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Research Article

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Application of regional citrate anticoagulation in patients at high risk of bleeding during intermittent hemodialysis: a prospective multicenter randomized controlled trial

Xiaoyan TANG[1,2], Dezheng CHEN[2], Ling ZHANG[1✉], Ping FU[1], Yanxia CHEN[3], Zhou XIAO[4], Xiangcheng XIAO[4], Weisheng PENG[4], Li CHENG[5], Yanmin ZHANG[5], Hongbo LI[5], Kehui LI[6], Bizhen GOU[7], Xin WU[7], Qian YU[7], Lijun JIAN[8], Zaizhi ZHU[9], Yu WEN[10], Cheng LIU[10], Hen XUE[11], Hongyu ZHANG[11], Xin HE[12], Bin YAN[13], Liping ZHONG[4], Bin HUANG[4], Mingying MAO[2]

[1]*Division of Nephrology, Kidney Research Institute, West China Hospital of Sichuan University, Chengdu 610041, China*
[2]*Department of Nephrology, People's Hospital of Jianyang City, Chengdu 641400, China*
[3]*Department of Nephrology, the Second Affiliated Hospital of Nanchang University, Nanchang 330006, China*
[4]*Department of Nephrology, Xiangya Hospital of Central South University, Changsha 410008, China*
[5]*Department of Nephrology, Wuhan No. 1 Hospital, Wuhan 430022, China*
[6]*Department of Blood Purification, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, China*
[7]*Department of Nephrology, Guiyang First People's Hospital, Guiyang 550002, China*
[8]*Department of Nephrology, West China Hospital Sichuan University Jintang Hospital, Jintang 610400, China*
[9]*Department of Nephrology, Meishan People's Hospital, Meishan 620020, China*
[10]*Department of Nephrology, Ziyang People's Hospital, Ziyang 641300, China*
[11]*Department of Nephrology, Ya'an People's Hospital, Ya'an 625000, China*
[12]*Department of Nephrology, Chengdu Kangfu Kidney Disease Hospital, Chengdu 610047, China*
[13]*Department of Nephrology, the First People's Hospital of Liangshan Yi Autonomous Prefecture, Xichang 615000, China*

Abstract: Objective: Safe and effective anticoagulation is essential for hemodialysis patients who are at high risk of bleeding. The purpose of this trial is to evaluate the effectiveness and safety of two-stage regional citrate anticoagulation (RCA) combined with sequential anticoagulation and standard calcium-containing dialysate in intermittent hemodialysis (IHD) treatment. Methods: Patients at high risk of bleeding who underwent IHD from September 2019 to May 2021 were prospectively enrolled in 13 blood purification centers of nephrology departments, and were randomly divided into RCA group and saline flushing group. In the RCA group, 0.04 g/mL sodium citrate was infused from the start of the dialysis line during blood draining and at the venous expansion chamber. The sodium citrate was stopped after 3 h of dialysis, which was changed to sequential dialysis without anticoagulant. The hazard ratios for coagulation were according to baseline. Results: A total of 159 patients and 208 sessions were enrolled, including RCA group (80 patients, 110 sessions) and saline flushing group (79 patients, 98 sessions). The incidence of severe coagulation events of extracorporeal circulation in the RCA group was significantly lower than that in the saline flushing group (3.64% vs. 20.41%, P<0.001). The survival time of the filter pipeline in the RCA group was significantly longer than that in the saline flushing group ((238.34±9.33) min vs. (221.73±34.10) min, P<0.001). The urea clearance index (Kt/V) in the RCA group was similar to that in the saline flushing group with no statistically significant difference (1.12±0.34 vs. 1.08±0.34, P=0.41). Conclusions: Compared with saline flushing, the two-stage RCA combined with a sequential anticoagulation strategy significantly reduced extracorporeal circulation clotting events and prolonged the dialysis time without serious adverse events.

Key words: Regional citrate anticoagulation; Intermittent hemodialysis; Calcium-containing dialysate; Saline flushing; Anticoagulation

1 Introduction

At present, unfractionated heparin, a systemic non-selective anticoagulant, is the most commonly used anticoagulant in hemodialysis. The 2002 European Best Practice Guidelines for Hemodialysis state

✉ Ling ZHANG, zhangling_crrt@163.com
Ling ZHANG, https://orcid.org/0000-0003-1155-4802
Xiaoyan TANG, https://orcid.org/0000-0002-3232-593X

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that systemic anticoagulation treatment should be avoided in patients at high risk of bleeding. The periodic washing of the peripheral circuit with saline or regional citrate anticoagulation (RCA) is recommended (European Best Practice Guidelines Expert Group on Hemodialysis and European Renal Association, 2002). Nonetheless, the incidence of coagulation with saline washing exceeds 50% (Richtrova et al., 2011; Guéry et al., 2014). Most patients do not achieve the desired anticoagulation effects because of the early termination of treatment. As an anticoagulant in the extracorporeal circuit, sodium citrate has been widely used in continuous renal replacement therapy (CRRT), and its safety and effectiveness have been fully verified (Schmitz et al., 2018; Zarbock et al., 2020). The 2012 Kidney Disease: Improving Global Outcomes (KDIGO) was recommended in clinical practice guidelines for acute kidney injury (AKI) (Khwaja, 2012). The classic method of RCA can achieve full anticoagulation effects by using a calcium-free dialysate. However, there is a risk of adverse reactions such as hypocalcemia; therefore, calcium needs to be continuously pumped into the venous return side (Faguer et al., 2017). Calcium-containing dialysate with a concentration gradient can make calcium ions diffuse from the dialysate into the blood. The serum calcium levels can also be maintained in the normal range in most patients without additional calcium supplementation, making the RCA procedure simpler and safer (von Brecht et al., 1986). Nevertheless, the premature termination rate of dialysis due to severe clotting was reported as up to 13%, and the clotting rate at the venous expansion chamber was 75.9% (Evenepoel et al., 2007). To improve this method, we increased the dose of citrate from the start of the dialysis line during blood draining and elevated the infusion of citrate at the venous expansion chamber to reduce the clotting of the peripheral circuit. At the same time, the content of citrate directly entering the body increased. Consequently, 1 h before the end of dialysis, we switched to heparin-free sequential anticoagulation (Buturović-Ponikvar et al., 2005; Buturovic et al., 2008) to eliminate the hidden danger of citrate accumulation and improve patient safety.

The aims of this study were to investigate the safety and effectiveness of two-stage RCA combined with sequential anticoagulation using common calcium-containing dialysate in intermittent hemodialysis (IHD) treatment for patients at high risk of bleeding, to explore the standard medication regimen of RCA, and to promote the application of RCA in common hemodialysis.

