 (78%)	> 0.001
Rising urine TIMP2.IGFBP7	6 (13%)	6 (75%)	> 0.001
Rising urine TIMP2/Urine Cr	25 (54%)	6 (75%)	0.27
Rising urine IGFBP7/Urine Cr	18 (39%)	7 (87%)	0.011
Rising urine TIMP2.IGFBP7/urine Cr	14 (30%)	7 (87%)	0.002
TIMP2.IGFBP7/1000 > 2	11 (23%)	6 (75%)	0.004

Note: Value presented as *n* (%). *p* value corresponds to Chi-square test.

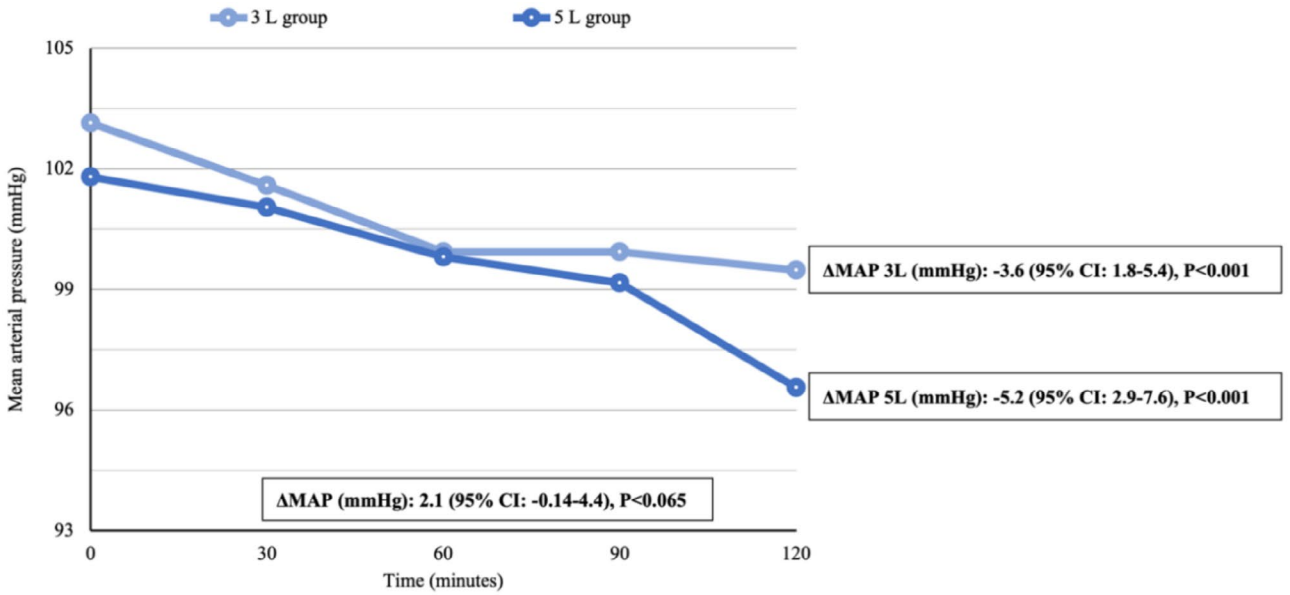
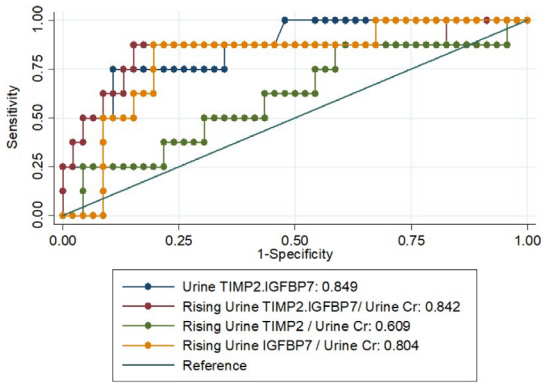


FIGURE 2 | Declined mean arterial blood pressure between two groups. Comparing mean arterial pressure between groups. *p value was less than 0.05.

