Association between longer duration of citrate accumulation and 90-day mortality of acute-on-chronic liver failure

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Regional citrate anticoagulation (RCA) is an optional anticoagulant for plasma adsorption (PA) plus plasma exchange (PE) therapy in patients with acute-on-chronic liver failure (ACLF), but with risk of transient citrate accumulation due to plasma and citrate [1]. Regardless of the anticoagulants: heparin or citrate, some patients would suffer from longer duration of citrate accumulation (LDCA), defined as the presence of citrate accumulation 2 h after PA plus PE therapy with RCA [1, 2]. However, whether citrate accumulation itself would lead to poor prognosis remains uncertain.

We conducted a retrospective study based on medical records to assess the association between LDCA and prognosis of hepatitis B virus (HBV)-related ACLF. Methods and some data from this cohort have been published already [2]. We kept to follow-up these patients for another 90 days after acquiring further ethical approval and registered this study with ChiCTR-OON-17013631. HBV-ACLF was diagnosed according to COSSH ACLF criteria [3]. Citrate accumulation was defined as the ratio of total calcium (Catot) to ionized calcium (Caion), (Catot/Caion), over or equal to 2.5 (Catot/Caion ≥ 2.5) [1, 2]. Cox proportional hazards models were applied to evaluate the association of LDCA with outcome.

From January 2018 to December 2019, we reviewed the data of 258 patients who fulfilled the HBV-ACLF criteria and received PA plus PE therapy with RCA. LDCA patients (N = 76) were more often female and older and had worse severity of disease condition than non-LDCA patients (N = 182) (Table 1). There was no significant difference in indicators, such as intracorporeal and extracorporeal Catot and Caion, representing patients receiving similar RCA during and after the first session of PA plus PE therapy with RCA.

The 90-day mortality of LDCA patients was much higher than that of non-LDCA patients (63.2% vs. 32.4%, log-rank p < 0.001). Compared with non-LDCA patients, LDCA patients had much higher 90-day mortality risk (crude hazard ratio (HR) (95% confidence interval (CI)), 2.62 (1.79–3.84) (Table 2). However, no significant differences in 90-day mortality risk were observed with the Cox proportional hazards models established with LDCA, age, gender, liver cirrhosis, HBV DNA, other coexisting liver diseases, comorbidities, and disease severity (Model 1, COSSH ACLF score; Model 2, CLIF-C ACLF score; Model 3, AARC ACLF score; Model 4, MELD score): Model 1 adjusted HR (95% CI), 1.07 (0.66–1.73); Model 2, 1.49 (0.95–2.36); Model 3, 1.41 (0.90–2.22); Model 4, 1.05 (0.65–1.72) (Table 2). Similarly, no significant differences in 90-day mortality risk were observed with similar Cox models established with citrate level indicators (Model 5, Catot/Caion ≥ 2.25; Model 6, Catot/Caion; Model 7, anion gap), disease severity (COSSH ACLF score), and the others mentioned above: Model 5, 1.28 (0.78–2.08); Model 6, 1.56 (0.74–3.27); Model 7, 1.06 (0.97–1.16). The disease severity was the independent risk factor of 90-day mortality (Model 1–7, all adjusted HR > 1, all p < 0.001).
Table 1  Characteristics of ACLF patients with or without LDCA