2 Patients and methods

2.1 Patients' characteristics

From September 2019 to May 2021, 170 patients were recruited in this study from 13 blood purification centers of nephrology departments, who had active bleeding disorders or were at high risk of bleeding requiring IHD. A total of 159 patients were finally enrolled, including 95 males and 64 females, 144 with end-stage renal disease (ESRD), 9 with AKI, and 6 with Phases 3–4 chronic kidney disease. The causes of active hemorrhagic disease and high risk of bleeding were hemorrhage in 89 cases (55.97%) and perioperation in 70 cases (44.03%). There were 40 cases (25.16%) of active gastrointestinal bleeding, 20 cases (12.58%) of arteriovenous fistulization, 16 cases (10.06%) of acute cerebral hemorrhage, 14 cases (8.81%) of parathyroidectomy, 7 cases (4.40%) of cuff catheterization, 7 cases (4.40%) of pulmonary hemorrhage, 7 cases (4.40%) of peritoneal dialysis catheterization, 7 cases (4.40%) of renal puncture, 5 cases (3.14%) of cystirrhagia, 5 cases (3.14%) of fundus hemorrhage, 4 cases (2.52%) of uterine bleeding, 4 cases (2.52%) of nasal hemorrhage, 4 cases (2.52%) of renal transplantation, 3 cases (1.89%) of cataract surgery, 3 cases (1.89%) of orthopedic surgery, 3 cases (1.89%) of renal hemorrhage, 3 cases (1.89%) of gingival hemorrhage, 2 cases (1.26%) of cholecystectomy and biliary drainage, 2 cases (1.26%) of abdominal hemorrhage, 1 case (0.63%) of calf intermuscular hematoma puncture, 1 case (0.63%) of right hemicolectomy, and 1 case (0.63%) of bloody abdominal abscess and puncture drainage.

2.2 Inclusion, exclusion, and withdrawal criteria

The inclusion criteria were (1) patients aged 18 years and above who needed IHD due to renal insufficiency, and (2) an active bleeding disease or high risk of bleeding in the body. Hemodialysis patients at high risk of bleeding were defined as follows: active bleeding has now ceased for no more than 3 d; surgical or traumatic wounds within the past 3 d; or acute

dialysis via an intravenous catheter (Swartz and Port, 1979). Other parameters were as follows: hemoglobin (Hb) 55–140 g/L, blood platelet (PLT) 100×10^9–350×10^9 L^{-1}, and total blood calcium 1.8–2.5 mmol/L. The exclusion criteria included: a history of sodium citrate allergy; liver function damage, total bilirubin >60 μmol/L; extensive muscle tissue injury or muscle tissue hypoperfusion (lactate>3 mmol/L); mask oxygen inhalation or oxygenation index of <300 mmHg (1 mmHg=0.133 kPa); unable to cooperate with the treatment due to mental disorders. The withdrawal criteria were as follows: in case of apparent citrate accumulation toxicity or untreatable hypocalcemia during the study, the patient could withdraw and continue the treatment with 0.009 g/mL saline flushing; the patient's bleeding risk disappeared and had completed three treatments or they had been discharged.

2.3 Randomization and interventions

This trial included two parts. Patients were randomized to the RCA or saline flushing group by the central computer. In the RCA group, 0.04 g/mL sodium citrate was infused from the start of the dialysis line during blood draining at 240–260 mL/h and from the venous expansion chamber at 80–100 mL/h. The level of ionized calcium (iCa^{2+}) dialyzer inlet was maintained at 0.25–0.35 mmol/L and the level of arterial iCa^{2+} was maintained at 1.0–1.2 mmol/L. If necessary, 0.10 g/mL calcium gluconate was supplemented from the intravenous end (10–20 mL/h), the blood flow was set at 150 mL/min, the dialysate flow rate was 300 mL/min, and the base excess (BE) of −10 to −8 mmol/L or bicarbonate ion (HCO_3^-) of 22–26 mmol/L was adjusted online. After 3 h, the infusion of sodium citrate was stopped and changed to no anticoagulant mode. At the same time, the blood flow was adjusted to 200–300 mL/min, the dialysate flow rate was 500 mL/min, the BE recovered to −3 to +3 mmol/L or the HCO_3^- recovered to 30–34 mmol/L, and the treatment time was 1 h. The parameters were adjusted according to the iCa^{2+} level at different points of the pipeline, from the clotting of filter and pipeline to pH and other indicators. When the iCa^{2+} dialyzer inlet was greater than 0.4 mmol/L or the clotting of the dialyzer was obvious, the citrate infusion rate at the start of the dialysis line during blood draining was increased. If the clotting at the venous expansion chamber was obvious, the citrate infusion rate at the venous expansion chamber was increased. If the arterial iCa^{2+} was less than 0.9 mmol/L, the citrate infusion rate was reduced. The actual speed was increased or decreased by about 10% each time. If the arterial pH>7.45 or BE>+3 mmol/L, the concentration of BE or bicarbonate was reduced online (Fig. 1). In the saline flushing group, 200 mL of 0.009 g/mL saline was given every half hour to flush the pipeline and dialyzer. The blood flow was 200–300 mL/min, the dialysate flow rate was 500 mL/min, and the treatment time was 2–4 h. If premature termination of dialysis occurred due to severe clotting within 2 h, the pipeline and dialyzer were replaced to continue the treatment. If the treatment time exceeded 2 h, it was terminated.

2.4 Hemodialysis parameters, main instruments and reagents

Standard calcium-containing dialysate was used for hemodialysis. After mixing with BiBag (Fresenius Medical Care, Bad Homburg, Germany) or BiCart (Gambro Lundia AB, Lund, Sweden) bicarbonate

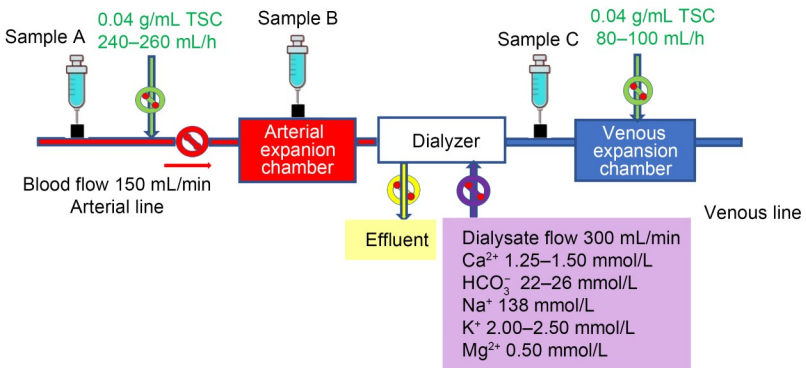

Fig. 1 Schematic diagram of two-stage regional citrate anticoagulation with ordinary calcium-containing dialysate. TSC: trisodium citrate.

