

Pro-adrenomedullin in acute decompensation of liver cirrhosis: relationship with acute-on-chronic liver failure and short-term survival

Running title: A new biomarker in acute decompensation of cirrhosis and ACLF

Huadong Yan¹²³ Susu Jin³ Lili Liang³ Jingyuan Du³ Guruprasad P. Aithal⁴⁵ Lanjuan Li^{1*}

1. State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Centre for Infectious Diseases, Collaborative Innovation Centre for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, 310003, China.
2. Department of Hepatology, Key Laboratory of Diagnosis and Treatment of Digestive System Tumors of Zhejiang Province, Hwamei Hospital, Ningbo No.2 Hospital, University of Chinese Academy of Sciences., Ningbo 315010, China
3. Department of Hepatology, Hwamei Hospital, Ningbo No.2 Hospital, Ningbo University School of Medicine, Ningbo, China
4. NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and University of Nottingham, Nottingham, UK.
5. Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham UK.

*Corresponding author: Dr. Lanjuan Li, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Centre for Infectious Diseases, Collaborative Innovation Centre for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, 310003, China. Tel: 86-0571-87236458, fax:86-0571-87236459, Email address: ljli@zju.edu.cn.

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Abstract

Background and aim: Acute-on-chronic liver failure (ACLF) is characterized by the presence of acute decompensation (AD) of cirrhosis, organ failures, and high short-term mortality rates. In present study, we explored whether Pro-adrenomedullin (Pro-ADM), a biomarker of sepsis, is a potential marker of outcome in patients admitted for AD or ACLF and whether it might be of additional value to conventional prognostic scoring systems in these patients.

Methods: 332 consecutive patients with AD of cirrhosis were prospectively enrolled. Pro-ADM was measured for all patients at baseline. Cox regression analysis was used to evaluate the impact of pro-ADM on short-term survival and developing ACLF during hospital stay.

Results: Serum pro-ADM levels were significantly high in non-survivors ($p < 0.001$) and showed significant correlation with ALT ($r = 0.181$, $p = 0.001$), INR ($r = 0.144$, $p = 0.009$), TB ($r = 0.368$, $p < 0.001$), Creatinine ($r = 0.145$, $p = 0.004$), MELD score ($r = 0.334$, $p < 0.001$) and CLIF-C OF score ($r = 0.375$, $p < 0.001$). Serum pro-ADM at admission was shown to be a predictor of 28-day mortality independently of MELD and CLIF-C OF scores. Prognostic models incorporating pro-ADM achieved high C index for predicting 28-day mortality in AD patients of cirrhosis. Moreover, baseline pro-ADM was found to be predictive of ACLF development during hospital stay.

Conclusions: Serum pro-ADM levels correlate with multiorgan failure and are independently associated with short-term survival and ACLF development in patients admitted for AD or ACLF.

Key words: Acute-on-chronic liver failure; Acute decompensation of cirrhosis; Organ failure; Biomarker; pro-adrenomedullin;

Introduction

Acute decompensation (AD) of liver cirrhosis is characterized by the development of major complications of the underlying liver disease and currently represents one of the most common causes of admission to hospital in patients with cirrhosis [1-2]. Acute-on-chronic liver failure (ACLF) is a complex syndrome that occurs in patients with AD, and is characterized by organ failures and high short term mortality [2-4].

Adrenomedullin (ADM), a peptide with 52 amino acids, is released from multiple tissues in response to physiological stress or after infection [5-6]. It has vasodilatory anti-inflammatory and antimicrobial properties, and can be enhanced by the regulation of the complement system [5]. It is found elevated in several critical diseases, including cardiovascular and renal disorders, sepsis, cirrhosis, cancer, and diabetes [6-8]. However, ADM is quickly cleared from circulation, making the measurement unreliable [9]. The midregional fragment of pro-adrenomedullin (pro-ADM), comprising amino acids 45-92, is more stable than the active ADM peptide and it has been detected in plasma of patients [10]. Recently, pro-ADM has been found useful as a marker of organ damage and predictor of mortality in patients with sepsis [11]. It has also been associated with increased mortality in patients with chronic heart failure (CHF) and can be used as a powerful new prognostic marker of death or heart failure in patients with acute myocardial infarction (AMI) [12-13]. However, the prognostic value of pro-ADM and its correlation with organ failure and ACLF in patients with AD of cirrhosis have not been evaluated so far.

In the present study, we aimed to evaluate pro-ADM as a potential marker of outcome in patients admitted for AD or ACLF and whether it might be of additional value to conventional prognostic

scoring systems in these patients.