sensitivity and specificity of changes in creatinine levels. This delays intervention and potentially worsens patient outcomes. Recent advancements offer promise, with a combined biomarker test (IGFBP7 and TIMP2) showing potential for assessing AKI risk in critically ill patients [9, 43, 44]. A combination of TIMP2•IGFBP7, urinary markers of cell cycle arrest reflecting cellular stress preceding tissue damage, has been shown to improve the diagnostic and prognostic values for AKI. Approved by the American Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2014, these markers offer advantages in terms of earlier diagnosis and prognosis compared to traditional methods relying on creatinine rise or reduced urine output [30, 45]. Urinary TIMP2•IGFBP7/1000 values greater than 2 have been associated with a higher risk of AKI in several studies, while values less than 0.3 have been associated with a lower risk [30–32], but there have been no studies in cirrhotic patients. The study by Chindarkar NS et al. [46] suggests that a [IGFBP7]•[TIMP2] value of 0.3 falls within the normal reference range (0.04–2.22 ng/mL) and has a good balance between sensitivity and specificity for detecting AKI in critically ill patients. Additionally, a value of 2.0 or higher indicates a significantly higher risk of AKI with high specificity.

The hypothesis of this study is presented in Figure 4.

This first randomized, prospective, single-center study investigated the predictive value of both novel biomarkers, TIMP2 and IGFBP7, in decompensated liver cirrhotic patients who had been diagnosed with diuretic-resistance or diuretic-intractable ascites. This study aimed to assess their ability to predict rapid GFR decline, hemodynamic events, 90-day readmission, and 90-day mortality in these patients randomly assigned to receive moderate volume paracentesis for 3 and 5 L groups. For patients presenting with tense ascites, the recommended first-line therapy is large-volume paracentesis (LVP) combined with albumin infusion. This combined approach effectively reduced ascites while minimizing the risk of complications like post-paracentesis circulatory dysfunction through intravascular volume maintenance. Smaller-volume paracentesis combined with albumin infusion could be utilized in patients with diuretic-resistance or diuretic-intractable ascites to minimize further complications.



Variables	Cut-off value	AUC	95% CI	Sensitivity	Specificity	*p*-value
Urine TIMP2.IGFBP7/1000	3.435	0.85	0.72-0.99	75%	89%	0.002
Rising Urine TIMP2.IGFBP7/ Urine Cr	0.0027	0.84	0.65-1	88%	85%	0.002
Rising Urine IGFBP7/Urine Cr	0.0025	0.80	0.06-0.95	88%	80%	0.006

FIGURE 3 | ROC curve for predicting hemodynamic event.

In this study, alcohol was the leading cause of cirrhosis (50%), followed by chronic HBV (20%), chronic HCV (11%), MASH (13%), and autoimmune hepatitis (3.5%). The mean CTP score was 8.9, which is similar to the study of Belcher et al. [11], which aimed to evaluate structural (neutrophil gelatinase–associated lipocalin, IL-18, kidney injury molecule-1 [KIM-1], liver-type fatty acid–binding protein [L-FABP], and albuminuria) and functional (fractional excretion of sodium [FENa]) urinary biomarkers as predictors of AKI progression and in-hospital mortality.

The median dose of spironolactone and furosemide was 50 and 20 mg, respectively. The dose of spironolactone and furosemide was not at a maximum dose due to the fact that most decompensated cirrhotic patients in this study had diuretic-intractable problems. At this point, the dosage of diuretics could not significantly affect the kinetics of kidney function in this context.

In our study, AKI did not develop within 2 weeks of the first abdominal paracentesis. However, 55% and 56% of cirrhotic patients who received 3 and 5 L of abdominal paracentesis, respectively, experienced a rapid decline in GFR within 90 days during multiple ascites releases. These results suggest that while a single abdominal paracentesis may not increase creatinine within 2 weeks, repeated procedures can lead to a decline in GFR over time. Urinary TIMP2.IGFBP7/1000 > 2 and rising TIMP2 levels were significantly associated with higher volumes of ascites release, particularly in the 5-L group. However, our findings were not directly comparable to the study by Bihorac et al. [47], which demonstrated urine TIMP2.IGFBP7/1000 > 2 predicted AKI in ICU patients who received major operations and nephrotoxic agents. This difference may be attributed to the fact that our study excluded cirrhotic patients receiving contrast agents or suffering from congestive heart failure.

