Journal Pre-proof

Dual Biomarker Strategy with [TIMP-2]•[IGFBP7] and Copeptin Enhances Early Diagnosis and Risk Prediction in HRS-AKI

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Please cite this article as: EL-Adawy NM, Abdel-Aleem MS, Abdel-Gelil RA-Aziz, Zaki ZM, Keryakos HKH, Dual Biomarker Strategy with [TIMP-2]•[IGFBP7] and Copeptin Enhances Early Diagnosis and Risk Prediction in HRS-AKI (2026), doi: https://doi.org/10.1016/j.nefro.2026.501486

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Title Page

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Dual Biomarker Strategy with [TIMP-2]•[IGFBP7] and Copeptin Enhances Early Diagnosis and Risk Prediction in HRS-AKI

Abstract

Background: Acute kidney injury (AKI) in liver cirrhosis, particularly hepatorenal syndrome (HRS), is a life-threatening complication that requires early diagnosis for effective treatment. HRS can potentially be reversed with the early initiation of vasoconstrictors. This study evaluated the diagnostic and prognostic value of cell cycle arrest biomarkers [TIMP-2]•[IGFBP7] and serum copeptin in HRS among Egyptian patients with HCV-related liver cirrhosis.

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Aims: The primary objectives of this study were to determine whether serum copeptin and urinary [TIMP-2]•[IGFBP7] can distinguish HRS-AKI from HRS-CKD and from non-HRS decompensated cirrhosis, and to evaluate whether these biomarkers, alone and in combination with MELD 3.0, improve short-term mortality risk prediction in patients with HRS-AKI.

Methods: This single-center observational study included 80 patients with HCV-related liver cirrhosis (20 compensated, 30 decompensated, 30 HRS) and 20 healthy controls. Urinary [TIMP-2]•[IGFBP7] and serum copeptin levels were measured and analyzed for association with HRS and mortality.

Results: Levels of both biomarkers were significantly elevated in HRS patients, particularly in HRS-AKI compared to HRS-CKD ($p < 0.01$). Serum copeptin showed a strong correlation with [TIMP-2]•[IGFBP7] (r = 0.72, $p < 0.01$). For HRS-AKI diagnosis, [TIMP-2]•[IGFBP7] (cutoff 0.25 [ng/mL]²/1000) demonstrated 93% sensitivity and 78% specificity, while copeptin (cutoff 9.97 pmol/L) showed 89% sensitivity and 83% specificity. The mortality rate in HRS-AKI was 66.7%, with both biomarkers significantly higher in non-survivors ($p < 0.01$). [TIMP-2]•[IGFBP7] (cutoff 0.52 [ng/mL]²/1000) predicted mortality with 92% sensitivity and 84% specificity.

Conclusion: In patients with decompensated HCV-related cirrhosis, combined assessment of serum copeptin and urinary [TIMP-2]•[IGFBP7] distinguishes HRS-AKI from HRS-CKD and, when added to MELD 3.0, significantly improves short-term mortality risk stratification beyond conventional clinical scoring alone. Mortality prediction and proposed cutoffs are exploratory and require validation in larger cohorts before routine clinical implementation.

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List of Abbreviations

AKI - Acute Kidney Injury

ATI - Acute Tubular Injury

AUC - Area Under the Curve

BMI - Body Mass Index

CI - Confidence Interval

CKD - Chronic Kidney Disease

COPD - Chronic Obstructive Pulmonary Disease

CPT - Child-Pugh-Turcotte (score)

ELISA - Enzyme-Linked Immunosorbent Assay

eGFR - Estimated Glomerular Filtration Rate

FeNa - Fractional Excretion of Sodium

HBV - Hepatitis B Virus

HCV - Hepatitis C Virus

HRS - Hepatorenal Syndrome

HRS-AKI - Hepatorenal Syndrome-Acute Kidney Injury

HRS-CKD - Hepatorenal Syndrome-Chronic Kidney Disease

ICA - International Club of Ascites

IGFBP7 - Insulin-like Growth Factor Binding Protein 7

INR - International Normalized Ratio

KDIGO - Kidney Disease: Improving Global Outcomes

LC - Liver Cirrhosis

MAP - Mean Arterial Pressure

MELD - Model for End-Stage Liver Disease

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NGAL - Neutrophil Gelatinase-Associated Lipocalin

NYHA - New York Heart Association

OR - Odds Ratio

ROC - Receiver Operating Characteristic

SD - Standard Deviation

TIMP-2 - Tissue Inhibitor of Metalloproteinases-2

UACR - Urine Albumin-to-Creatinine Ratio

Introduction

The incidence of acute kidney injury (AKI) in liver cirrhosis is increasing, affecting up to 40% of cases and making it the most common organ failure in decompensated cirrhosis (1). The primary causes of AKI in liver cirrhosis include prerenal azotemia, acute tubular injury (ATI), and the hepatorenal syndrome-acute kidney injury (HRS-AKI) subtype, though multiple etiologies can coexist (2). Early and accurate diagnosis is crucial as HRS carries the worst prognosis among the causes of AKI in liver cirrhosis, and treatment strategies vary significantly (3, 4). The 90-day mortality rate for HRS-AKI is approximately 50% (5), and the dynamic overlap of AKI etiologies further complicates the clinical management.

HRS is a functional, prerenal AKI characterized by impaired autoregulation, minimal proteinuria, and low urinary sodium excretion, progressing with the severity of liver disease (6). Distinguishing potentially reversible HRS from ATI remains challenging, yet essential: HRS requires vasoconstrictor therapy (e.g., terlipressin) with albumin to restore renal perfusion (targeting a ≥15 mmHg increase in mean arterial pressure [MAP]), whereas ATI necessitates supportive care or dialysis (4). Despite its functional nature—evidenced by preserved kidney integrity on postmortem examination and post-transplant recovery—HRS diagnosis relies on serum creatinine, a suboptimal marker in cirrhosis. Reduced creatinine

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production (due to hepatic/muscle atrophy, low protein intake), volume overload, and bilirubin interference delay diagnosis (7). The International Club of Ascites (ICA) criteria, though improved, still reflect advanced renal dysfunction, potentially postponing vasoconstrictor initiation by 3–4 days (8). Notably, higher baseline creatinine correlates with poorer terlipressin response, underscoring the need for earlier biomarkers. While urinary neutrophil gelatinase-associated lipocalin (NGAL) and fractional sodium excretion (FeNa) aid in assessing tubular injury, their routine use remains limited globally.

Kidney biopsy, the gold standard for most renal diseases, shows poor correlation with kidney function and urinalysis in liver disease (9-11), highlighting the unmet diagnostic needs in HRS. Serum copeptin, a stable surrogate for vasopressin, rises with liver disease severity and complications (12, 13), while urinary cell cycle arrest biomarker [TIMP-2]•[IGFBP7] reflects renal tubular stress and injury. However, their diagnostic and prognostic utility in HRS remains underexplored (14).

Therefore, we aimed to evaluate whether combining serum copeptin, a marker of hemodynamic and vasopressin axis activation, with urinary [TIMP-2]•[IGFBP7], a marker of tubular stress, can improve the clinical assessment of kidney injury in decompensated cirrhosis. Specifically, our objectives were: (1) to determine whether these biomarkers distinguish HRS-AKI from HRS-CKD and non-HRS decompensated cirrhosis, and (2) to assess whether copeptin and [TIMP-2]•[IGFBP7], alone and in combination with MELD 3.0, refine short-term mortality risk stratification in HRS-AKI.

To date, serum copeptin and urinary [TIMP-2]•[IGFBP7] have been evaluated separately in cirrhosis and AKI, but, to our knowledge, their combined diagnostic and prognostic performance in patients with decompensated cirrhosis and HRS has not previously been reported (12-14).