powder, the final components were 138 mmol/L sodium ion (Na^+), 2.00–2.50 mmol/L potassium ion (K^+), 0.50 mmol/L magnesium ion (Mg^{2+}), 110 mmol/L chlorine ion (Cl^-), 30–34 mmol/L HCO_3^-, and 1.25–1.50 mmol/L calcium ion (Ca^{2+}). During the dialysis treatment, conventional hemodialysis machines were mainly used, including Fresenius (4008S type, Fresenius Medical Care), Gambro (AK200S type, Gambro Lundia AB), B. Braun Dialog$^+$ machine (BB; B. Braun, Melsungen, Germany), and polysulfone membrane dialyzers adopting Fresenius FX8 (Fresenius Medical Care) and Gambro 14L (Gambro Dialysatoren GmbH, Hechingen, Germany). Blood gas was detected and analysed using an Abbott i-STAT portable blood gas analyzer (Abbott Point of Care Inc., Princeton, NJ, USA) and its test pieces $EG7^+$, $CG8^+$, $CG4^+$. A 0.04 g/mL sodium citrate solution was used (200 mL/bag; Chengdu Qingshan Likang Pharmaceutical Co., Ltd., Chengdu, China).

2.5 Blood collection

At the beginning and the end of dialysis, blood samples from the two patient groups were collected in the arterial line (sample A shown in Fig. 1) to analyze serum creatinine (Scr), urea, Hb, PLT, prothrombin time (PT), international normalized ratio (INR), electrolytes, and blood gas. In the RCA group, blood samples were collected from the arterial line, dialyzer inlet, and dialyzer outlet (sample A, sample B, and sample C, respectively, as shown in Fig. 1) at 1 and 3 h after dialysis. Blood was collected directly at sample B and sample C. At sample A, sodium citrate and ultrafiltration were suspended for 5 min before blood collection to reduce the impact of extracorporeal recirculation on the test indices.

2.6 Interpretation of results

The primary outcomes were the clotting scores and the survival time of extracorporeal circulation. The clotting of the dialyzer and the arteriovenous expansion chamber was determined by semi-quantitative measurement. The clotting scores were rated 0, 1, 2, and 3 (clean, mild, moderate, and serious, respectively). The detailed scoring methods were as follows: (1) for the arterial and venous expansion chambers, a score of 0 indicated no visible clotting, a score of 1 indicated a little clotting affecting no more than 1/2 of expansion chamber, a score of 2 indicated clotting between 1/2 and 2/3 of the expansion chamber, and a score of 3 indicated clotting in more than 2/3 of the expansion chamber; (2) for the dialyzer, a score of 0 indicated no visible coagulation or the coagulation of several fibers, and a score of 1, 2, or 3 indicated a small degree of fiber coagulation in no more than 1/2, between 1/2 and 2/3, or in more than 2/3 of the expansion chamber, respectively. Serious clotting events (clotting score=3) in extracorporeal circuits were defined as volume of clotting in more than 2/3 of the expansion chamber or area of fibrin coagulation in more than 2/3 of total or venous expansion chamber with pressure of >200 mmHg or transmembrane pressure of >300 mmHg.

Secondary outcomes included counts of Hb and PLT, PT, INR, electrolytes, blood gas before and after dialysis, and dialysis adequacy evaluations. The dialysis adequacy assessment was calculated by urea clearance index (Kt/V, where K is dialyzer clearance of urea, t is dialysis time, and V is volume of distribution of urea) and urea reduction ratio (URR). Kt/V refers to the single-pool Kt/V (spKt/V). The calculation formula employed (Daugirdas, 1993) was $spKt/V=-\ln(R-0.008\times t)+(4-3.5\times R)\times UFV/W$, where R is the ratio of blood urea post-dialysis/blood urea pre-dialysis, t is dialysis time (h), UFV is ultrafiltration volume (L), and W is bodyweight after dialysis (kg).

2.7 Statistical analysis

The normally distributed continuous variables were presented as mean±standard deviation (SD), and the categorical variables were presented as frequency and proportion. A Chi-square test was used for rate comparison. The normally distributed continuous variables were analyzed with the t-test between two groups. A paired t-test was used to compare normally distributed quantitative data before and after treatment. A Kaplan-Meier survival curve was used for pipeline survival time comparison as well as the log-rank test. Repeated measurement analysis of variance was used for the blood gas monitoring indices in the RCA group. The statistical analysis of the data was performed by SPSS (Version 24.0; IBM Corp., Armonk, New York, USA). A two-sided $P<0.05$ was considered statistically significant. The survival curves and other graphs were plotted using GraphPad Prism (Version 9.0.0; GraphPad Software, San Diego, CA, USA). The hazard ratios for coagulation were according to

the baseline. Subgroup effects were assessed by testing the interaction of treatment group and subgroup strata, with $P<0.10$ considered as indicative of statistical significance. R (Version 4.1.2) was used for forest mapping.

3 Results

3.1 Patients and baseline characteristics

Eventually, a total of 159 patients were included, in which 80 patients in the RCA group completed 110 treatment sessions and 79 patients in the saline flushing group completed 98 treatment sessions (Fig. 2). There were statistically significant differences ($P<0.05$) in vascular access, total protein, and albumin levels between the two groups. Other baseline laboratory indices, such as age, dialyzer, causes of high-risk bleeding, Hb, PLT, iCa^{2+}, PT, activated partial thromboplastin time (APTT), blood urea nitrogen (BUN), and Scr, showed no statistical significance ($P>0.05$; Table 1).

3.2 Primary outcomes

The incidence of premature termination due to severe clotting in extracorporeal circulation in the RCA group was significantly lower than that in the saline flushing group (3.64% vs. 20.41%, $P<0.001$; Fig. 3). The survival time of the filter pipeline in the RCA group was significantly longer than that in the saline flushing group, namely, (238.34±9.33) min vs. (221.73±34.10) min, $P<0.001$ (Fig. 4). The incidences of severe clotting in the dialyzer, venous expansion chamber, and arterial expansion chamber in the RCA group were significantly lower than those in the saline flushing group (0.91% vs. 14.28%, $P<0.001$; 2.73% vs. 15.31%, $P<0.001$; 0% vs. 2.04%, $P<0.01$; Table 2). RCA had a significant protective effect in various subgroups (including different age groups, gender groups, access type groups, etc.). No interaction between intervention and the above covariates was observed (Fig. 5).