Urine TIMP2.IGFBP7 demonstrated higher performance for predicting the rapid GFR decline compared to serum creatinine. Cirrhotic patients with ascites who have urinary TIMP2.IGFBP7 levels above the cutoff value of 3.435 should be aware of the risk of hemodynamic events during abdominal paracentesis, even if less than 5 L of ascites are removed. Additionally, elevated urinary TIMP2.IGFBP7 levels might indicate renal tubular injury, even in the absence of increased serum creatinine. Low-volume abdominal paracentesis might be safe at this time, or considering the addition of a volume expander like intravenous albumin could be reasonable for this patient even though moderate ascites release was done. Further repeated abdominal paracentesis in these patients might lead to a further decline in GFR within 3 months.

Urine TIMP2.IGFBP7/1000, rising urine TIMP2.IGFBP7/Cr, and rising urine IGFBP7/urine Cr all demonstrated strong potential for predicting hemodynamic events with AUCs of 0.85 (95% CI 72–0.99), 0.84 (95% CI 0.65–1), and 0.80 (95% CI 0.06–0.95), respectively. The predictive performance of TIMP2.IGFBP7 was as good as the other biomarkers such as urine NGAL (AUC 0.77; 95% CI: 0.68–0.85), urine IL-18 (AUC 0.71; 95% CI 0.61–0.81), urine KIM-1 (AUC 0.66; 95% CI 0.56–0.76), and urine L-FABP (AUC 0.76; 95% CI; 0.66–0.85) as reported in the study of Belcher et al. [11].

In both the 3 and 5-L groups, patients with elevated urine IGFBP7, elevated urine TIMP2.IGFBP7, elevated urine IGFBP7/urine Cr, elevated urine TIMP2.IGFBP7/urine Cr, and urine TIMP2.IGFBP7/1000 > 2 exhibited significantly higher rates of hemodynamic events than those without these increases. This study confirmed that the risk of renal tubular injury would increase even with abdominal paracentesis of less than 5 L in patients with decompensated cirrhosis. This is the strength of this study that defines the performance of two novel biomarkers,