Subjects and Methods

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Study Design and Population

This prospective observational case-control study was conducted at Minia University Hospital between August 2022 and December 2024. The primary objectives of this study were to determine whether serum copeptin (hemodynamic/vasopressin axis) and urinary [TIMP-2]•[IGFBP7] (tubular stress) can distinguish HRS-AKI from HRS-CKD and from non-HRS decompensated cirrhosis, and to evaluate whether these biomarkers, alone and in combination with MELD 3.0, improve short-term mortality risk prediction in patients with HRS-AKI. A secondary objective was to explore clinically actionable cutoff values for copeptin and [TIMP-2]•[IGFBP7] that could support early recognition and risk-guided management in routine practice. The study enrolled 80 patients with HCV-related liver cirrhosis and 20 age- and sex-matched healthy controls. Approval was obtained from the local ethics committee, and the study adhered to the Helsinki Declaration. All participants provided informed written consent.

We prospectively enrolled adult patients with HCV-related cirrhosis who were followed at our tertiary liver unit. All enrolled patients had previously received direct-acting antiviral therapy and had achieved sustained virologic response (SVR12), confirmed by undetectable HCV RNA at enrollment. To minimize etiologic heterogeneity, we restricted inclusion to hepatitis C–related cirrhosis and excluded other causes of chronic liver disease (e.g., metabolic dysfunction–associated steatotic liver disease, alcoholic liver disease, autoimmune or cholestatic liver disease). This restriction was chosen to avoid confounding from differing comorbidity profiles and pathophysiologic mechanisms, particularly the higher prevalence of hypertension, diabetes, obesity, and intrinsic kidney disease in non-HCV etiologies, which could influence baseline copeptin and [TIMP-2]•[IGFBP7] levels and obscure biomarker–outcome relationships.

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Patients with HCV-related liver cirrhosis (LC) were stratified into three groups: (1) the compensated LC group (n=20) with no clinically or radiologically detectable ascites; (2) the decompensated LC group (n=30) presenting with diuretic-responsive ascites; and (3) the HRS group (n=30), which was further subdivided into HRS-AKI (n=15) meeting ADQI/ICA criteria, and HRS-CKD (n=15) with eGFR < 60 mL/min/1.73 m^2 for $\geq$ 3 months (Fig. 1). Inclusion criteria required HCV-positive liver cirrhosis confirmed by liver biopsy or by clinical, radiographic, and biochemical investigations, with HRS diagnosis following the revised ADQI/ICA criteria. Exclusion criteria comprised: (1) serious comorbidities (NYHA class IV heart failure, O_2-dependent COPD, advanced cancer); (2) non-HCV cirrhosis etiologies; (3) shock; (4) structural kidney disease (presence of **significant proteinuria** (e.g., >500 mg/day or equivalent spot UACR), **persistent hematuria** suggestive of glomerular disease, and **abnormal renal imaging** (e.g., small echogenic kidneys or obstructive uropathy); (5) nephrotoxic drug use within 30 days; (6) active urinary tract infection; (7) anuria >12 hours; (8) current renal replacement therapy; or (9) history of solid organ transplantation.

Definition of Hepatorenal Syndrome

Hepatorenal syndrome is defined according to ADQI/ICA criteria, with HRS-AKI diagnosis requiring: (a) cirrhosis with ascites; (b) AKI meeting KDIGO criteria (serum creatinine increase $\geq$0.3 mg/dL [$\geq$26.5 μmol/L] within 48 hours or $\geq$50% increase from baseline within 7 days and/or urine output $\leq$0.5 mL/kg/h for $\geq$6 hours); (c) no improvement after 24-hour volume resuscitation with albumin (1 g/kg/day); and (d) exclusion of alternative AKI causes (8), where underlying kidney disease does not exclude superimposed HRS-AKI; HRS-CKD requires eGFR <60 mL/min/1.73 m² for $\geq$3 months without structural kidney disease (e.g., proteinuria <500 mg/day, normal renal imaging) (8, 15). Refractory ascites, as

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defined by the EASL clinical practice guidelines on ascites management (16), was frequent in this cohort but was not required for inclusion.

Clinical Data Collection and Laboratory Methods

Demographic data were collected for all patients, and abdominopelvic ultrasound examinations were performed at enrollment using an ultrasound machine with a 3.5 megahertz (MHz) transducer (**GE Healthcare, USA**). Whole citrated blood samples were used to measure complete blood counts (CBC) using an automated hematology analyzer (*Sysmex Corporation, Kobe, Japan*). A fully automated clinical chemistry auto-analyzer system, Konelab 60i (*Thermo Electron Corporation, Finland*) was utilized to measure fasting blood glucose, liver and renal function tests using commercial kits. Complete urine analysis including examination of urine sediment was conducted along with an assessment of the urine albumin-to-creatinine ratio. Child-Pugh-Turcotte (CPT) scores and MELD 3.0 scores were calculated for all patients. The MELD 3.0 score was calculated according to the recently updated model by Kim et al., which incorporates serum bilirubin, creatinine, INR, serum sodium, serum albumin, and sex (17). This choice reflects our current local practice, where MELD 3.0 is used for prognostic assessment and transplant prioritization.

Sampling Timeline and Biomarker Kinetics

All biomarkers were measured at the time of HRS diagnosis, following volume resuscitation and confirmation of non-responsiveness to albumin challenge per ADQI/ICA criteria. Importantly, this timing reflects a clinically actionable window just prior to vasoconstrictor therapy initiation, ensuring the relevance of biomarker levels for both diagnostic and prognostic purposes.

While both copeptin and [TIMP-2]•[IGFBP7] were sampled concurrently, their biological trajectories likely differ. Copeptin—a stable surrogate for arginine vasopressin (AVP)—is expected to rise early in response to splanchnic vasodilation and systemic

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hypoperfusion, consistent with its role as a marker of hemodynamic dysfunction. In contrast, [TIMP-2]•[IGFBP7], which reflects G1 cell cycle arrest in renal tubular epithelial cells, may increase slightly later as structural tubular stress ensues. This temporal distinction supports a pathophysiological sequence: circulatory dysfunction marked by copeptin surge, followed by tubular stress detected via [TIMP-2]•[IGFBP7].

Urinary tissue inhibitor of metalloproteinases-2 (TIMP-2) was measured on baseline urine samples collected at enrollment, centrifuged to remove particulate matter, aliquoted, and stored at −80 °C until analysis. TIMP-2 concentrations were determined using a commercially available sandwich ELISA kit (SinoGeneClon Biotech Co., Ltd., Hangzhou, China) according to the provider's instructions, and are reported in ng/mL. All measurements were performed in duplicate, and the mean value was used for analysis. The composite index [TIMP-2]•[IGFBP7] was subsequently calculated as the product of the individual concentrations and expressed as (ng/mL)²/1000 for consistency with prior literature. This yields a single numerical value expressed in **units of (ng/mL)²/1000**, the standardized unit for [TIMP-2]•[IGFBP7] .

Urinary insulin-like growth factor binding protein-7 (IGFBP7) was quantified from the same baseline urine samples using a commercial sandwich ELISA (SinoGeneClon Biotech Co., Ltd., Hangzhou, China). Samples were thawed once, gently mixed, and, when necessary, diluted with assay buffer to fall within the linear range of the standard curve. IGFBP7 concentrations are reported in ng/mL. Each sample was assayed in duplicate, and the average of the two readings was used. Together with urinary TIMP-2, IGFBP7 values were used to derive the composite [TIMP-2]•[IGFBP7] index as described above.

Plasma copeptin was measured on EDTA (or serum) samples obtained at the same time point as the urine collection, immediately centrifuged, and stored at −80 °C until batch analysis. Copeptin levels were determined using a commercial two-site second-generation

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ELISA kit (SinoGeneClon Biotech Co., Hangzhou, China) following the manufacturer's

protocol, and are expressed in pmol/L (or ng/mL, as applicable). All samples were analyzed

in duplicate, and the mean concentration was used for statistical analyses.