3.3 Secondary outcomes

3.3.1 Dialysis adequacy evaluations, electrolytes, and coagulation routine

The Kt/V and URR in the RCA group were similar to those in the saline flushing group, with no statistically significant differences (1.12±0.34 vs. 1.08±0.34, $P=0.41$; (59.39±11.24)% vs. (58.83±12.62)%, $P=0.74$; Table 3). There was no statistical difference in ultrafiltration volume between the two groups ((1913.43±966.76) mL vs. (1690.16±950.55) mL, $P=0.10$; Table 3). The iCa^{2+} concentration after dialysis in the RCA group was lower than that in the saline flushing group ((1.10±0.14) mmol/L vs. (1.16±0.17) mmol/L, $P<0.01$; Fig. 6a and Table 3), and the Na^{+} concentration

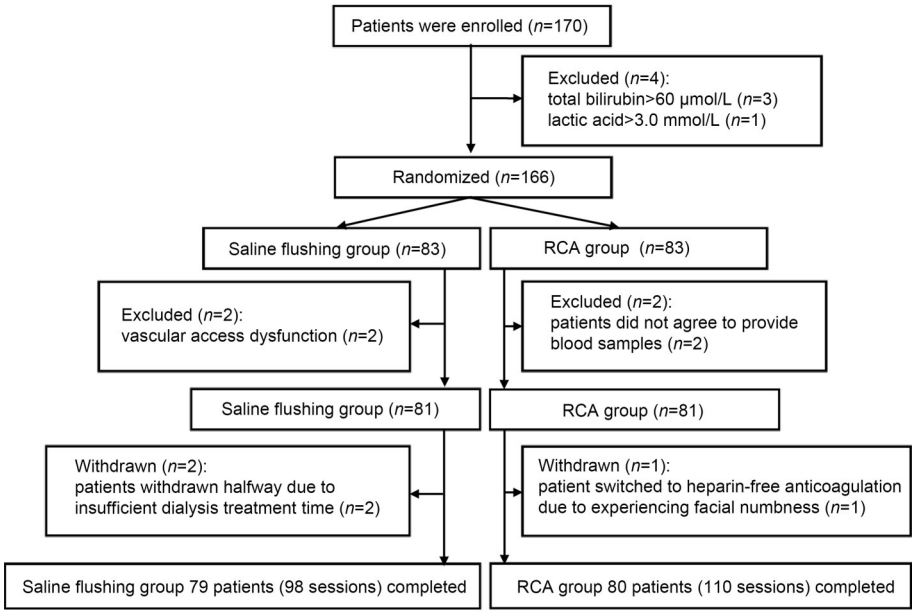

Fig. 2 Patient enrolment flowchart. RCA: regional citrate anticoagulation.

Table 1 Baseline characteristics of included patients

Factor	RCA (n=80)	Saline flushing (n=79)	P value
Male	49 (61.25%)	46 (58.23%)	0.70
Access type-AV fistula/catheter	30/50	44/35	0.02
Age (years)	55.48±13.08	54.99±15.22	0.83
Hypertension	57 (71.25%)	66 (83.54%)	0.06
Diabetes	22 (27.50%)	21 (26.58%)	0.90
Dialyzer 14L	26 (32.50%)	22 (27.85%)	0.52
Dialyzer FX8	18 (22.50%)	21 (26.58%)	0.55
Causes of high risk of bleeding			
Active gastrointestinal bleeding	21 (26.25%)	19 (24.05%)	0.75
Acute cerebral hemorrhage	9 (11.25%)	7 (8.86%)	0.62
Arteriovenous fistulization	12 (15.00%)	8 (10.13%)	0.35
Hb (g/L)	88.25±18.70	93.96±21.44	0.08
PLT (10^9 L^{-1})	178.52±68.22	172.48±66.62	0.57
TP (g/L)	61.81±10.91	65.42±9.68	0.03
ALB (g/L)	34.07±6.77	37.13±6.94	0.01
BUN (mmol/L)	22.13±9.34	22.95±9.80	0.59
Scr (μmol/L)	750.68±318.28	804.63±325.31	0.29
Na^+ (mmol/L)	138.73±3.34	138.63±4.34	0.87
TCa (mmol/L)	2.12±0.22	2.19±0.22	0.07
iCa^{2+} (mmol/L)	1.10±0.12	1.09±0.17	0.71
CO_2CP (mmol/L)	21.04±4.24	21.29±4.13	0.73
PT (s)	12.86±7.35	11.89±1.41	0.25
APTT (s)	31.65±8.28	31.08±6.32	0.63
Fibrinogen (g/L)	3.91±1.49	4.11±1.51	0.43
D-Dimer (μg/L)	2.28±3.03	1.64±2.57	0.23

The normally distributed continuous variables were presented as mean±standard deviation (SD), and the categorical variables were characterized by frequency and proportion (n (%)). A Chi-square test was used for rate comparison. The variables were analyzed with the t-test between two groups. P<0.05 was considered statistically significant. RCA: regional citrate anticoagulation; AV: arteriovenous; Hb: hemoglobin; PLT: platelet; TP: total protein; ALB: albumin; BUN: blood urea nitrogen; Scr: serum creatinine; Na^+: sodium; TCa: total calcium; iCa^{2+}: ionized calcium; CO_2CP: carbon dioxide-combining power; PT: prothrombin time; APTT: activated partial thromboplastin time.

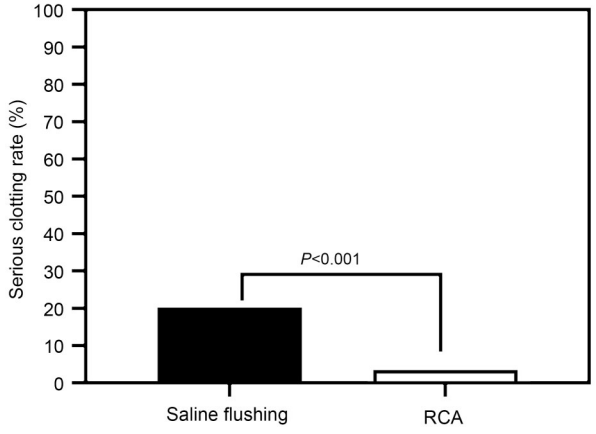

Fig. 3 Serious clotting events in extracorporeal circuits between the RCA group and the normal saline flushing group. RCA: regional citrate anticoagulation.