Patients and Methods

Study Design

Patients were prospectively screened and enrolled from January 2016 to June 2017 in hepatology department, Hwamei Hospital, Ningbo No.2 Hospital, University of Chinese Academy of Sciences. All acute decompensated cirrhosis patients were assessed and diagnosed by liver biopsy, a composite of clinical signs and findings provided by laboratory tests, endoscopic signs of portal hypertension, radiological evidence of liver nodularity or clinical evidence of prior hepatic decompensation. The study fulfilled the principles of the Declaration of Helsinki and was approved by the ethics committee of Ningbo No.2 Hospital. Written informed consent was obtained from each participant or their legal representatives before enrollment. All authors had access to the study data and had reviewed and approved the final manuscript.

Patients

Patients with a new episode of acute decompensation (AD) were prospectively screened and enrolled. Events of acute decompensation included overt ascites, upper gastrointestinal bleeding, hepatic encephalopathy and bacterial infection. Causes of exclusion were summarized in Figure 1: Patients were excluded based on the following criteria as previous described [14]: (i) age < 18 years; (ii) pregnancy; (iii) hepatocellular carcinoma (HCC) outside Milan criteria or other types of tumors; (iv) chronic renal diseases [estimated glomerular filtration rate (eGFR) < 90 ml/min] and other severe co-morbidities such as myocardial infarction, subarachnoid or cerebral hemorrhage; (v) HIV infection.

Data collection

We collected the following clinical and demographic information in a pre-specified datasheet: age, sex, etiologies of cirrhosis, admission events, medical history for previous hospitalization, anti-viral treatment, and other treatment, laboratory parameters, events of organ failures, and prognosis.

ACLF was defined by the EASL-ACLF criteria, CLIF-C OF score was used to define six organ failures including liver, coagulation, kidney, circulation, lung and brain failure[2,15]. Bacterial or fungal infections were diagnosed as previously described(3): (i) pneumonia: new pulmonary infiltrate with fever, respiratory symptoms, findings on auscultation, or WBC count $>10,000/\text{mm}^3$ or $<4000/\text{mm}^3$; (ii) spontaneous bacterial peritonitis (SBP): poly-morphonuclear cells in ascitic fluid $>250/\text{mL}$; (iii) bacteremia: positive blood cultures without a source of infection; (iv) urinary tract infection: urine WBC $>10/\text{high power field}$ with positive culture and symptom of urinary irritation; (v) others bacterial infections included skin infection and intra-abdominal infection; (vi) fungal infection: positive fungal culture.

The management of enrolled patients was first focusing on acute precipitating events and complications to support organ dysfunction and failure. Patients with bacterial infection were immediately treated with empirical antibiotic therapy and then based on bacteria culture and antibiotics sensitivity test. In addition, weight-based intravenous albumin was used in patients with SBP and AKI. Diagnostic abdominocentesis were performed in patients with ascites to examine the whether SBP was present. Those with moderate ascites were treated with restriction of sodium intake and/or diuretics (aldosterone antagonist and/or furosemide). Paracentesis combining with intravenous albumin was used in those with large or refractory ascites. All patients with acute variceal bleeding received intravenous somatostatin, proton pump inhibitors (PPI) and antibiotic prophylaxis. For those with uncontrolled hemorrhage due to pharmacological therapy,

Sengstaken-Blackmore tube and/or urgent therapeutic endoscopy were performed. Patients with renal failure were treated with intravenous albumin, vasoconstrictors (such as dopamine, noradrenaline or terlipressine) or renal replacement therapy, and potential precipitating events were investigated. Patients with hepatic encephalopathy were given lactulose, antibiotics and L-ornithine aspartate, and potential precipitating events were investigated; Fluid replacement was performed in patients with MAP<70mmHg, and vasoactive agents were used when necessary. Oxygen therapy was performed in patients with decreased PaO₂ or SpO₂; nasal catheter, mask or venture mask oxygen inhalation or mechanical ventilation were chosen according to the severity of respiratory dysfunction. Lastly, intravenous high glucose, albumin or plasma were used as clinically indicated, glutathione, adenosylmethionine or branched-chain amino acids were used as nutritional support. The follow-up of patients started at onset of hospital admission. For patients discharged from hospital, prognostic information was obtained from medical records, telephone contact or visiting. The primary endpoint of the study was 28-day, 90-day transplant-free mortality.