For all biomarker assays, incubation, washing, and color development procedures

followed the respective manufacturers' standardized protocols; optical density was read at

450 ± 2 nm with reference wavelength correction, and concentrations were interpolated from

4-parameter logistic standard curves by operators blinded to clinical data and outcomes.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 23 (IBM,

Armonk, NY, USA). Continuous variables were assessed for normality using the

Kolmogorov-Smirnov test and presented as mean ± standard deviation (SD) for parametric

data or median (interquartile range, IQR) for non-parametric data, while categorical variables

were expressed as frequencies (percentages). Group comparisons were conducted using

independent samples t-tests or one-way ANOVA (with post-hoc Tukey's HSD testing) for

normally distributed data, and the Mann-Whitney U or Kruskal-Wallis tests for non-

parametric data. Categorical variables were analyzed using chi-square or Fisher's exact tests,

as appropriate. Correlation analyses between biomarkers were performed using Pearson's

(linear relationships) or Spearman's (non-linear relationships) correlation coefficients, with

strength interpreted per Cohen's criteria: 0.00-0.24: negligible, 0.25-0.49: weak, 0.50-0.74:

strong, ≥0.75: very strong. Receiver operating characteristic (ROC) curve analysis

determined optimal biomarker cutoff values by maximizing Youden's index. Multivariate

logistic regression (adjusted for age and sex) assessed independent biomarker associations

with HRS-AKI, reporting adjusted odds ratios (aOR) with 95% confidence intervals (CI). All

tests were two-tailed, with statistical significance set at $p < 0.05$.

Sample size and Power Calculation

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The sample size was determined a priori using G*Power (version 3.1) to detect a clinically meaningful difference in urinary [TIMP-2]•[IGFBP7] between patients with hepatorenal syndrome (HRS) and healthy controls. Based on prior reports of large biomarker gradients between AKI and non-AKI populations, we assumed a standardized effect size (Cohen's *d*) of 0.80 for the primary comparison, with a two-sided type I error of 0.05 and 80% power. Under these assumptions, a minimum of 26 participants per group was required for a two-sample *t* test. Because the primary a priori power calculation was based on the main biomarker contrast between HRS and controls, comparisons between the HRS subtypes (HRS-AKI vs HRS-CKD; n=15 each) should be interpreted as exploratory. We have therefore framed subtype analyses as hypothesis-generating and highlighted the need for external validation.

To account for potential exclusions or missing samples and to allow for multivariable modeling, we planned to enroll 80 patients with HCV-related cirrhosis (distributed across compensated, decompensated, and HRS strata) and 20 healthy controls (total $n = 100$). This final sample size exceeded the minimum per-group requirement and provided ≥80% power to detect the anticipated between-group differences in [TIMP-2]•[IGFBP7] and serum copeptin, as well as to evaluate associations between these biomarkers and HRS-AKI using logistic regression models adjusted for key covariates.

Results

Demographic, Laboratory, and Clinical Characteristics

The study groups were well-matched for age and sex (p = 0.36 and 0.91 respectively; **Table 1**). HRS patients had lower body mass index compared with compensated cirrhotics (p < 0.01) and showed progressive hematologic abnormalities, with declining hemoglobin and pronounced thrombocytopenia relative to controls (both p < 0.01).

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Liver function tests demonstrated marked synthetic impairment in HRS, with higher bilirubin (p < 0.01), lower albumin (p = 0.03), and more prolonged INR compared with compensated cirrhosis (p = 0.02), whereas aminotransferase levels did not clearly distinguish between cirrhosis stages (p > 0.05). Renal dysfunction was most evident in HRS, where serum creatinine was approximately doubled relative to other groups (p < 0.01), while urine albumin-to-creatinine ratio remained similar across groups (p = 0.24).

Standard prognostic scores increased in parallel with disease severity, with Child-Pugh and MELD 3.0 scores lowest in compensated cirrhosis and highest in HRS (p < 0.01), consistent with HRS as a terminal complication of portal hypertension.

Urinary [TIMP-2]•[IGFBP7] and serum copeptin levels showed a significant stepwise rise from healthy controls through compensated and decompensated cirrhosis to HRS (both p < 0.01; **Table 1, Fig. 2**). Biomarker levels were lowest in controls, increased modestly in compensated cirrhosis, and were highest in HRS. Copeptin showed a modest decrease in decompensated versus compensated cirrhosis, before increasing again in HRS.

Within the HRS subgroups, HRS-AKI patients had substantially 41% higher [TIMP-2]•[IGFBP7] and 28% higher copeptin levels than HRS-CKD patients (both p < 0.01; **Table 1**), indicating more pronounced acute tubular stress and circulatory disturbances in HRS-AKI.

Correlation Analysis Between Serum Copeptin and Urinary [TIMP-2]•[IGFBP7]:

Serum copeptin and urinary [TIMP-2]•[IGFBP7] were strongly correlated (Pearson r = 0.72, 95% CI 0.61-0.80; p < 0.001; Fig. 3).

Mortality Outcomes

Thirty-Day Mortality Outcomes:

Thirty-day mortality increased sharply with disease severity (**Table S1**). Thirty-day mortality was 0/20 (0%) in controls and 0/20 (0%) and 0/30 (0%) in compensated and

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decompensated cirrhosis, respectively; it was 4/15 (26.7%) in HRS-CKD and 10/15 (66.7%) in HRS-AKI (p = 0.012 for HRS-CKD vs HRS-AKI), underscoring the prognostic distinction between acute and chronic kidney injury in decompensated cirrhosis.

Biomarker Levels in Non-Survivors

Among patients with HRS-AKI, non-survivors had markedly higher copeptin and [TIMP-2]•[IGFBP7] levels than survivors (both p < 0.001; **Table 2**), supporting their use as prognostic markers in this high-risk subgroup.

Diagnostic Performance

HRS-AKI Prediction:

Both serum copeptin and urinary [TIMP-2]•[IGFBP7] showed excellent diagnostic accuracy for identifying HRS-AKI among cirrhotic patients (**Table 3, Fig. 4A**). Copeptin achieved an AUC of 0.90 (95% CI 0.84-0.96) and urinary [TIMP-2]•[IGFBP7] an AUC of 0.91 (95% CI 0.86-0.97), both p < 0.001. At cutoffs of 9.97 pmol/L for copeptin and 0.25 (ng/mL)²/1000 for [TIMP-2]•[IGFBP7], copeptin provided stronger rule-in specificity, whereas [TIMP-2]•[IGFBP7] offered higher sensitivity for ruling out HRS-AKI.

Mortality Prediction in HRS-AKI:

For 30-day mortality prediction among HRS-AKI patients, both biomarkers again performed well (**Table 4, Fig. 4B**). Urinary [TIMP-2]•[IGFBP7] yielded an AUC of 0.92 (95% CI 0.86-0.98) and serum copeptin an AUC of 0.87 (95% CI 0.79-0.95), each p < 0.001. At the optimal cutoff of >0.52 (ng/mL)²/1000, [TIMP-2]•[IGFBP7] achieved high sensitivity and specificity, while copeptin >14.6 pmol/L provided similar sensitivity with somewhat lower specificity.

Multivariate Analysis of HRS-AKI Risk Factors

Biomarker associations with HRS-AKI Risk:

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In multivariable logistic regression adjusted for age and sex, both serum copeptin and urinary [TIMP-2]•[IGFBP7] were strongly and independently associated with HRS-AKI (**Table S2**). Each standard unit increase in these biomarkers was associated with more than doubling of HRS-AKI odds (adjusted ORs >2.5 for both; p < 0.001).

Biomarker Performance After MELD Adjustments

When further adjusted for MELD 3.0 score, copeptin and [TIMP-2]•[IGFBP7] remained independently predictive of HRS-AKI, and MELD 3.0 score itself was a powerful predictor (**Table 5**). The combined model showed excellent discrimination (C-statistic = 0.92), a significant 7% improvement over MELD 3.0 alone (Δ +0.07, $P = 0.02$), with good calibration (Hosmer-Lemeshow $P = 0.83$).

Discussion:

In this prospective case–control study of Egyptian patients with HCV-related cirrhosis, we found that urinary [TIMP-2]•[IGFBP7] and serum copeptin were markedly elevated in patients with HRS, particularly in HRS-AKI, and provided excellent diagnostic and prognostic performance. Both biomarkers achieved AUCs >0.90 for HRS-AKI identification and showed strong, independent associations with HRS-AKI after adjustment for age, sex, and MELD 3.0. Integrating these markers with MELD 3.0 improved risk prediction by 7%, supporting their potential role as adjuncts to standard clinical assessment in cirrhosis-associated AKI.