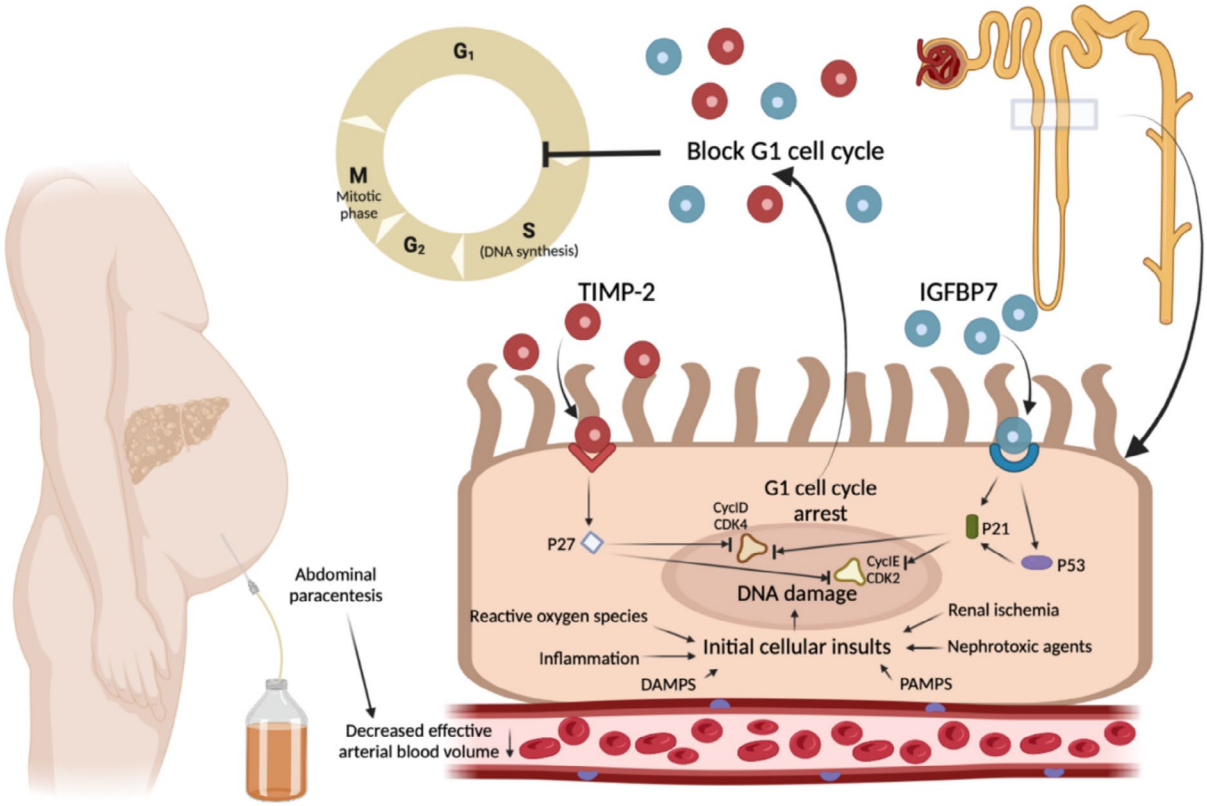


FIGURE 4 | The hypothesis of renal tubular injury with detection of TIMP2 and IGFBP7 in cirrhosis. Adapted from Kashani et al. [30] proposed mechanism of the novel biomarkers in AKI developing in patients with cirrhosis: Initial renal tubular injury by decreased effective arterial blood volume during abdominal paracentesis. Regarding to DNA of tubular cellular damage, IGFBP7 and TIMP2 are secreted in the tubular cells. IGFBP7 directly increases the expression of p53 and p21 and TIMP-2 stimulates p27 expression via IGFBP7 and TIMP2 receptors. Theses p proteins then block the effect of the cyclin-dependent protein kinase complexes (CyclD-CDK4 and CyclE-CDK2) on the cell cycle promotion, thereby resulting in G_1 cell cycle arrest for short periods of time presumably to avoid cells with possible damage from dividing.

which the previous two studies from Bihorac et al. [31] and Hoste et al. [32] did not perform. This finding confirmed that TIMP2 and IGFBP7 are known to be involved in the cellular response to various abuses, such as inflammation, ischemia, oxidative stress, or receiving renal toxicity agents [27–29]. This observation provides a mechanistic explanation for the increased risk of AKI in patients with decompensated cirrhosis. AKI is one of the clinical manifestations of post-paracentesis induced circulatory dysfunction (PICD) [36]. According to the European Association for the Study of the Liver (EASL) clinical practice guidelines for the management of patients with decompensated cirrhosis, the risk of developing PICD is low in patients undergoing paracentesis of less than 5 L of ascites. Therefore, the guidelines recommend albumin replacement only for patients who release more than 5 L of ascites in a single session. In Thailand, there are reimbursement challenges related to albumin infusion for patients who release less than 5 L of ascites. Additionally, including albumin in this study might have reflected the hypothesis of early tubular injury, as novel biomarkers are released only in the early phase of injury. However, according to a recent study of Tergast et al., performing repeated low-volume paracentesis of 1.5 L/d or more without albumin infusion is associated with a higher risk for hyponatremia, AKI, and increasing inflammation [48]. This study also revealed renal tubular injury even with small-volume abdominal paracentesis, suggesting vulnerability in these patients. This finding raises the possibility that albumin infusion might play a role in preventing or mitigating the severity of PICD and protecting renal function.

Most patients in this study demonstrated hemodynamic events (15%) and rapid decline of GFR events (55%), and there were not statistically significant differences between the two groups. This study's findings suggest that even small-volume (less than 5 L) abdominal paracentesis may be associated with a decline in mean arterial pressure and potential for further renal injury. This is in contrast to the previous study by Peltekian et al. [33], which demonstrated a single 5 L paracentesis in patients with cirrhosis and tense, diuretic-resistant ascites without albumin infusion caused no disturbances in systemic and renal hemodynamics 48 h after the procedure. This study's findings could support the conclusion that although short-term hemodynamic and renal outcomes were not achieved, the rapid GFR decline in 90 days could be displayed even if an abdominal paracentesis of less than 5 L was performed.