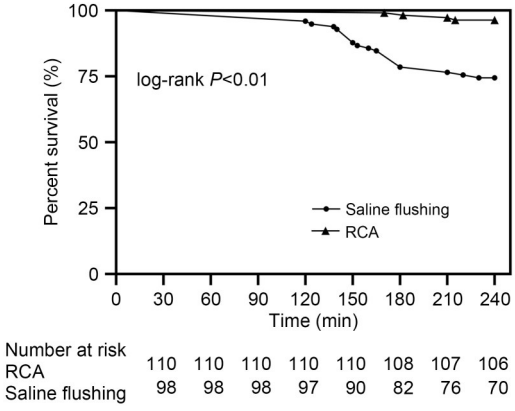

Fig. 4 Kaplan-Meier curves for pipeline survival time between the RCA and saline groups. The log-rank test was used for the survival analyses. RCA: regional citrate anticoagulation.

Table 2 Comparison of different clotting between the RCA and normal saline flushing groups

Clotting site	Clotting score	Incidence		*P* value
		RCA (*n*=110)	Saline flushing (*n*=98)	
Extracorporeal circuits	3	4 (3.64%)	20 (20.41%)	<0.001
Dialyzer	0	88 (80.00%)	16 (16.33%)	<0.001
	1	17 (15.45%)	38 (38.78%)	
	2	4 (3.64%)	30 (30.61%)	
	3	1 (0.91%)	14 (14.28%)	
Venous expansion chamber	0	83 (75.45%)	19 (19.39%)	<0.001
	1	20 (18.18%)	23 (23.47%)	
	2	4 (3.64%)	41 (41.83%)	
	3	3 (2.73%)	15 (15.31%)	
Arterial expansion chamber	0	105 (95.45%)	62 (63.27%)	<0.01
	1	5 (4.55%)	29 (29.59%)	
	2	0 (0%)	5 (5.10%)	
	3	0 (0%)	2 (2.04%)	

The incidences of different clotting scores were characterized by frequency and proportion (*n* (%)), and analyzed with Pearson's Chi-square or Fisher's exact test. The clotting scores were rated 0, 1, 2, and 3, representing clean, mild, moderate, and serious, respectively.

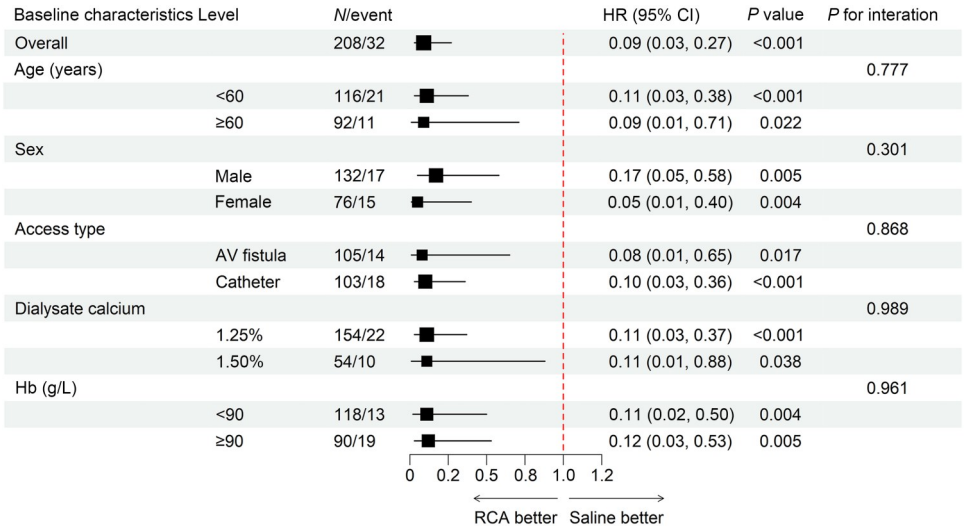

Fig. 5 Hazard ratios (HRs) for coagulation according to the baseline characteristics. *P* values are for the interaction between the RCA group and baseline. There was no significant interaction between RCA and subgroup variables, as defined according to the prespecified threshold level of significance for interaction (*P*=0.10). RCA: regional citrate anticoagulation; Saline: saline flushing; Hb: hemoglobin; AV: arteriovenous; *N*: number of patients; CI: confidence interval.

after dialysis in the RCA group was higher than that in the saline flushing group ((139.79±3.41) mmol/L vs. (138.28±3.07) mmol/L, $P<0.01$; Fig. 6b and Table 3). There was no statistical difference in Hb, PLT, INR, PT, APTT, pH, or HCO_3^- between the two groups before or after dialysis ($P>0.05$; Figs. 6c and 6d, Table 3). The difference in changes of Hb, PLT, Na^+, iCa^{2+}, pH, HCO_3^-, INR, PT, or APTT before and after dialysis was not statistically significant between the two groups ($P>0.05$; Table 3). In addition, there was no statistical difference in PLT, INR, PT, or APTT between pre-hemodialysis (pre-HD) and post-hemodialysis (post-HD) within each group ($P>0.05$; Table 3). The concentrations of Hb, Na^+, and HCO_3^-, and pH value in each group were significantly higher post-HD than pre-HD ($P<0.001$; Figs. 6b–6d and Table 3). The concentration of iCa^{2+} in the saline flushing group was significantly higher post-HD than pre-HD ((1.16±0.17) mmol/L vs. (1.11±0.19) mmol/L, $P<0.01$; Fig. 6a and Table 3).

3.3.2 Blood gas analysis in the RCA group

In the RCA group, the concentration of arterial iCa^{2+} initially decreased, and it stabilized at the (1.00±