Pathophysiological Insights

The strong correlation between serum copeptin and urinary [TIMP-2]•[IGFBP7] (r = 0.72, *p < 0.001*) suggests a tight coupling between hemodynamic stress and tubular injury in advanced cirrhosis. Both biomarkers showed progressive elevation from compensated cirrhosis to HRS-AKI, reflecting the underlying pathophysiology of cirrhosis-associated renal injury. However, the modest decrease in copeptin observed in decompensated versus

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compensated cirrhosis was small in magnitude and may reflect clinical heterogeneity (e.g., differences in diuretic exposure, plasma osmolality/sodium, and timing relative to acute stress) rather than a true reduction in vasopressin-system activation; copeptin increased again in HRS, consistent with progressive effective arterial underfilling and neurohormonal activation.

[TIMP-2] and IGFBP7 are expressed by tubular epithelial cells and upregulated in response to ischemic or inflammatory stress, where they induce G1 cell-cycle arrest to prevent cell division under injurious conditions. Their product, [TIMP-2]•[IGFBP7], therefore reflects early tubular stress before overt decline in glomerular filtration (18). This highly statistically significant relationship indicates that as copeptin levels rise, [TIMP-2]•[IGFBP7] levels increase proportionally, with the correlation coefficient falling within the "strong" range (0.50-0.74). This is stronger than typically seen between traditional renal markers (e.g., creatinine/cystatin C correlations average r=0.60-0.65).

Copeptin is a stable surrogate for arginine vasopressin release and integrates non-osmotic neurohormonal activation driven by splanchnic vasodilation and effective arterial underfilling in advanced cirrhosis. Rising copeptin levels across disease stages and their further increase in HRS-AKI mirror the hemodynamic crisis that characterizes this syndrome, with activation of the vasopressin, sympathetic, and renin–angiotensin–aldosterone systems and progressive renal vasoconstriction (13).

Taken together, these patterns support a unified pathophysiologic model of HRS-AKI in which circulatory dysfunction (captured by copeptin) and structural tubular stress (captured by [TIMP-2]•[IGFBP7]) act synergistically. The higher [TIMP-2]•[IGFBP7] levels in HRS-AKI versus HRS-CKD are consistent with more intense acute tubular injury in the AKI phenotype, while copeptin quantifies the severity of the hemodynamic insult. This dual-marker framework aligns with contemporary ICA-AKI concepts emphasizing the interaction

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between splanchnic vasodilation, neurohormonal activation, and ischemic tubular damage, and provides a biologic rationale for combining hemodynamic and tubular stress biomarkers in risk stratification models (8). This complements prior work by Zhang et al. (14), though our larger cohort and inclusion of HRS-CKD provide novel stratification. Notably, preserved UACR across groups supports the functional nature of HRS.

Diagnostic Advancements

Both urinary [TIMP-2]•[IGFBP7] and serum copeptin showed excellent discrimination for HRS-AKI with AUCs of 0.91 and 0.90, respectively. At their optimal cutoffs, [TIMP-2]•[IGFBP7] provided higher sensitivity, whereas copeptin offered slightly greater specificity, suggesting complementary diagnostic roles.

Importantly, these diagnostic associations remained strong after adjusting for age and sex, and persisted after further adjustment for MELD 3.0. In multivariable models, both biomarkers independently contributed to HRS-AKI prediction: every 1-unit increase in copeptin (pmol/L) was associated with a 2.78-fold increase in the odds of HRS-AKI, and each 1-unit rise in [TIMP-2]•[IGFBP7] ($(ng/mL)^2/1000$) was associated with a 2.58-fold higher odds. These effect sizes surpass many conventional AKI biomarkers (e.g., NGAL typically shows aOR 1.5-2.0) (19). These associations persisted after MELD 3.0 score adjustment, indicating that the biomarkers capture pathophysiologic information not fully reflected in liver disease severity scores. Clinically, this supports using [TIMP-2]•[IGFBP7] as an early, sensitive screen for HRS-AKI and copeptin as a confirmatory marker that strengthens rule-in decisions when elevated. However, the observation that copeptin and [TIMP-2]•[IGFBP7] may facilitate earlier detection of HRS-AKI should be interpreted with caution, as it is based on a relatively small HRS-AKI subgroup (n = 15) and remains promising but preliminary. These early-detection signals require confirmation in

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larger, multicenter cohorts before they can be translated into firm clinical decision thresholds or routine screening strategies.

Prognostic implications and Clinical Utility

Short-term mortality in HRS-AKI was high in our cohort (about two-thirds of patients died within 30 days), and clearly exceeding reported averages (50-60%) (20, 21). This elevated mortality may reflect (1) delayed diagnosis in our cohort, (2) HCV-specific disease progression, and (3) limited terlipressin availability. In our cohort, short-term mortality clustered in HRS-AKI, whereas compensated and decompensated non-HRS groups did not experience deaths over 30 days. However, we cannot definitively conclude that renal failure is the dominant mortality driver without longer follow-up and cause-of-death data. The striking 40% absolute mortality difference between HRS subtypes (AKI vs CKD) highlights the time-sensitive nature of AKI management. These findings, consistent with ICA 2024 guidelines but showing higher-than-expected HRS-AKI mortality, strongly support the clinical utility of early biomarkers such as [TIMP-2]•[IGFBP7] and copeptin to identify high-risk patients when interventions may still alter outcomes, particularly given the 3.5-fold greater mortality risk for HRS-AKI versus HRS-CKD patients in this cohort (8).

In practical terms, copeptin and [TIMP-2]•[IGFBP7] could be incorporated as add-on tests in patients with decompensated cirrhosis who present with AKI and already undergo routine blood and urine sampling. A single time-point measurement of urinary [TIMP-2]•[IGFBP7] and serum copeptin at presentation would complement, rather than replace, serum creatinine and MELD 3.0: [TIMP-2]•[IGFBP7] could serve as a sensitive screen for HRS-AKI and early tubular stress, while elevated copeptin would help confirm a hemodynamic HRS phenotype and flag patients who may benefit from earlier vasoconstrictor therapy, closer monitoring, and expedited transplant evaluation.

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Within HRS-AKI, non-survivors displayed significantly higher copeptin and urinary [TIMP-2]•[IGFBP7] levels than survivors, and both biomarkers showed excellent prognostic performance for 30-day mortality. Urinary [TIMP-2]•[IGFBP7] (AUC 0.92) slightly outperformed copeptin (AUC 0.87), aligning with its role in detecting subclinical tubular injury before functional decline (4). At an exploratory cutoff of >0.52 (ng/mL)²/1000, [TIMP-2]•[IGFBP7] achieved high sensitivity and specificity for mortality, while copeptin >14.6 pmol/L provided similar sensitivity with lower specificity.

These findings suggest a pragmatic approach in which combined assessment of copeptin and [TIMP-2]•[IGFBP7] helps identify HRS-AKI patients at highest short-term risk who may benefit from early vasoconstrictor therapy, intensive monitoring, and timely consideration of liver transplantation. The relatively narrow copeptin range among non-survivors (15.6-19.4 pmol/L) suggests a possible "critical threshold" above 15 pmol/L beyond which mortality escalates dramatically. Urinary [TIMP-2]•[IGFBP7] >0.65 (ng/mL)²/1000 captured all non-survivors, potentially serving as a marker of irreversible injury. The strong performance of both markers in predicting HRS-AKI mortality supports their combined use in prognostic models. Given the limited number of fatal events, these proposed cutoffs should be considered hypothesis-generating and require external validation in larger, prospective cohorts before routine clinical use.

Our prognostic analyses were based on MELD 3.0, which incorporates sodium, albumin, and sex in addition to bilirubin, creatinine, and INR. While this reflects contemporary practice in our center, the incremental prognostic contribution of copeptin and [TIMP-2]•[IGFBP7] may differ in settings that still use earlier MELD formulations (MELD or MELD-Na). Future studies should therefore evaluate these biomarkers alongside the specific MELD-based model used in each transplant program.

Strengths and Novelty

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Our study is, to our knowledge, the first to assess the joint use of serum copeptin and urinary [TIMP-2]•[IGFBP7] in cirrhotic patients with HRS, integrating hemodynamic and tubular stress signals into a single risk framework. While this dual-biomarker strategy appears to add prognostic information beyond MELD 3.0, external validation in larger, multi-etiology cohorts is needed before routine clinical implementation.