Pro-ADM measurements

Blood samples were collected under standardized conditions in plasma collection tubes containing ethylenediaminetetraacetic acid. All samples clotted for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 × g. Then the serum was removed and stored at 80°C until it was assayed. Samples are centrifuged again after thawing before the assay. In order to avoid measurement bias, the pro-ADM level was determined by quantitative sandwich enzyme immunoassay technique as extensively described [16], according to the manufacturer's recommendations (all kits from Cusabio Biotech Co., Ltd., Wuhan, China).

Statistical analysis

Continuous variables were expressed as the mean \pm standard deviation (SD) or the median with the interquartile range. Binary or nominal variables were expressed as a number and percentage of the total patient population. Groups were compared using Student's t-test, the Mann–Whitney U-test, or a Chi-squared test. Linear regression analysis was used to compare the correlation between groups. Survival curves were drawn to describe subgroups of patients; the cumulative incidence of death was compared using the Log–rank test. Cox regression analysis was performed to identify factors associated with multiple outcomes. For all multivariate analyses, univariate analysis was performed to screen the potential risk factors of poor prognosis. Then, candidate variables (P-value < 0.10) were entered into multivariate analysis. The discrimination of several prognostic models was measured by C-index. Changes of pro-ADM prior mortality were visualized via locally weighted scatterplot smoothing (LOESS) plot. Confidence intervals were given for the 95% level. Differences were considered significant if the P-value was less than 0.05. Statistical analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, IL,USA) or GraphPad Prism 5 (GraphPad Software, San Diego, CA,USA).

Results

Patients

As shown in Figure1, 469 patients with cirrhosis of the liver were candidates for enrollment, out of which 332 patients with AD were included in the study and 133 patients were excluded as they fulfilled either one or more of exclusion criteria. Another 30 compensated cirrhosis patients and 30 healthy people were included as control groups. The baseline characteristics of the study cohort were shown in Tables 1. There were 231 (69.6%) males and mean age of the patients was 57 ± 13 . The etiology of cirrhosis was related to hepatitis B in 194 (58.4%), alcohol in 46

(13.9%), hepatitis B plus alcohol in 32(9.6%), others in 42(12.7%) and cryptogenic in 18 (5.4%). 98(29.5%) patients experienced previous decompensation. Ascites was seen in 233(70.2%) patients and 32(9.6%) patients had HE. Bacterial was present in 60(18.1%) patients and 54(16.3%) patients had GI bleeding. The median pro-ADM concentration was 11.8(12.9) nmol/L. 36(10.8%) patients had ACLF at admission. Characteristics of patients with or without ACLF were shown in supplement tables 1.

Correlation between serum pro-ADM with diseases severity

As shown in figure 2, the pro-ADM in AD patients was significantly high than those in healthy control group and the compensated cirrhosis group (both $P < 0.001$). Furthermore, The pro-ADM in ACLF patients was significant high than those in AD without bacterial infection ($P < 0.001$). The difference of pro-ADM between ACLF patients and AD with bacterial infection was not statistically significant ($p = 0.056$). Baseline characteristics of patients based on their pro-ADM concentration (pro-ADM ≤ 11.8 nmol/L vs pro-ADM > 11.8 nmol/L) were shown in supplement tables 2. The high pro-ADM patients had significantly high levels of ALT, AST, TB, INR, WBC, and CRP as well as disease severity scores such as CTP score, MELD score and CLIF-C OF score. The high pro-ADM patients had significantly high 28-day mortality and 90-day mortality.

As shown in figure 3, pro-ADM correlated with ALT ($r = 0.181$, $p = 0.001$), INR ($r = 0.144$, $p = 0.009$), TB ($r = 0.368$, $p < 0.001$), Creatinine ($r = 0.145$, $p = 0.004$), MELD score ($r = 0.334$, $p < 0.001$) and CLIF-C OF score ($r = 0.375$, $p < 0.001$). Pro-ADM was correlated with bacterial infection in cirrhotic patients ($r = 0.108$, $p = 0.048$). As shown in supplement table 3, pro-ADM was a predictors of bacterial infection on univariate logistic regression analysis ($p = 0.050$). But on multivariate logistic regression analysis, WBC count and CRP were independent predictors of bacterial infection. The

performances of CRP and WBC count for predicting bacterial infection were 0.696 (AUROC; 95% CI: 0.643–0.745) and 0.674 (AUROC; 95% CI: 0.621–0.724), significantly higher than pro-ADM (AUROC,0.576, 95% CI: 0.521–0.630),($p < 0.001$). The WBC and CRP value in ACLF patients and AD with bacterial infection were all significantly higher than those in AD without bacterial infection (both $P < 0.001$). But the WBC and CRP value in ACLF patients were significant high than those in AD without bacterial infection ($P < 0.001$). WBC and CRP value between ACLF patients and AD with bacterial was not significant ($p = 0.34$ and 0.51 , respectively).