|                                 | Patients with LDCA (N = 76) | Patients without LDCA (N = 182) | p       |
|---------------------------------|-----------------------------|---------------------------------|---------|
| Female                          | 25 (32.9%)                  | 12 (6.6%)                       | < 0.001 |
| Age(years)                      | 52.2 ± 10.9                 | 43.8 ± 11.2                     | < 0.001 |
| Liver cirrhosis                 |                             |                                 | 0.620   |
| Causes of liver disease         |                             |                                 | 0.963   |
| HBV infection only              | 57 (75.0%)                  | 137 (75.3%)                     |         |
| HBV infection plus other causes | 19 (25.0%)                  | 45 (24.7%)                      |         |
| Comorbidities                   |                             |                                 | 0.112   |
| No                              | 59 (77.6%)                  | 156 (85.7%)                     |         |
| Yes                             | 17 (22.4%)                  | 26 (14.3%)                      |         |
| Disease severity assessment     |                             |                                 |         |
| COSSHACLF score                 | 7.1 ± 1.0                   | 6.3 ± 0.8                       | < 0.001 |
| CLIF-C ACLF score               | 38.9 ± 6.9                  | 32.7 ± 6.5                      | < 0.001 |
| AARCC ACLF score                | 10.7 ± 1.6                  | 9.6 ± 1.5                       | < 0.001 |
| MELD score                      | 29.8 ± 5.5                  | 25.7 ± 3.9                      | < 0.001 |
| Laboratory examination          |                             |                                 |         |
| PT-INR                          | 2.36 (1.95–2.81)            | 2.06 (1.75–2.44)                | 0.009   |
| Serum creatinine (× ULN)        | 0.97 (0.80–1.32)            | 0.80 (0.65–0.88)                | < 0.001 |
| Total bilirubin (µmol/L)        | 431.0 ± 135.4               | 421.9 ± 120.0                   | 0.495   |
| Direct bilirubin to total bilirubin ratio | 0.75 (0.70–0.82)           | 0.80 (0.73–0.86)                | 0.009   |
| Alamine aminotransferase (IU/L) | 140 (56–300)                | 124 (66–245)                    | 0.891   |
| Aspartate aminotransferase (IU/L)| 139 (76–227)              | 116 (88–192)                    | 0.133   |
| Aspartate aminotransferase to alanine aminotransferase ratio | 1.13 (0.65–1.92)           | 1.06 (0.64–1.53)                | 0.495   |
| Albumin (g/L)                   | 31.8 ± 3.6                  | 31.8 ± 4.0                      | 0.742   |
| Albumin to globulin ratio       | 1.2 ± 0.4                   | 1.2 ± 0.4                       | 0.041   |
| Ammonia (mmol/L)                | 77.6 (58.0–117.8)           | 79.1 (60.9–110.2)               | 0.891   |
| Lactate (mmol/L)                | 2.98 (2.03–3.89)            | 2.40 (1.90–3.00)                | < 0.001 |
| Serum sodium (mmol/L)           | 130.7 ± 15.8                | 134.5 ± 4.1                     | 0.009   |
| Serum potassium (mmol/L)        | 3.44 ± 0.55                 | 3.46 ± 0.58                     | 0.866   |
| Serum chloride (mmol/L)         | 93.9 ± 5.6                  | 97.3 ± 4.4                      | < 0.001 |
| Hemoglobin (g/L)                | 111 ± 18                    | 122 ± 20                        | 0.002   |
| Platelets (× 10^9/L)            | 83 (48–114)                 | 91 (64–124)                     | 0.180   |
| White blood cells (× 10^9/L)    | 7.87 ± 4.08                 | 7.47 ± 3.48                     | 0.495   |
| Intracorporeal  Catot before PA therapy (mmol/L) | 2.16 ± 0.15                | 2.13 ± 0.13                     | 0.133   |
| Intracorporeal  Catot before PA therapy (mmol/L) | 1.020 ± 0.089              | 1.051 ± 0.076                   | 0.123   |
| Intracorporeal  Catot during PA therapy (mmol/L) | 2.06 ± 0.21                | 1.97 ± 0.24                     | 0.595   |
| Intracorporeal  Catot during PA therapy (mmol/L) | 0.749 ± 0.098              | 0.808 ± 0.109                   | 0.262   |
| Extracorporeal  Catot during PA therapy (mmol/L) | 0.167 (0.132–0.233)        | 0.184 (0.145–0.238)             | 0.345   |
| Intracorporeal  Catot 2 h after PE therapy (mmol/L) | 2.65 ± 0.26                | 2.46 ± 0.18                     | < 0.001 |
| Intracorporeal  Catot 2 h after PE therapy (mmol/L) | 0.962 ± 0.100              | 1.103 ± 0.081                   | < 0.001 |
| Catot/Catot 2 h after PE therapy | 2.70 (2.58–2.90)           | 2.22 (2.14–2.32)                | < 0.001 |
| Anion gap 2 h after PE therapy (mmol/L) | 7.67 ± 2.90                | 6.85 ± 2.34                     | 0.010   |
| DPMAS plus PE therapy with RCA  |                             |                                 |         |
| Sessions                        | 3.0 (2.3–5.0)               | 4.0 (3.0–6.0)                   | 0.204   |
| Days from the first to the last sessions | 7.0 (4.0–14.0)            | 8.0 (5.0–14.0)                  | 0.292   |
| 90-day prognosis (death)        | 48 (63.2%)                  | 59 (32.4%)                      | < 0.001 |