Limitations and Future Directions

This study has several limitations. First, it was conducted at a single center with a modest sample size and included only Egyptian patients with predominantly HCV-related cirrhosis. As a result, our findings are most directly applicable to this etiologic group. They may not fully generalize to other causes of cirrhosis, such as MASLD or alcohol-related liver disease, in which hypertension, diabetes, and intrinsic chronic kidney disease are more prevalent and could modify copeptin and [TIMP-2]•[IGFBP7] profiles and their associations with HRS-AKI and mortality. External validation in larger, multi-etiology cohorts is therefore essential before these thresholds can be adopted in routine clinical practice.

Second, the number of fatal events was relatively small, which limits the precision of our mortality estimates and the strength of the proposed prognostic cutoffs. We did not formally evaluate the time interval between biomarker measurement and death or analyze cause-specific mortality, and we are therefore unable to determine whether particular biomarker patterns are linked to specific modes or timing of death. These questions should be addressed in larger, prospectively designed studies with systematic collection of time-to-event and cause-of-death data.

Third, we did not examine precipitating factors (such as infections or gastrointestinal bleeding) or response to vasoconstrictor therapy in relation to biomarker levels. We were also unable to define threshold values predictive of treatment response, which would be highly relevant for guiding clinical decision-making. Future work should integrate serial biomarker

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measurements with detailed data on precipitating events, treatment strategies, and therapeutic response.

Finally, we did not directly compare these biomarkers against other causes of AKI in cirrhosis, such as acute tubular necrosis or prerenal azotemia, and we did not have kidney biopsy data. Although histological severity often correlates poorly with functional impairment in cirrhotic patients, the absence of tissue-level correlation remains a limitation. Additional studies are needed to clarify how copeptin and [TIMP-2]•[IGFBP7] perform in differentiating HRS from other AKI phenotypes and to define their role as adjuncts to, rather than replacements for, established clinical diagnostic criteria.

Prospective multicenter studies in diverse cirrhosis populations should be prioritized to validate and extend our findings. Research should focus on evaluating these biomarkers in pre-HRS populations to assess their early predictive value, as well as investigating their integration with novel functional tests (e.g., furosemide stress test) to enhance diagnostic accuracy. These investigations will be critical for establishing evidence-based protocols incorporating these biomarkers into the management of this life-threatening complication of advanced liver disease.

Conclusion

Urinary [TIMP-2]•[IGFBP7] and serum copeptin appear to be promising tools for early HRS diagnosis and short-term risk stratification. Their strong correlation (r = 0.72) supports combined use to capture both functional (hemodynamic/vasopressin-related, via copeptin) and structural (tubular stress, via [TIMP-2]•[IGFBP7]) components of renal injury, providing a more complete assessment of HRS than either marker alone. In practice, both biomarkers can be measured from the same initial blood and urine samples at the time HRS is first suspected, so any "sequential" use refers to interpretation rather than additional sampling or treatment delay. Larger multicenter studies are needed to validate the proposed cutoffs,

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compare performance across different cirrhosis etiologies and MELD models, and clarify how these biomarkers can be integrated into routine clinical decision pathways.

Statements and Declarations

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author contributions:

N.M.A. and H.K.H.K. equally contributed to the conception and design of the research; M.S.A. contributed to the design of the research; R.A.A. and Z.M.Z. contributed to the acquisition and analysis of the data; R.A.A. and H.K.H.K. contributed to the interpretation of data; and all authors drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

Ethics approval and consent to participate

The research was conducted in accordance with the ethical standards of the Minia University, Faculty of Medicine, Institutional Review Board "MUFMIRB" on human experimentation and with the Declaration of Helsinki of 1975, as revised in 2008. Informed written consent was obtained from all patients included in the study.

Clinical trial number: not applicable

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Data availability: The datasets generated and/or analyzed during the current study are

available from the corresponding author on reasonable request.

Consent for publication: Not applicable.

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References:

1. Desai AP, Knapp SM, Orman ES, Ghabril MS, Nephew LD, Anderson M, et al. Changing epidemiology and outcomes of acute kidney injury in hospitalized patients with cirrhosis - a US population-based study. J Hepatol. 2020;73(5):1092-9.

2. Velez JCQ. Hepatorenal Syndrome Type 1: From Diagnosis Ascertainment to Goal-Oriented Pharmacologic Therapy. Kidney360. 2022;3(2):382-95.

3. Martin-Llahi M, Guevara M, Torre A, Fagundes C, Restuccia T, Gilabert R, et al. Prognostic importance of the cause of renal failure in patients with cirrhosis. Gastroenterology. 2011;140(2):488-96 e4.

4. Parikh CR, Mansour SG. Perspective on Clinical Application of Biomarkers in AKI. J Am Soc Nephrol. 2017;28(6):1677-85.

5. Wong F, Pappas SC, Curry MP, Reddy KR, Rubin RA, Porayko MK, et al. Terlipressin plus Albumin for the Treatment of Type 1 Hepatorenal Syndrome. N Engl J Med. 2021;384(9):818-28.

6. Abuelo JG. Normotensive ischemic acute renal failure. N Engl J Med. 2007;357(8):797-805.

7. Wilson FP, Sheehan JM, Mariani LH, Berns JS. Creatinine generation is reduced in patients requiring continuous venovenous hemodialysis and independently predicts mortality. Nephrol Dial Transplant. 2012;27(11):4088-94.

8. Nadim MK, Kellum JA, Forni L, Francoz C, Asrani SK, Ostermann M, et al. Acute kidney injury in patients with cirrhosis: Acute Disease Quality Initiative (ADQI) and International Club of Ascites (ICA) joint multidisciplinary consensus meeting. J Hepatol. 2024;81(1):163-83.

9. Jouet P, Meyrier A, Mal F, Callard P, Guettier C, Stordeur D, et al. Transjugular renal biopsy in the treatment of patients with cirrhosis and renal abnormalities. Hepatology. 1996;24(5):1143-7.

10. Wadei HM, Geiger XJ, Cortese C, Mai ML, Kramer DJ, Rosser BG, et al. Kidney allocation to liver transplant candidates with renal failure of undetermined etiology: role of percutaneous renal biopsy. Am J Transplant. 2008;8(12):2618-26.

11. Trawale JM, Paradis V, Rautou PE, Francoz C, Escolano S, Sallee M, et al. The spectrum of renal lesions in patients with cirrhosis: a clinicopathological study. Liver Int. 2010;30(5):725-32.

12. Sola E, Kerbert AJ, Verspaget HW, Moreira R, Pose E, Ruiz P, et al. Plasma copeptin as biomarker of disease progression and prognosis in cirrhosis. J Hepatol. 2016;65(5):914-20.

13. Kerbert AJC, Verspaget HW, Navarro AA, Jalan R, Sola E, Benten D, et al. Copeptin in acute decompensation of liver cirrhosis: relationship with acute-on-chronic liver failure and short-term survival. Crit Care. 2017;21(1):321.

14. Zhang CC, Hoffelt DAA, Merle U. Urinary cell cycle arrest biomarker [TIMP-2].[IGFBP7] in patients with hepatorenal syndrome. Biomarkers. 2019;24(7):692-9.

15. Nadim MK, Garcia-Tsao G. Acute Kidney Injury in Patients with Cirrhosis. N Engl J Med. 2023;388(8):733-45.

16. European Association for the Study of the L. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. J Hepatol. 2010;53(3):397-417.

17. Kim WR, Mannalithara A, Heimbach JK, Kamath PS, Asrani SK, Biggins SW, et al. MELD 3.0: The Model for End-Stage Liver Disease Updated for the Modern Era. Gastroenterology. 2021;161(6):1887-95 e4.

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18.   Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, et al. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care. 2013;17(1):R25.

19.   Cruz DN, de Cal M, Garzotto F, Perazella MA, Lentini P, Corradi V, et al. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. Intensive Care Med. 2010;36(3):444-51.

20.   Kaewput W, Thongprayoon C, Dumancas CY, Kanduri SR, Kovvuru K, Kaewput C, et al. In-hospital mortality of hepatorenal syndrome in the United States: Nationwide inpatient sample. World J Gastroenterol. 2021;27(45):7831-43.