Recent studies have demonstrated the beneficial use of these two biomarkers in ICU patients [30–32]; therefore, this may influence the interpretation of their levels in cirrhotic patients in an outpatient department (OPD) setting. The hemodynamics affecting the kidneys in critically ill patients in the ICU setting share some similarities with those seen in cirrhotic patients but also exhibit important differences. Both groups can experience renal

hypoperfusion due to systemic hypotension, sepsis-induced circulatory dysfunction, and decreased effective arterial blood volume. Inflammatory mediators play a significant role in AKI pathogenesis in both settings. Cirrhosis introduces unique hemodynamic alterations, including splanchnic vasodilation, portal hypertension, and the development of hepatorenal syndrome (HRS). The renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system are often chronically activated in cirrhosis, leading to renal vasoconstriction. The hemodynamics of a large volume paracentesis and the reaccumulation of ascites are very specific to cirrhotic patients. ICU patients often experience more profound and acute hemodynamic instability due to sepsis, septic shock, and the use of vasoactive medications. ICU patients frequently have multiple organ system failure that impacts renal function. Cirrhotic patients admitted to the ICU with sepsis or bleeding exhibit a combination of these hemodynamic profiles. It is very important to consider the baseline hemodynamics of a cirrhotic patient when comparing them to a patient that does not suffer from cirrhosis.

While our study primarily focused on the risk of AKI following paracentesis, it is important to note that frequent hospitalizations, increased mortality, and a high number of paracentesis procedures within a short timeframe may also impact patient outcomes. These factors can contribute to systemic inflammation, malnutrition, and other complications that can exacerbate kidney dysfunction. There were some limitations to this study. First, this was a single-center study design and focus on a Thai population; the applicability of this study may be limited to diverse patient populations, particularly in regions with different causes of cirrhosis such as MASH or chronic hepatitis C. Future research should include multicenter trials with larger sample sizes to validate these findings and explore the long-term impact of these factors on renal function in patients with cirrhosis and ascites. Second, this study was limited to outpatients with cirrhosis and ascites, so its findings may not be applicable to all cirrhotic patients, including those with acute decompensated cirrhosis, acute-on-chronic liver failure, or underlying heart or renal disease. Third, the urine biomarker data collections were not performed in cirrhotic patients who received more than 5 L of ascites release. Finally, this study did not validate the model to predict the risk of a rapid decline in GFR, hemodynamic event, or 90-day mortality in an external cohort. However, this study is the first to demonstrate the potential of urine TIMP2 and IGFBP7 as diagnostic biomarkers and confirmed that those who received moderate volume paracentesis could develop renal tubular injury. Future research could quantify the key hormones involved in the pathophysiology of ascites accumulation by measuring plasma renin and aldosterone levels, which may correlate with the volume of ascites removed and the rise in urinary TIMP2 and IGFBP7. To accurately assess the temporal relationship between paracentesis-induced hemodynamic changes and renal injury, serial measurements of MAP and urinary biomarkers are necessary. Extending serial urinary TIMP2 and IGFBP7 measurements to Day 7 and 14 post-paracentesis would further enhance the capture of renal injury and PICD temporal dynamics in this patient population. Finally, a more comprehensive assessment of potential confounding factors, combined with more frequent serum creatinine monitoring, would strengthen the correlation between early biomarkers and delayed renal outcomes.

5 | Conclusions

In patients undergoing 5 L of ascites removal, urinary TIMP2.IGFBP7 levels exceeding 2 were not associated with an increased likelihood of acute renal failure. This observation suggests that even smaller volumes of ascites removal may pose a risk to renal function in cirrhotic patients with ascites.

Acknowledgments

Our study was funded by the Gastroenterological Association of Thailand and the Department of Medicine, Phramongkutklao Hospital.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. G. Garcia-Tsao, C. R. Parikh, and A. Viola, "Acute Kidney Injury in Cirrhosis," *Hepatology* 48 (2008): 2064–2077.

2. F. Wong, J. G. O'Leary, K. R. Reddy, et al., "New Consensus Definition of Acute Kidney Injury Accurately Predicts 30-Day Mortality in Patients With Cirrhosis and Infection," *Gastroenterology* 145 (2013): 1280–1288.