Evaluate the impact of pro-ADM on short-term survival

At 28 days after enrolment in the study, 19 (5.7%) patients died and 2 (0.6%) received a liver transplantation. After 90 days of follow-up, 37(11.1%) patients died and 8(2.4%) were transplanted. The main causes of death at 28 and 90 days were listed in Supplementary table 5. Serum pro-ADM was found to be significantly higher in patients who died or were transplanted as compared to those who were still alive without liver transplantation at 28 days follow-up.(2.1(1.3) vs. 1.1(1.2) nmol/L, $p < 0.001$).Figure. 4 shows the estimated probability of death after 28 and 90 days of follow-up according to serum pro-ADM concentration, showing that high serum pro-ADM concentrations have an additional negative impact on mortality risk.

The univariate cox regression analysis of various predictors with 28 day survival are also listed in table 2. The predictors which were significant on univariate analysis were pro-ADM ($p < 0.001$), bacterial infection ($P = 0.018$), hepatic encephalopathy ($p < 0.001$), AST ($p = 0.003$), albumin ($p < 0.001$), total bilirubin ($p < 0.001$), INR ($p < 0.001$), Serum sodium ($p = 0.005$), WBC count ($p < 0.001$), creatinine ($p < 0.001$), CRP ($p = 0.004$), CTP score ($p < 0.001$), MELD score ($p < 0.001$), MELD-Na score ($p < 0.001$), CLIF-C OF score ($p < 0.001$).

Of all the significant predictors on univariate analysis, further multivariate cox regression analysis based on CLIF-C OF score or MELD score was done. Two different prognostic models were developed each incorporating pro-ADM (as selection variable) (Table 3) along with different combinations of WBC count, CLIF-C OF score, MELD score. Moreover, prognostic models incorporating pro-ADM achieved high C index for predicting 28-day mortality in AD patients of cirrhosis (MELD+pro-ADM+WBC vs MELD, 0.846 vs 0.753, P=0.026; MELD+pro-ADM vs MELD, 0.797 vs 0.753, P=0.096; CLIF-C OF+pro-ADM+WBC vs CLIF-C OF, 0.871 vs 0.787, P=0.020; CLIF-C OF+pro-ADM vs CLIF-C OF, 0.837 vs 0.787, P=0.046; Table 4).

Kaplan-Meier curves showed highest mortality for patients with high pro-ADM (>11.8nmol/mL) and presence of ACLF (Fig.5AB). Similarly patients with high pro-ADM (>11.8nmol/mL) and high MELD score (>12.2) had the worst survival (Fig. 5CD).

Finally, as shown in supplement table 4, a Univariate cox regression analysis and multivariate cox regression analysis was performed in order to identify the independent predictive factors for ACLF development during hospital stay (n = 34). Serum pro-ADM, together with WBC and MELD score was found to be an independent predictive factor for ACLF development.

Discussion

AD of liver cirrhosis continues to be a major cause of mortality and is directly related to the severity of organ damage in many cirrhotic patients [2-3]. In present study, we assessed the prognostic ability of pro-ADM in patients admitted with AD or ACLF. The results demonstrate that pro-ADM is significantly correlated with organ failure and short-term mortality and can be used as a biomarker for prognosis in patients with AD or ACLF.

Previous reports had underlined that pro-ADM shows good predictive power in several critical diseases, including sepsis, CHF and AMI [11-13]. A study with 213 consecutive hospitalized patients with cirrhosis and ascites showed that pro-ADM may be a promising marker to identify patients with bacterial infections as well as patients at risk of short-term mortality[17]. However, little is known about the prognostic value of pro-ADM in patients with AD and ACLF.. In present study, we assessed pro-ADM as a potential marker of early mortality in a cohort of 332 AD patients of cirrhosis. Compared with healthy control group and stable cirrhotic patients, pro-ADM concentration was significantly high in patients with AD and even higher in ACLF patients. Pro-ADM had a significant positive correlation of pro-ADM with several liver damage indexes, including ALT, TB and INR. We also found a significant difference in MELD and CLIF-SOFA scores which are already established prognostic scores in patients with decompensated cirrhosis between the two groups. However, there was no significant difference in bacterial infection between the two groups. Pro-ADM was less specific in predicting bacterial infection or sepsis than CRP and WBC count in patients with decompensated cirrhosis in our cohort. Furthermore, we found serum pro-ADM predicted mortality independently after adjusting for all these predictors on multivariate analysis. Prognostic models incorporating pro-ADM achieved high C index for predicting 28-day mortality in patients with decompensated cirrhosis (Table 4). Kaplan-Meier curves showed an increased mortality with high pro-ADM concentration and presence of ACLF or high MELD scores (Fig.5). In addition, pro-ADM was also an independent risk factor for the development of ACLF.