21.   Allegretti AS, Ortiz G, Wenger J, Deferio JJ, Wibecan J, Kalim S, et al. Prognosis of Acute Kidney Injury and Hepatorenal Syndrome in Patients with Cirrhosis: A Prospective Cohort Study. Int J Nephrol. 2015;2015:108139.

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Tables

Table 1. Demographic, Laboratory, and Clinical Characteristics by Study Group.

Parameter	Compensated Cirrhosis (n=20)	Decompensated Cirrhosis (n=30)	HRS (n=30)	Controls (n=20)	*p*-value
Demographics					
Age (years), mean ± SD	52.1 ± 8.7	53.1 ± 10.5	51.9 ± 8.8	49.9 ± 9.5	0.36
Male sex, n (%)	11 (55%)	17 (57%)	19 (63%)	11 (55%)	0.91
BMI (kg/m²), mean ± SD	28.9 ± 2.4	28.6 ± 1.9	26.7 ± 2.7	28.1 ± 2.6	<0.01**
Smoking, n (%)	7 (35.0%)	5 (16.7%)	9 (30.0%)	7 (35.0%)	0.40
Hematology					
Hemoglobin (g/dL), mean ± SD	$12.4^B \pm 1.2$	$10.6^C \pm 1.1$	$9.8^D \pm 0.7$	$13.9^A \pm 1.2$	<0.01**
Platelets (×10⁹/L), mean ± SD	$182^B \pm 49$	$108^C \pm 61$	$81^D \pm 57.7$	$248^A \pm 79$	<0.01**
WBCs (x10⁹/L)	$5.93^{AB} \pm 1.33$	$5.70^{AB} \pm 1.71$	$5.28^B \pm 1.10$	$6.48^A \pm 1.56$	0.04*
Liver Function					
Total Bilirubin (mg/dL), mean ± SD	$1.16^C \pm 0.74$	$2.22^B \pm 0.62$	$3.25^A \pm 0.85$	$0.98^D \pm 0.17$	< 0.01**
Direct Bilirubin (mg/dL), mean ± SD	$0.96^C \pm 0.55$	$1.62^B \pm 0.47$	$1.99^A \pm 0.66$	$0.64^D \pm 0.13$	0.05*
AST (IU/L)	$58.1^A \pm 18.1$	$48.2^A \pm 24.6$	$49.6^A \pm 18.5$	$37.0^B \pm 15.4$	< 0.01*
ALT (IU/L)	$51.1^A \pm 20.1$	$44.9^A \pm 17.1$	$52.4^A \pm 20.4$	$36.8^B \pm 13.0$	< 0.01*
Albumin (g/dL), mean ± SD	$3.92^B \pm 0.44$	$2.82^C \pm 0.48$	$2.1^D \pm 0.61$	$4.35^A \pm 0.87$	0.03*
INR, mean ± SD	$1.22^{ C} \pm 0.14$	$1.85^B \pm 0.20$	$2.14^A \pm 0.15$	$1.03^D \pm 0.12$	0.02
Renal Function					
Creatinine (mg/dL), mean ± SD	$1.01^B \pm 0.38$	$0.91^B \pm 0.23$	$2.08^A \pm 0.63$	$0.88^B \pm 0.14$	<0.01*
Urea (mg/dL)	$37.2^{AB} \pm 3.0$	$35.5^{AB} \pm 3.3$	$40.9^A \pm 11.8$	$30.3^B \pm 6.6$	<0.01*
UACR (mg/g)	7.8 ± 3.0	6.1 ± 3.1	7.1 ± 3.45	7.5 ± 3.4	0.24

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Parameter	Compensated Cirrhosis (n=20)	Decompensated Cirrhosis (n=30)	HRS (n=30)	Controls (n=20)	*p*-value
Disease Severity					
Child-Pugh score, mean ± SD	$5.6^C \pm 0.15$	$8.1^B \pm 1.8$	$13.2^A \pm 1.9$	-	<0.01*
MELD 3.0 score, mean ± SD	$8.1^C \pm 2.2$	$19.7^B \pm 3.6$	$27.9^A \pm 4.2$	-	<0.01*
Biomarker Profile					
[TIMP-2]•[IGFBP7] (ng/mL)²/1000	0.27 ± 0.07^C	0.28 ± 0.06^C	0.39 ± 0.12^B	0.13 ± 0.06^D	< 0.01*
Serum Copeptin (pmol/L)	9.78 ± 1.52^C	8.38 ± 2.34^D	11.28 ± 2.12^B	6.86 ± 1.16^E	< 0.01*

Abbreviations: **BMI**, body mass index; **Hb**, hemoglobin; **WBCs**, white blood cells; **AST**, aspartate aminotransferase; **ALT**, alanine aminotransferase; **INR**, international normalized ratio; **UACR**, urine albumin-to-creatinine ratio; **CPT**, Child–Pugh–Turcotte score (range 5–15); **MELD 3.0**, Model for End-Stage Liver Disease score (range 6–40). **TIMP-2**, tissue inhibitor of metalloproteinase-2; **IGFBP7**, insulin-like growth factor binding protein 7. Data are presented as mean ± SD or n (%). Continuous variables were compared using one-way ANOVA with Duncan's multiple range post hoc test; categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. A two-sided p < 0.05 was considered statistically significant. Superscript letters (A–D) indicate statistically significant between-group differences on Duncan's post hoc testing; groups sharing at least one letter are not significantly different (p ≥ 0.05). Liver disease severity scores (CPT and MELD 3.0) were not applicable to controls and are reported only for cirrhotic patient groups.

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Table 2. Biomarker Levels and Mortality in HRS-AKI.

Parameter	Survivors (n=5)	Non-Survivors (n=10)	Mean Difference (95% CI)	p-value
Serum copeptin (pmol/L)				
Mean ± SD	11.9 ± 2.72	16.4 ± 0.90	+4.5 (2.8 to 6.2)	<0.001**
Urinary [TIMP-2]•[IGFBP7] (ng/mL)²/1000				
Mean ± SD	0.43 ± 0.11	0.77 ± 0.09	+0.34 (0.25 to 0.43)	<0.001**

Data are presented as mean ± standard deviation (SD). Mean differences are calculated as

non-survivors minus survivors and reported with 95% confidence intervals (CI). p-values are

derived from independent-samples t-tests with Welch's correction. HRS-AKI, hepatorenal

syndrome–acute kidney injury. $p < 0.001$ indicates a highly significant difference.

Table 3. Diagnostic Performance of Biomarkers for HRS-AKI Prediction.

Biomarker	Serum copeptin (pmol/L)	Urinary [TIMP-2]•[IGFBP7] (ng/mL)²/1000
AUC (95% CI)	0.90 (0.84-0.96)	0.91 (0.86-0.97)
Cutoff Value	9.97	0.25
Sensitivity	89%	93%
Specificity	83%	78%
PPV	83.7%	86.5%
NPV	75.8%	79.8%
p-value	< 0.001	< 0.001

AUC values are derived from receiver operating characteristic (ROC) curve analysis for

discrimination of HRS-AKI versus other study groups. Optimal cutoff values were

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determined by maximizing the Youden index; sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are calculated at these thresholds. p-values test the null hypothesis that AUC = 0.50. AUC = area under the ROC curve; HRS-AKI = hepatorenal syndrome–acute kidney injury; [TIMP-2]•[IGFBP7] = tissue inhibitor of metalloproteinases-2 × insulin-like growth factor–binding protein 7.

Table 4. Prognostic Performance of Biomarkers for HRS-AKI Mortality.

Biomarker	Serum copeptin (pmol/L)	Urinary [TIMP-2]•[IGFBP7] (ng/mL)²/1000
AUC (95% CI)	0.87 (0.79-0.95)	0.92 (0.86-0.98)
Cutoff Value	14.6	0.52
Sensitivity	91%	92%
Specificity	73%	84%
PPV	78.7%	83.5%
NPV	69.5%	72.8%
p-value	< 0.001	< 0.001

AUC values are derived from receiver operating characteristic (ROC) curve analysis for prediction of 30-day mortality among patients with HRS-AKI. Optimal cutoff values were determined by maximizing the Youden index; sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are calculated at these thresholds. Ninety-five percent confidence intervals (CIs) for AUC were obtained using bootstrap resampling with 2,000 replicates, and p-values test the null hypothesis that AUC = 0.50; pairwise AUC comparisons were performed using the DeLong method. AUC = area under the ROC curve; CI = confidence interval; HRS-AKI = hepatorenal syndrome–acute kidney injury; [TIMP-2]•[IGFBP7] = tissue inhibitor of metalloproteinases-2 × insulin-like growth factor–binding protein 7; PPV = positive predictive value; NPV = negative predictive value.