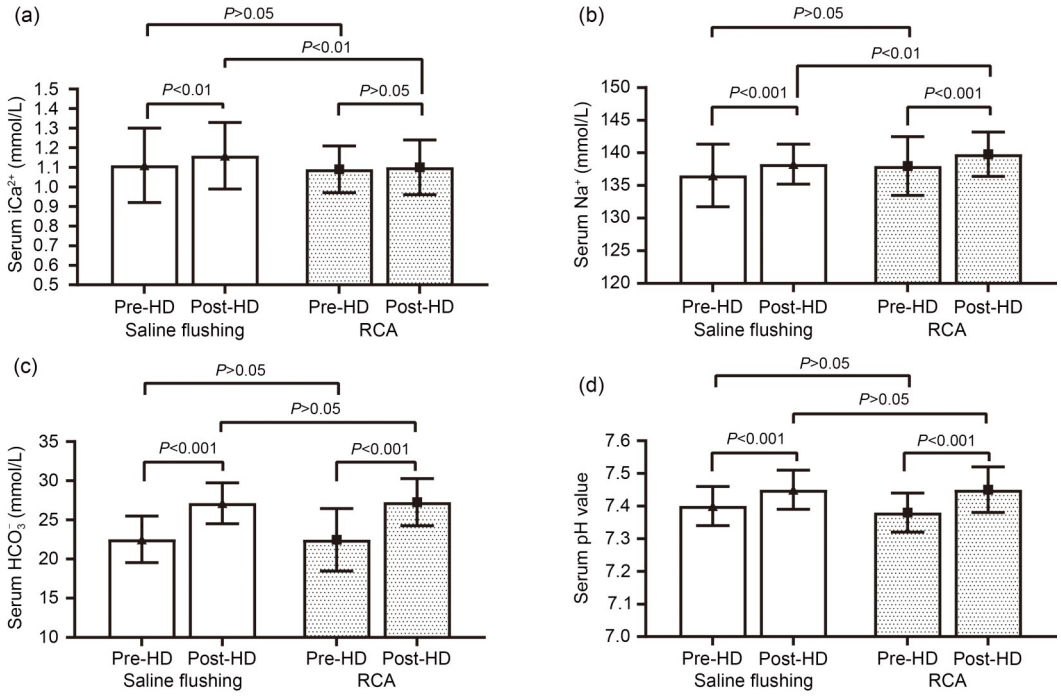

Fig. 6 Concentrations of ionized calcium (iCa^{2+}) (a), sodium ion (Na^+) (b), and bicarbonate ion (HCO_3^-) (c), and the pH value (d) before and after dialysis in the RCA (*n*=110) and normal saline flushing (*n*=98) groups. The variables were analyzed with the *t*-test between two groups and with a paired *t*-test before and after dialysis in each group. Data are expressed as mean±standard deviation (SD). Pre-HD: pre-hemodialysis; Post-HD: post-hemodialysis; RCA: regional citrate anticoagulation.

0.15) mmol/L level in the first 3 h and gradually increased after switching to sequential therapy without anticoagulant after 3 h. There were no statistical differences in the concentration of arterial iCa^{2+} before and after dialysis ((1.09±0.12) mmol/L vs. (1.10±0.14) mmol/L, P>0.05), and the results showed that the concentration of arterial iCa^{2+} after dialysis had returned to the pre-dialysis level. The arterial iCa^{2+} concentration from the beginning to the end of dialysis was in the range of 1.00–1.15 mmol/L, reaching the goal of maintaining the arterial iCa^{2+} level at 1.00–1.20 mmol/L (Fig. 7a). The concentration curves of iCa^{2+} for 1 and 3 h in the RCA group were almost the same (Fig. 8). The concentration of iCa^{2+} before the dialyzer was (0.30±0.07) mmol/L and (0.32±0.10) mmol/L at 1 and 3 h, respectively, reaching the target range of 0.25–0.35 mmol/L anticoagulation. The concentration of iCa^{2+} was close to the arterial level after the dialyzer at 1 and 3 h (P>0.05; Fig. 8). The arterial Na^+ concentration and pH value increased gradually in the first 3 h in the RCA group and decreased slightly after 3 h (Figs. 7b and 7d). The arterial HCO_3^- showed a gradual upward trend from the beginning to the end of dialysis (Fig. 7c). After dialysis, the arterial Na^+, HCO_3^-, or pH values did not exceed the standard high limit in all patients (Figs. 7b–7d).

3.4 Adverse events

In the RCA group, facial numbness occurred in two cases (2/110, 1.82%), and transient hypotension occurred in two cases (2/110, 1.82%). Besides, transient hypotension occurred in two cases (2/98, 2.04%) in the saline flushing group, and blood loss due to severe coagulation occurred in one case (1/98, 1.02%). The vital signs of patients in the two groups were stable during treatment, and no serious adverse events were reported.

4 Discussion

Citrate as an anticoagulant is only applied in vitro rather than as a systemic anticoagulant. Therefore, it is especially suitable for hemodialysis patients at high risk of bleeding. Morita et al. (1961) first reported the application of sodium citrate anticoagulation in the hemodialysis of patients; nevertheless, this method

Table 3 Comparisons of blood routine, coagulation routine, and electrolyte before and after dialysis, ultrafiltration and dialysis adequacy evaluations between the RCA and normal saline flushing groups

Factor	Detection time	RCA (n=110)	Saline flushing (n=98)	P value
Hb (g/L)	Pre-HD	86.33±19.31	91.94±22.41	0.05
	Post-HD	90.83±21.37***	95.78±26.07***	0.14
	Pre−Post	−4.50±9.04	−3.84±9.69	0.62
PLT ($\times10^9$ L^{-1})	Pre-HD	172.30±59.05	164.44±58.28	0.35
	Post-HD	174.67±64.64	163.47±57.71	0.35
	Pre−Post	−2.22±23.65	0.95±33.34	0.43
INR	Pre-HD	1.04±0.24	1.03±0.13	0.78
	Post-HD	1.02±0.31	1.06±0.41	0.48
	Pre−Post	0.02±0.30	−0.02±0.40	0.38
PT (s)	Pre-HD	12.64±5.96	12.74±8.26	0.92
	Post-HD	11.93±3.28	12.13±3.87	0.69
	Pre−Post	0.72±6.41	0.61±8.95	0.92
APTT (s)	Pre-HD	35.21±22.53	33.70±15.34	0.58
	Post-HD	30.09±7.48	32.20±12.50	0.14
	Pre−Post	5.11±22.09	1.56±16.95	0.20
iCa^{2+} (mmol/L)	Pre-HD	1.09±0.12	1.11±0.19	0.45
	Post-HD	1.10±0.14	1.16±0.17**	<0.01
	Pre−Post	−0.02±0.16	−0.06±0.17	0.07
Na^+ (mmol/L)	Pre-HD	137.99±4.50	136.54±4.80	0.06
	Post-HD	139.79±3.41***	138.28±3.07***	<0.01
	Pre−Post	−1.92±3.86	−1.78±3.29	0.78
HCO_3^- (mmol/L)	Pre-HD	22.46±3.99	22.52±2.97	0.91
	Post-HD	27.27±3.01***	27.13±2.61***	0.74
	Pre−Post	−5.20±4.67	−4.60±3.03	0.29
pH	Pre-HD	7.38±0.06	7.40±0.06	0.08
	Post-HD	7.45±0.07***	7.45±0.06***	0.93
	Pre−Post	−0.07±0.07	−0.06±0.05	0.16
URR (%)		59.39±11.24	58.83±12.62	0.74
Kt/V		1.12±0.34	1.08±0.34	0.41
UFV (mL)		1913.43±966.76	1690.16±950.55	0.10