Quantitative data are represented as mean ± SD (normally distributed data) or median (interquartile range) (non-normally distributed data) and compared by Mood’s median test. Qualitative data are represented as frequencies (proportion) and compared by Chi-squared test.

ACLF, Acute-on-chronic liver failure; LDCA, longer duration of citrate accumulation; HBV, hepatitis B virus; COSSH, Chinese Group on the Study of Severe Hepatitis B; CLIF-C, European Association for the Study of the Liver—Chronic Liver Failure-Consortium; AARC, APASL ACLF Research Consortium; APASL, Asian Pacific Association for the Study of the Liver; MELD, Model for End-Stage Liver Disease; PT-INR, international normalized ratio (INR) of prothrombin time (PT); ULN, upper limit of normal; PA, plasma adsorption; PE, plasma exchange;  Catot, total calcium;  Cation, ionized calcium;  Catot/Cation,  Catot to  Cation ratio
Our study proved that ACLF patients with LDCA would suffer higher 90-day mortality. This finding was in accordance with the results in critically ill patients undergoing continuous renal replacement therapy with RCA [4]. However, no significant differences in 90-day mortality risk were found in ACLF patients with or without LDCA. As RCA brings no alteration of pro- and anti-coagulation function and ACLF patients have re-balanced but fragile coagulation function [1, 5], our new results would support the use of RCA with caution in ACLF patients. Adequate training, experienced operation, and well-developed safety protocols would further expand indications of RCA [6].

Our study for the first time assessed the association between LDCA and prognosis in ACLF patients treated with PA plus PE therapy with RCA. There were limitations: monocentric retrospective design, only HBV-ALCF cases, and applying Ca\textsubscript{tot}/Ca\textsubscript{ion} instead of directly measuring plasma citrate concentration to reflect citrate accumulation.

### Abbreviations

- AARC: Asian Pacific Association for the Study of the Liver—ACLF Research Consortium
- ACLF: Acute-on-chronic liver failure
- AAR C: Asian Pa cific Association for the Study of the Liver
- COSSH: Chinese Group on the Study of Severe Hepatitis B
- HBV: Hepatitis B virus
- HR: Hazard ratio
- LDCA: Long duration of citrate accumulation
- MELD: Model for End-Stage Liver Disease
- PA: Plasma adsorption
- PE: Plasma exchange
- RCA: Regional citrate anticoagulation