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Table 5. Multivariable Logistic Regression for HRS-AKI Prediction.

Variable	Adjusted OR	95% CI	p-value	VIF
Copeptin (per 5 pmol/L)	2.15	1.72-2.68	<0.001	1.8
[TIMP-2]•[IGFBP7] (per 0.1 unit)	1.89	1.45-2.47	<0.001	2.1
MELD 3.0 (per 5 points)	3.02	2.33-3.91	<0.001	3.4
Age (per decade)	1.12	0.97-1.29	0.12	1.2
Male sex	1.45	0.88-2.39	0.14	1.1

Multivariable logistic regression model for prediction of HRS-AKI including serum copeptin, urinary [TIMP-2]•[IGFBP7], MELD 3.0, age, and sex. Adjusted odds ratios (ORs) are reported per 5 pmol/L increase in copeptin, per 0.1-unit increase in [TIMP-2]•[IGFBP7], and per 5-point increase in MELD 3.0. Variance inflation factors (VIFs) indicate no problematic multicollinearity among predictors. Overall model discrimination was excellent (c-statistic 0.92; 95% CI 0.88–0.96) and significantly higher than MELD 3.0 alone (c-statistic 0.85; $\Delta c = +0.07$, p = 0.02). Calibration by Hosmer–Lemeshow (HL) goodness-of-fit test was good ($\chi^2 = 4.32$, p = 0.83). HRS-AKI = hepatorenal syndrome–acute kidney injury; MELD 3.0 = Model for End-stage Liver Disease 3.0; OR = odds ratio; CI = confidence interval; VIF = variance inflation factor.

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Figure Legends

Fig. 1. Study enrollment and patient allocation.

Fig. 2. Biomarker levels across cirrhosis stages.

Fig. 3. Correlation between serum copeptin and urinary [TIMP-2]•[IGFBP7].

Fig. 4. Receiver operating characteristic (ROC) curves for the diagnostic and prognostic performance of serum copeptin and urinary [TIMP-2]•[IGFBP7] in cirrhotic patients
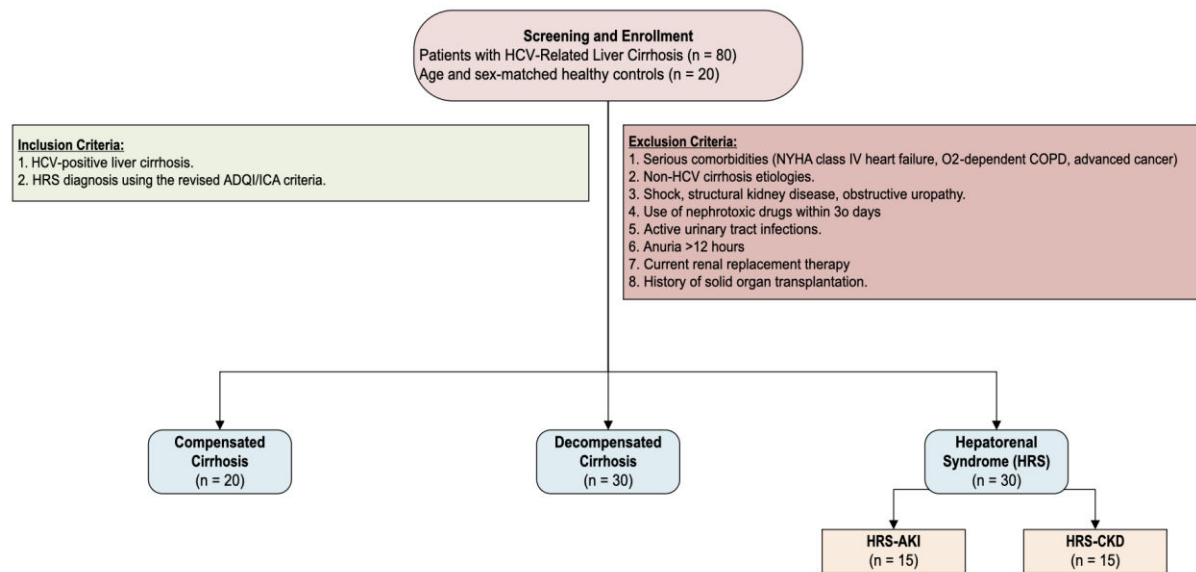
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Fig. 1. Study enrollment and patient allocation**.** A total of 80 patients with HCV-related liver cirrhosis were enrolled and stratified into three groups: compensated cirrhosis (n = 20), decompensated cirrhosis (n = 30), and hepatorenal syndrome (n = 30). The flow diagram outlines patient eligibility, exclusions, and final group assignment.
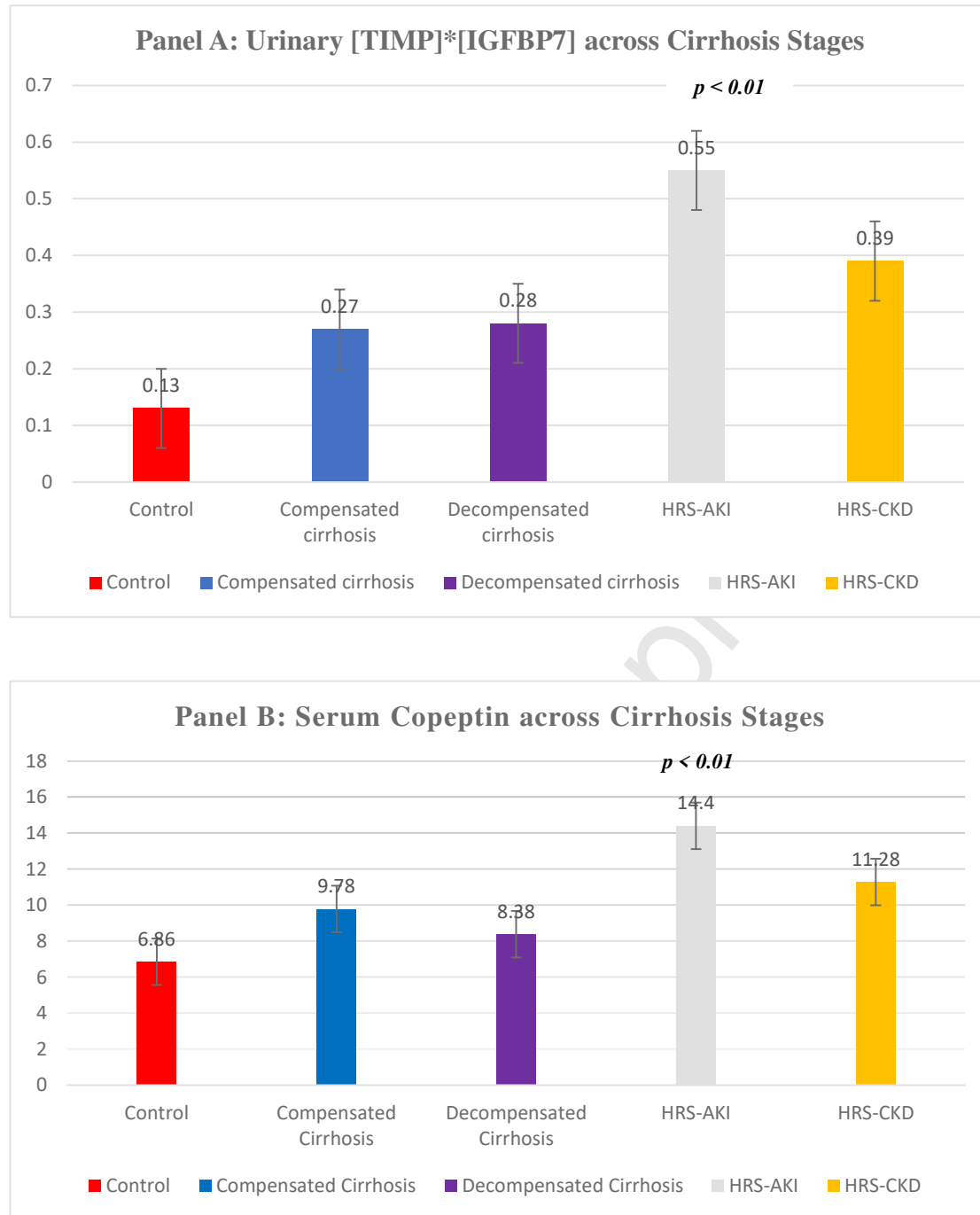
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Fig. 2. Biomarker Levels across Cirrhosis Stages. **Panel A** illustrates urinary [TIMP-2]•[IGFBP7] (ng/mL)2/1000 levels, which progressively increase from healthy controls to HRS-AKI. **Panel B** shows serum copeptin levels (pmol/L), demonstrating a similar rising trend with disease severity. The p-values displayed on the figure represent pairwise comparisons between the HRS group (reference) and each of the other study groups (healthy controls, compensated cirrhosis, decompensated cirrhosis, and the alternate HRS subtype). Pairwise p-values were derived from one-