3. M. Martin-Llahi, M. Guevara, A. Torre, et al., "Prognostic Importance of the Cause of Renal Failure in Patients With Cirrhosis," *Gastroenterology* 140 (2011): 488–496.

4. P. Gines, L. Tito, V. Arroyo, et al., "Randomized Comparative Study of Therapeutic Paracentesis With and Without Intravenous Albumin in Cirrhosis," *Gastroenterology* 94 (1988): 1493–1502.

5. D. M. Simon, J. R. McCain, H. L. Bonkovsky, J. O. Wells, D. K. Hartle, and J. T. Galambos, "Effects of Therapeutic Paracentesis on Systemic and Hepatic Hemodynamics and on Renal and Hormonal Function," *Hepatology* 7, no. 3 (1987): 423–429, https://doi.org/10.1002/hep.1840070302.

6. R. H. Wiesner, S. V. McDiarmid, P. S. Kamath, et al., "MELD and PELD: Application of Survival Models to Liver Allocation," *Liver Transplantation* 7, no. 7 (2001): 567–580, https://doi.org/10.1053/jlts.2001.25879.

7. C. Francoz, D. Prie, W. Abdelrazek, et al., "Inaccuracies of Creatinine and Creatinine-Based Equations in Candidates for Liver Transplantation With Low Creatinine: Impact on the Model for End-Stage Liver Disease Score," *Liver Transplantation* 16 (2010): 1169–1177.

8. E. Cholongitas, L. Marelli, A. Kerry, et al., "Female Liver Transplant Recipients With the Same GFR as Male Recipients Have Lower MELD Scores—A Systematic Bias," *American Journal of Transplantation* 7, no. 3 (2007): 685–692, https://doi.org/10.1111/j.1600-6143.2007.01666.x.

9. P. T. Murray, R. L. Mehta, A. Shaw, et al., "Potential Use of Biomarkers in Acute Kidney Injury: Report and Summary of Recommendations From the 10th Acute Dialysis Quality Initiative Consensus Conference," *Kidney International* 85 (2014): 513–521.

10. C. Francoz, M. K. Nadim, and F. Durand, "Kidney Biomarkers in Cirrhosis," *Journal of Hepatology* 65 (2016): 809–824.

11. J. M. Belcher, G. Garcia-Tsao, A. J. Sanyal, et al., "Urinary Biomarkers and Progression of AKI in Patients With Cirrhosis," *Clinical Journal of the American Society of Nephrology* 9 (2014): 1857–1867.

12. J. Puthumana, X. Ariza, J. M. Belcher, et al., "Urine Interleukin 18 and Lipocalin 2 Are Biomarkers of Acute Tubular Necrosis in Patients With Cirrhosis: A Systematic Review and Meta-Analysis," *Clinical Gastroenterology and Hepatology* 15 (2017): 1003–1013.

13. A. L. Gerbes, V. Gulberg, M. Bilzer, et al., "Evaluation of Serum Cystatin C Concentration as a Marker of Renal Function in Patients With Cirrhosis of the Liver," *Gut* 50 (2002): 106–110.

14. L. A. Inker, C. H. Schmid, H. Tighiouart, et al., "Estimating Glomerular Filtration Rate From Serum Creatinine and Cystatin C," *New England Journal of Medicine* 367 (2012): 20–29.

15. A. L. Mindikoglu, T. C. Dowling, M. R. Weir, S. L. Seliger, R. H. Christenson, and L. S. Magder, "Performance of Chronic Kidney Disease Epidemiology Collaboration Creatinine-Cystatin C Equation for Estimating Kidney Function in Cirrhosis," *Hepatology* 59 (2014): 1532–1542.

16. A. Suksamai, A. Chaiprasert, and S. Chirapongsathorn, "Serum Cystatin C as a Predictor of 90-Day Mortality Among Patients Admitted With Complications of Cirrhosis," *JGH Open* 5 (2021): 607–613.

17. J. M. Belcher, A. J. Sanyal, A. J. Peixoto, et al., "Kidney Biomarkers and Differential Diagnosis of Patients With Cirrhosis and Acute Kidney Injury," *Hepatology* 60 (2014): 622–632.