The variables were analyzed with the t-test between two groups and with a paired t-test before and after dialysis in each group. Intra-group comparison with pre-dialysis: *** P<0.001, ** P<0.01. All data are presented as mean±SD. Pre-HD: pre-hemodialysis; Post-HD: post-hemodialysis; Pre−Post: difference between pre-HD and post-HD; RCA: regional citrate anticoagulation; Hb: hemoglobin; PLT: platelet; INR: international normalized ratio; PT: prothrombin time; APTT: activated partial thromboplastin time; iCa^{2+}: ionized calcium; Na^+: sodium ion; HCO_3^-: bicarbonate ion; URR: urea reduction ratio; Kt/V: urea clearance index; UFV: ultrafiltration volume; SD: standard deviation.

could not be popularized due to the risks of hypocalcemia or metabolic alkalosis. In recent decades, citrate anticoagulation has been widely used in CRRT, its safety and effectiveness have been fully verified, and citrate has been increasingly applied in IHD. Citrate forms calcium citrate through free calcium in the extracorporeal circulation plasma of the complex, which reduces the level of free calcium (coagulation factor IV) in vitro, preventing the conversion of prothrombin to thrombin.

In this study, the uniform and constant initial blood flow was 150 mL/min, and the infusion rates of 0.04 g/mL citrate from the start of the dialysis line during blood draining and venous expansion chamber were 240–260 mL/h and 80–100 mL/h, respectively. The changes of calcium and sodium ions and alkali load caused by 0.04 g/mL citrate maintained a relatively constant predicted value. The iCa^{2+} concentrations at the dialyzer inlet were 0.31 and 0.32 mmol/L after 1 and 3 h of dialysis, respectively, reaching the anticoagulant target range of 0.25–0.35 mmol/L. The concentrations of iCa^{2+} at the dialyzer outlet were 0.92 and 0.91 mmol/L after 1 and 3 h of dialysis, respectively, which were close to the arterial iCa^{2+} level. This was the key to achieving one-stage RCA at the venous expansion chamber. Therefore, sodium citrate was injected at the venous expansion chamber at 80–100 mL/h to achieve full anticoagulation in all peripheral circuits.

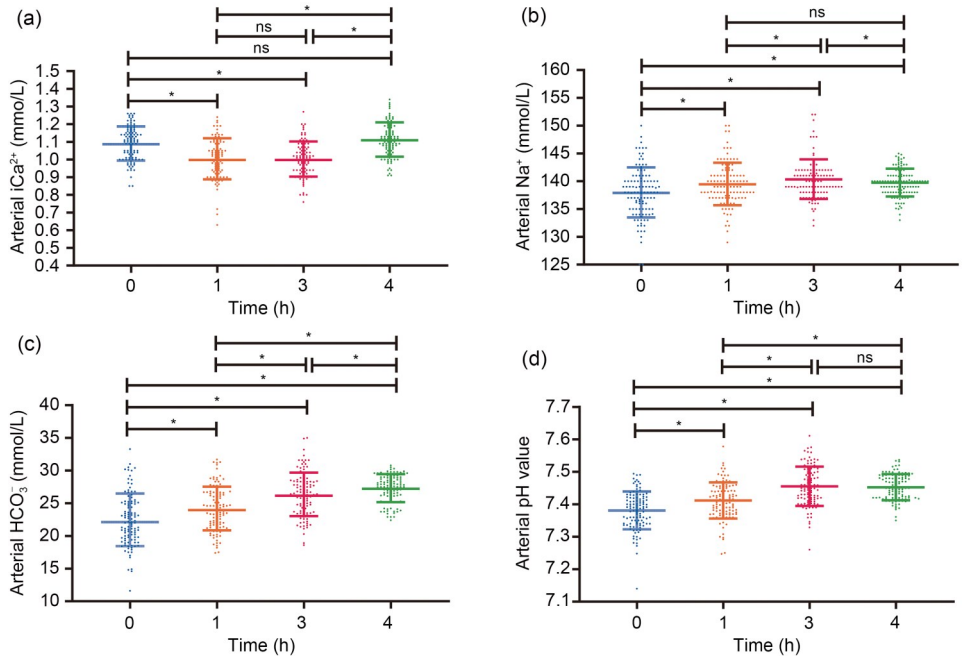

Fig. 7 Concentrations of iCa^{2+} (a), Na^+ (b), HCO_3^- (c), and the pH value (d) trend from the beginning to the end of dialysis in the RCA group. Comparisons of iCa^{2+}, Na^+, HCO_3^-, and pH value at different time points using repeated measurement analysis of variance (ANOVA). * P<0.05 is defined as statistical difference and ns indicates no significant statistical difference. RCA: regional citrate anticoagulation; iCa^{2+}: ionized calcium; Na^+: sodium ion; HCO_3^-: bicarbonate ion.

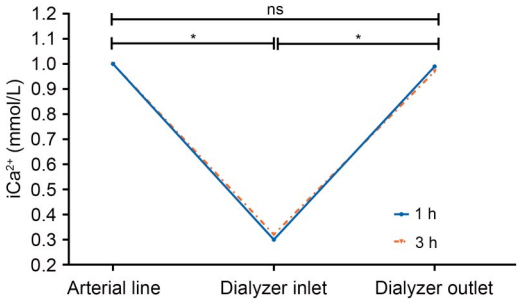

Fig. 8 Course of ionized calcium (iCa^{2+}) levels at different sampling sites (arterial line; dialyzer inlet, and dialyzer outlet) during citrate dialysis at 1 and 3 h. All values are presented as mean. * P<0.05 was defined as statistical difference, and ns indicates no significant statistical difference.

The severe clotting rates of the arterial expansion chamber, the dialyzer, and the venous expansion chamber were 0%, 0.91%, and 2.73% respectively, which meant that the ideal anticoagulant effect had been reached.