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way ANOVA followed by Duncan's multiple range post hoc testing (two-sided), with p < 0.05 considered statistically significant.
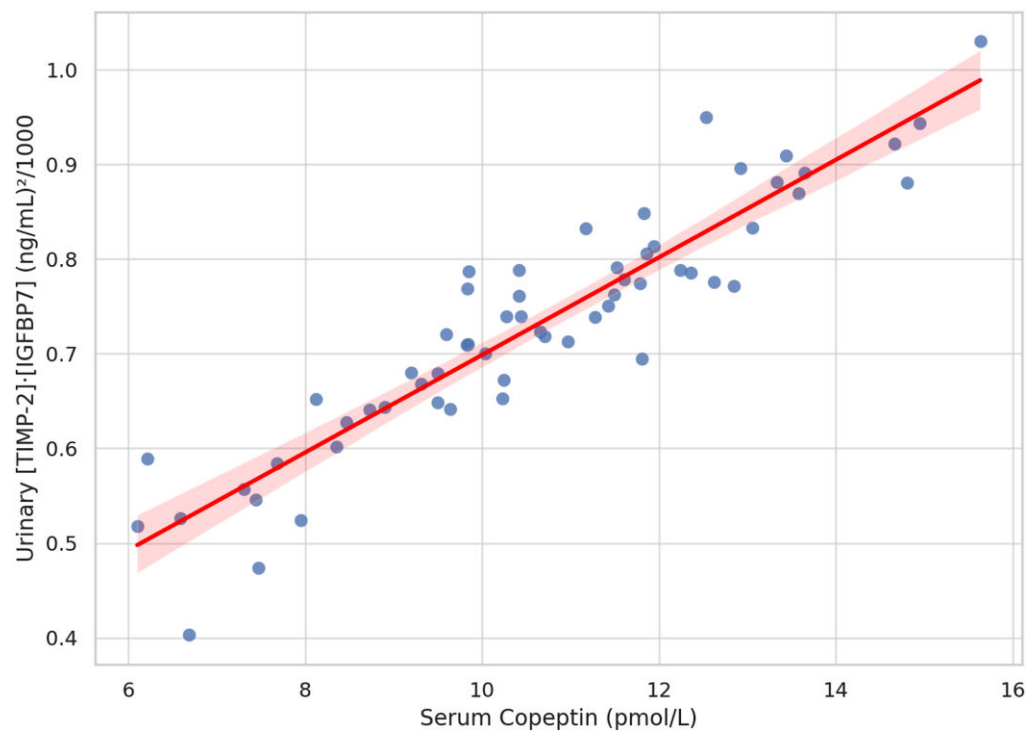

Fig. 3. Correlation between serum copeptin and urinary [TIMP-2]•[IGFBP7]. This scatter plot illustrates the strong positive correlation between serum copeptin and urinary [TIMP-2]•[IGFBP7] levels (r = 0.72, p < 0.001), supporting their combined use as mechanistically complementary biomarkers for HRS-AKI.
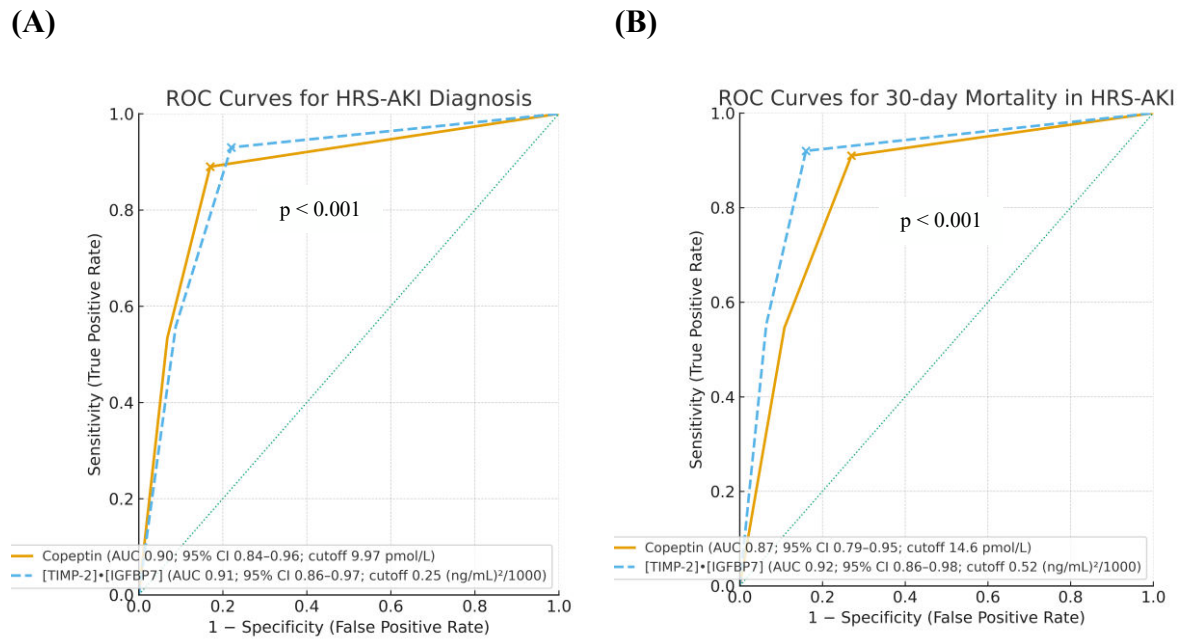
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(A)

(B)

Fig. 4. Receiver operating characteristic (ROC) curves for the diagnostic and prognostic performance of serum copeptin and urinary [TIMP-2]•[IGFBP7] in cirrhotic patients.

(A) ROC curves for serum copeptin and urinary [TIMP-2]•[IGFBP7] in discriminating HRS-AKI from other cirrhotic subgroups. Copeptin yielded an AUC of 0.90 (95% CI 0.84–0.96) with an optimal cutoff of 9.97 pmol/L (sensitivity 89%, specificity 83%), while [TIMP-2]•[IGFBP7] (expressed as $(ng/mL)^2/1000$) showed an AUC of 0.91 (95% CI 0.86–0.97) with an optimal cutoff of 0.25 $(ng/mL)^2/1000$ (sensitivity 93%, specificity 78%). **(B)** ROC curves for 30-day mortality prediction among patients with HRS-AKI. Copeptin achieved an AUC of 0.87 (95% CI 0.79–0.95) with a cutoff of 14.6 pmol/L (sensitivity 91%, specificity 73%), whereas [TIMP-2]•[IGFBP7] achieved an AUC of 0.92 (95% CI 0.86–0.98) with a cutoff of 0.52 $(ng/mL)^2/1000$ (sensitivity 92%, specificity 84%). The diagonal line represents the line of no discrimination. Dots on each curve indicate the optimal cut-off points determined by the Youden index. HRS-AKI, hepatorenal syndrome–acute kidney injury; ROC, receiver operating characteristic.

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