18. C. Fagundes, M. N. Pepin, M. Guevara, et al., "Urinary Neutrophil Gelatinase-Associated Lipocalin as Biomarker in the Differential Diagnosis of Impairment of Kidney Function in Cirrhosis," *Journal of Hepatology* 57 (2012): 267–273.

19. J. Mishra, Q. Ma, A. Prada, et al., "Identification of Neutrophil Gelatinase-Associated Lipocalin as a Novel Early Urinary Biomarker for Ischemic Renal Injury," *Journal of the American Society of Nephrology* 14, no. 10 (2003): 2534–2543, https://doi.org/10.1097/01.asn.0000088027.54400.c6.

20. W. K. Han, V. Bailly, R. Abichandani, R. Thadhani, and J. V. Bonventre, "Kidney Injury Molecule-1 (KIM-1): A Novel Biomarker for Human Renal Proximal Tubule Injury," *Kidney International* 62 (2002): 237–244.

21. Y. Liu, W. Guo, J. Zhang, et al., "Urinary Interleukin 18 for Detection of Acute Kidney Injury: A Meta-Analysis," *American Journal of Kidney Diseases* 62 (2013): 1058–1067.

22. C. R. Parikh and P. Devarajan, "New Biomarkers of Acute Kidney Injury," *Critical Care Medicine* 36 (2008): 59–65.

23. E. D. Siew, T. A. Ikizler, T. Gebretsadik, et al., "Elevated Urinary IL-18 Levels at the Time of ICU Admission Predict Adverse Clinical Outcomes," *Clinical Journal of the American Society of Nephrology* 5 (2010): 1497–1505.

24. H. J. Anders, B. Banas, and D. Schlondorff, "Signaling Danger: Toll-Like Receptors and Their Potential Roles in Kidney Disease," *Journal of the American Society of Nephrology* 15, no. 4 (2004): 854–867, https://doi.org/10.1097/01.asn.0000121781.89599.16.

25. K. Doi, E. Noiri, R. Maeda-Mamiya, et al., "Urinary L-Type Fatty Acid-Binding Protein as a New Biomarker of Sepsis Complicated With Acute Kidney Injury," *Critical Care Medicine* 38, no. 10 (2010): 2037–2042, https://doi.org/10.1097/CCM.0b013e3181eedac0.

26. N. Shah, F. E. Mohamed, M. Jover-Cobos, et al., "Increased Renal Expression and Urinary Excretion of TLR4 in Acute Kidney Injury Associated With Cirrhosis," *Liver International* 33 (2013): 398–409.

27. D. R. Emlet, N. Pastor-Soler, A. Marciszyn, et al., "Insulin-Like Growth Factor Binding Protein 7 and Tissue Inhibitor of Metalloproteinases-2: Differential Expression and Secretion in Human Kidney Tubule Cells," *American Journal of Physiology. Renal Physiology* 312 (2017): F284–F296.

28. P. M. Price, R. L. Safirstein, and J. Megyesi, "The Cell Cycle and Acute Kidney Injury," *Kidney International* 76 (2009): 604–613.

29. D. W. Seo, H. Li, C. K. Qu, et al., "Shp-1 Mediates the Antiproliferative Activity of Tissue Inhibitor of Metalloproteinase-2 in Human Microvascular Endothelial Cells," *Journal of Biological Chemistry* 281 (2006): 3711–3721.

30. K. Kashani, A. Al-Khafaji, T. Ardiles, et al., "Discovery and Validation of Cell Cycle Arrest Biomarkers in Human Acute Kidney Injury," *Critical Care* 17 (2013): R25.

31. A. Bihorac, L. S. Chawla, A. D. Shaw, et al., "Validation of Cell-Cycle Arrest Biomarkers for Acute Kidney Injury Using Clinical Adjudication," *American Journal of Respiratory and Critical Care Medicine* 189 (2014): 932–939.

32. E. A. Hoste, P. A. McCullough, K. Kashani, et al., "Derivation and Validation of Cutoffs for Clinical Use of Cell Cycle Arrest Biomarkers," *Nephrology, Dialysis, Transplantation* 29 (2014): 2054–2061.