Contrary to our results, Evenepoel et al. (2002) conducted 203 treatments on 45 patients in a cross-over study and found that the clotting rate in the dialyzer was 8.87%. Buturovic-Ponikvar et al. (2008) found that the clotting rate in the dialyzer was 16% in a randomized trial, and 24% of the patients exhibited a change in the venous line. These contrasting results are related not only to the difference in citrate dosage but also to the different methods of citrate infusion. In the studies by Evenepoel et al. (2002) and Buturovic-Ponikvar et al. (2008), citrate was infused at the arterial end, while in our study, we infused citrate at the arterial line and venous expansion chamber. By changing the infusion method with a two-stage regional citrate infusion, a better anticoagulation effect can be achieved in vitro, as reported by Lin et al. (2019). In their randomized controlled study, the infusion velocities of 0.04 g/mL citrate before blood pumping and at the venous expansion chamber were 170 and 50 mL/h, respectively. In contrast with our study, Lin et al. (2019) used a smaller dose of citrate; however, the incidence of treatment interruption due to severe clotting after dialysis was 3.03%, slightly lower than the 3.64% reported in our study, and was ultimately related to the use of sequential anticoagulation. In the RCA group, only two cases (2/110, 1.82%) were interrupted due to clotting in the first 3 h, and the other two cases (2/110, 1.82%) occurred without anticoagulant within 1 h.

In our study, the total dose of citrate was 320–360 mL/h. Whether there was risk of citrate accumulation is a valid question. The majority of the 240–260 mL/h blood transfused before the blood pump

had been removed by the dialyzer via diffusion. The remaining 80–100 mL/h blood transfusion from the venous expansion chamber participated in the tricarboxylic acid cycle and was metabolized in the liver, skeletal muscles, and other parts of the body. Throughout the dialysis process, the concentration of arterial line calcium ions is maintained above 1.0 mmol/L, indicating that citrate can be completely metabolized at this dose. To increase the safety of citrate treatment and eliminate the potential hidden dangers of citrate accumulation, the sequential anticoagulation of citrate was performed 1 h before the end of the dialysis process (Buturović-Ponikvar et al., 2005; Buturovic et al., 2008). Before the patient could experience citrate accumulation, metabolic alkalosis, and hypernatremia, these would have been resolved immediately, as confirmed by the research results. The concentrations of bicarbonate and sodium ions showed a gradual upward trend within the first 3 h. Within 3 h, 15 cases (13.64%) of >30 mmol/L bicarbonate and 7 cases (6.36%) of >135 mmol/L sodium were reported. After 3 h, anticoagulant-free dialysis was performed. Subsequently, the concentrations of bicarbonate and sodium ions did not exceed the normal raised limit. In the previous studies of Buturović-Ponikvar et al. (2005) and Buturovic et al. (2008), the authors adopted sequential anticoagulation with citrate and shortened the time of anticoagulant-free dialysis to half an hour. After dialysis, the citrate concentration decreased to the level before dialysis, but the time required to correct possible adverse reactions such as metabolic alkalosis was not considered.

The most common complication of citrate was hypocalcemia. In our study, calcium-containing dialysate was used, and calcium ions diffused from the dialysate to the blood, increasing blood iCa^{2+}. Most patients did not need extra calcium supplementation, except for five patients (4.55%). Another two patients (1.82%) had arterial iCa^{2+} concentrations of 0.89 and 0.93 mmol/L after 1 h, respectively, accompanied by perioral and facial numbness. In their case, citrate infusion was stopped, and the calcium-containing dialysate concentration was adjusted to 1.5%, with dialysate flow to 500 mL/h and blood flow to 220–250 mL/min. Their symptoms were relieved after approximately 10 min. When mild hypocalcemia occurred during the RCA, the dialysate calcium concentration and related parameters could be adjusted online, and the therapeutic effect could be achieved without intravenous calcium infusion, which further simplified the treatment process.

In the present study, although the blood and dialysis flow rates were lower in the RCA group than in the saline flushing group 3 h before dialysis, the Kt/V was not statistically significant between the two groups, which was attributed to the better anticoagulant effect in the RCA group, the lower incidence of dialyzer coagulation, the larger effective membrane area of the dialyzer, and the longer dialysis time. However, the failure of solute clearance in the RCA group to meet the requirements of dialysis adequacy is related to the fact that in this study, we paid more attention to treatment safety, and when the patient's bleeding risk disappeared, the dialysis adequacy could be increased by changing the mode of anticoagulation and dialysis.

Our study has certain limitations. Firstly, the sample size of patients to be included in the study was small, which may have caused data bias. Therefore, data based on larger sample size need to be analyzed in further studies. Secondly, our study targeted dialysis patients receiving short-term RCA treatment, and dialysis adequacy for long-term patients was not considered, which will be explored in our follow-up experimental research.

5 Conclusions

The use of standard calcium-containing dialysate for two-stage infusion of citrate combined with sequential anticoagulation in IHD can significantly reduce the occurrence of coagulation events in the extracorporeal circulation, and has no serious adverse effects; thereby it is safe, effective, and suitable for widespread clinical use.

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Author contributions

Ling ZHANG designed the trial and revised the manuscript. Xiaoyan TANG collected and analyzed the data, and wrote the manuscript. All authors contributed to the conduct of the trial and data collection. All authors have read and approved the final manuscript, and therefore, have full access to

all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Xiaoyan TANG, Dezheng CHEN, Ling ZHANG, Ping FU, Yanxia CHEN, Zhou XIAO, Xiangcheng XIAO, Weisheng PENG, Li CHENG, Yanmin ZHANG, Hongbo LI, Kehui LI, Bizhen GOU, Xin WU, Qian YU, Lijun JIAN, Zaizhi ZHU, Yu WEN, Cheng LIU, Hen XUE, Hongyu ZHANG, Xin HE, Bin YAN, Liping ZHONG, Bin HUANG, and Mingying MAO declare that they have no conflict of interest.

The ethical approval of this research protocol was obtained from the institutional review boards of the participating hospitals, including West China Hospital of Sichuan University (Project No. 2019 Annual Review (530)), the First Affiliated Hospital of Guangxi Medical University (Project No. 2020 Rapid Approval (035)), Xiangya Hospital of Central South University (Project No. Good Clinical Practice (GCP) Rapid Approval 202008263), Wuhan No. 1 Hospital (Project No. (2020)22), People's Hospital of Jianyang City (Project No. 2020042), Ziyang People's Hospital (Project No. 2020-K-2-11), Ya'an People's Hospital (Project No. 202020), Chengdu Kangfu Kidney Disease Hospital (Project No. 2019530), and the First People's Hospital of Liangshan Yi Autonomous Prefecture (Project No. 2020068). The trial was registered by the China Clinical Trial Registry with the identification of ChiCTR1900027425. The study has obtained written informed consent from all subjects, and it conforms to the declaration of the World Medical Association Declaration of Helsinki and the code of Good Clinical Practice (GCP).

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