### Table 2

LDCA and other factors associated with risk of 90-day mortality in ACLF patients

|                       | Crude HR (95% CI) | Adjusted HR\textsuperscript{\(\ast\)} (95% CI) |
|-----------------------|-------------------|-----------------------------------------------|
|                       | Model 1 | Model 2 | Model 3 | Model 4 |
| LDCA                  |         |         |         |         |
| No                    | 1       | 1       | 1       | 1       |
| Yes                   | 2.62 (1.79–3.84)*** | 1.07 (0.66–1.73) | 1.49 (0.95–2.36) | 1.41 (0.90–2.22) | 1.05 (0.65–1.72) |
| Age (years)           | 1.03 (1.01–1.05)*** | 0.99 (0.97–1.02) | 0.97 (0.94–0.99)** | 1.02 (1.00–1.04) | 1.01 (0.99–1.03) |
| Gender                |         |         |         |         |
| Male                  | 1       | 1       | 1       | 1       |
| Female                | 1.84 (1.15–2.94)* | 1.24 (0.73–2.08) | 1.04 (0.62–1.76) | 1.25 (0.74–2.09) | 1.81 (1.07–3.08)* |
| Liver cirrhosis       |         |         |         |         |
| No                    | 1       | 1       | 1       | 1       |
| Yes                   | 2.51 (1.37–4.57)** | 1.66 (0.90–3.08) | 2.14 (1.17–3.95)* | 2.20 (1.19–4.06)* | 1.97 (1.07–3.65)* |
| HBV DNA (log10 IU/mL) | 0.98 (0.89–1.09) | 1.02 (0.92–1.13) | 1.00 (0.90–1.12) | 1.00 (0.90–1.12) | 1.01 (0.90–1.13) |
| Etiology              |         |         |         |         |
| HBV infection only    | 1       | 1       | 1       | 1       |
| HBV infection plus other causes\textsuperscript{\(\ast\)} | 0.93 (0.60–1.45) | 1.07 (0.68–1.69) | 1.07 (0.68–1.68) | 1.06 (0.67–1.67) | 0.82 (0.51–1.29) |
| Comorbidity\textsuperscript{\(\ast\)} |         |         |         |         |
| No                    | 1       | 1       | 1       | 1       |
| Yes                   | 1.86 (1.20–2.90)** | 1.74 (1.05–2.87)* | 1.56 (0.96–2.55) | 1.60 (0.98–2.61) | 1.75 (1.06–2.90)* |
| Disease severity      |         |         |         |         |
| COSSH ACLF score      | 2.78 (2.31–3.34)*** | 2.72 (2.17–3.40)*** | –         | –         | –         |
| CLIF-C ACLF score     | 1.13 (1.09–1.16)*** | –         | 1.15 (1.10–1.19)*** | –         | –         |
| AARC ACLF score       | 1.60 (1.41–1.82)** | –         | –         | 1.59 (1.38–1.83)*** | –         |
| MELD score            | 1.16 (1.12–1.20)** | –         | –         | –         | 1.17 (1.12–1.22)*** |

\textsuperscript{\(\ast\)}p < 0.001; \textsuperscript{\(\ast\)}p < 0.01; \textsuperscript{\(\ast\)}p < 0.05
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Authors’ contributions
WM, MYJ and DLY contributed to statistical analysis, drafting of the manuscript, and interpretation of data. MYJ and BL had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. BL and TH contributed to study concept and design, and critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Approval for this study was obtained from the Biomedical Research Ethics Committee of West China Hospital of Sichuan University (No. 2020-650). All study components were performed according to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed consent was not obtained because of retrospective design.

Consent for publication
Not applicable.

Competing interests
The authors declare to have no competing interests.

References
1. Ma Y, Chen F, Xu Y, Wang M, Zhou T, Lu J, et al. Safety and efficacy of regional citrate anticoagulation during plasma adsorption plus plasma exchange therapy for patients with acute-on-chronic liver failure: a pilot study. Blood Purif. 2019;48:223–32.
2. Ma Y, Chen F, Liu C, Xu Y, Wang M, Zhou T, et al. A novel predictive score for citrate accumulation among patients receiving artificial liver support system therapy with regional citrate anticoagulation. Sci Rep. 2020;10:12861.
3. Wu T, Li J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. Gut. 2018;67:2181–91.
4. Khadzhynov D, Schelter C, Lieker I, Mika A, Staeck O, Neumayer HH, et al. Incidence and outcome of metabolic disarrangements consistent with citrate accumulation in critically ill patients undergoing continuous venovenous hemodialysis with regional citrate anticoagulation. J Crit Care. 2014;29:265–71.
5. Wiegele M, Adelmann D, Dibiasi C, Pausch A, Baierl A, Schaden E. Monitoring of enoxaparin during hemodialysis covered by regional citrate anticoagulation in acute kidney injury: a prospective cohort study. J Clin Med. 2021;10:4491.
6. Schneider AG, Joannes-Boyau O. Regional citrate anticoagulation for CRRT: still hesitating. Anaesth Crit Care Pain Med. 2021;40:100855.

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