33. K. M. Peltekian, F. Wong, P. P. Liu, A. G. Logan, M. Sherman, and L. M. Blendis, "Cardiovascular, Renal, and Neurohumoral Responses to Single Large-Volume Paracentesis in Patients With Cirrhosis and Diuretic-Resistant Ascites," *American Journal of Gastroenterology* 92 (1997): 394–399.

34. F. Salerno, S. Badalamenti, P. Incerti, et al., "Repeated Paracentesis and i.v. Albumin Infusion to Treat 'tense' Ascites in Cirrhotic Patients. A Safe Alternative Therapy," *Journal of Hepatology* 5 (1987): 102–108.

35. M. P. Moranville, K. D. Mieure, and E. M. Santayana, "Evaluation and Management of Shock States: Hypovolemic, Distributive, and Cardiogenic Shock," *Journal of Pharmaceutical Practice* 24 (2011): 44–60.

36. J. Sola-Vera and J. Such, "Understanding the Mechanisms of Paracentesis-Induced Crculatory Dysfunction," *European Journal of Gastroenterology & Hepatology* 16 (2004): 295–298.

37. L. S. Chawla, R. Bellomo, A. Bihorac, et al., "Acute Kidney Disease and Renal Recovery: Consensus Report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup," *Nature Reviews Nephrology* 13, no. 4 (2017): 241–257, https://doi.org/10.1038/nrneph.2017.2.

38. C. Ronco, R. Bellomo, and J. A. Kellum, "Acute Kidney Injury," *Lancet* 394 (2019): 1949–1964.

39. R. Moreau and D. Lebrec, "Acute Renal Failure in Patients With Cirrhosis: Perspectives in the Age of MELD," *Hepatology* 37 (2003): 233–243.

40. P. Gines and R. W. Schrier, "Renal Failure in Cirrhosis," *New England Journal of Medicine* 361 (2009): 1279–1290.

41. A. Krag, F. Bendtsen, J. H. Henriksen, and S. Moller, "Low Cardiac Output Predicts Development of Hepatorenal Syndrome and Survival in Patients With Cirrhosis and Ascites," *Gut* 59 (2010): 105–110.

42. M. Bernardi, R. Moreau, P. Angeli, B. Schnabl, and V. Arroyo, "Mechanisms of Decompensation and Organ Failure in Cirrhosis: From Peripheral Arterial Vasodilation to Systemic Inflammation Hypothesis," *Journal of Hepatology* 63 (2015): 1272–1284.

43. A. Lacquaniti, F. Ceresa, S. Campo, et al., "Acute Kidney Injury and Sepsis After Cardiac Surgery: The Roles of Tissue Inhibitor Metalloproteinase-2, Insulin-Like Growth Factor Binding Protein-7, and Mid-Regional Pro-Adrenomedullin," *Journal of Clinical Medicine* 12, no. 16 (2023): 5193, https://doi.org/10.3390/jcm12165193.

44. A. Lacquaniti and P. Monardo, "Acute Kidney Injury: Advances in Clinical Management," *Journal of Clinical Medicine* 11, no. 24 (2022): 7308, https://doi.org/10.3390/jcm11247308.

45. G. Ilaria, K. Kianoush, B. Ruxandra, et al., "Clinical Adoption of Nephrocheck(R) in the Early Detection of Acute Kidney Injury," *Annals of Clinical Biochemistry* 58 (2021): 6–15.

46. N. S. Chindarkar, L. S. Chawla, J. A. Straseski, et al., "Reference Intervals of Urinary Acute Kidney Injury (AKI) Markers [IGFBP7]•[TIMP2] in Apparently Healthy Subjects and Chronic Comorbid Subjects Without AKI," *Clinica Chimica Acta* 452 (2016): 32–37.

47. M. Ostermann, P. A. McCullough, L. G. Forni, et al., "Kinetics of Urinary Cell Cycle Arrest Markers for Acute Kidney Injury Following Exposure to Potential Renal Insults," *Critical Care Medicine* 46 (2018): 375–383.

48. T. L. Tergast, M. Griemsmann, L. Stockhoff, et al., "Daily Low-Volume Paracentesis and Clinical Complications in Patients With Refractory Ascites," *JAMA Network Open* 6 (2023): e2322048.