Pro-ADM release in patients with decompensated cirrhosis is most likely related to three possible mechanisms. First, ADM gene expression can be up-regulated by a variety of factors, including

endotoxin and cytokines [18-19]. Plasma levels of endotoxin and endotoxin-related cytokines are elevated in cirrhotic patients [20-21]. Therefore, endotoxemia or cytokines may stimulate ADM production in patients with decompensated cirrhosis. Second, ADM may participate in the hemodynamic derangement in patients with liver cirrhosis. Cirrhotic patients present with a hyperdynamic circulation, which is characterized by arterial hypotension, increased cardiac output and reduced systemic vascular resistance [22]. ADM has vasoactive functions by dilatation of resistance and capacitance vessels including mesentery vessels, stimulation of nitric oxide synthesis, and increase of cardiac output [19]. It can also play a natriuretic/ diuretic role via inhibiting the production of aldosterone [8]. Third, increased ADM concentration in patients with cirrhosis may be a result of decreased renal clearance. Previous reports have found that ADM concentration is elevated in patients with renal failure [23]. However, there are still numerous unknown mechanisms of ADM system in patients with decompensated cirrhosis and needing to be further explored.

There are several limitations to our study. This was a single-center study, and the results need to be replicated in larger multicenter studies. Number of patients with sepsis in our cohort is small. As a result, the association between pro-ADM and patients with sepsis might need further study to confirm. In addition, as the study was focused on examining the value of pro-ADM at the baseline on the short-term outcome of cirrhosis with acute decompensation, we are unable to investigate the effect of interventions during the hospital stay on pro-ADM levels. We have also presented the mean values of this biomarker in healthy controls and group of compensated cirrhosis. These latter groups aren't age and sex matched to the study group.

In summary, our findings demonstrated that serum pro-ADM levels correlate with multiorgan

failure in patients with decompensated cirrhosis. In addition, pro-ADM is a predictor of short-term mortality in cirrhotic patients admitted for AD, independently of MELD and CLIF-C OF scores. Serum pro-ADM shows the potential to add relevant prognostic value to these prognostic scoring systems. Altogether, these findings suggest that pro-ADM is an interesting potential prognostic marker in hospitalized cirrhotic patients with AD and ACLF.

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Disclosure statement

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest related to this manuscript.

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Figure legends

Figure.1 Flow chart of patient selection. AD: acute decompensation of cirrhosis; ACLF: acute-on-chronic liver failure.

Figure.2 Comparison of serum pro-ADM in subgroups:(3A) Comparison of pro-ADM in healthy control, compensated cirrhosis and AD patients ;(3B) (3C) and (3D) ,compare pro-ADM,WBC and CRP across groups of ACLF and AD with or without bacterial infection. ACLF: acute-on-chronic liver failure; AD: acute decompensation; WBC: white blood cell; CRP: C-reactive protein .

Figure.3 Correlation between serum pro-ADM with ALT, INR,TB, Creatinine,MELD and CLIF-C OF and. ALT: alanine aminotransferase; TB: total Bilirubin; INR: International normalized ratio ; WBC: white blood cell; MELD: Model for End-Stage Liver Disease; CLIF-C OF: The Chronic Liver Failure-consortium organ failure.

Figure.4 Changes of pro-ADM prior 28-day (4A) and 90-day (4B) mortality were visualized via locally weighted scatterplot smoothing (LOESS) plot. Shaded region depicts 95% confidence interval.

Figure.5 Kaplan Meier curves depicting the survival probability with respect to serum pro-ADM with presence or absence of ACLF and MELD score >12.2. (A) and (B): Patients with serum pro-ADM>11.8nmol/L and ACLF had the worst survival.(C) and (D): with serum pro-ADM >11.8nmol/L and MELD score >12.2 had the worst survival. The cumulative of 28 day and 90 day survival across the groups was compared using the log-rank test.