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Simplified Citrate Anticoagulation for CRRT Without Calcium Replacement

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Since 2012, citrate anticoagulation is the recommended anticoagulation strategy for continuous renal replacement therapy (CRRT). The main drawback using citrate as anticoagulant compared with heparin is the need for calcium replacement and the rigorous control of calcium levels. This study investigated the possibility to achieve anticoagulation while eliminating the need for calcium replacement. This was successfully achieved by including citrate and calcium in all CRRT solutions. Thereby the total calcium concentration was kept constant throughout the extracorporeal circuit, whereas the ionized calcium was kept at low levels enough to avoid clotting. Being a completely new concept, only five patients with acute renal failure were included in a short, prospective, intensely supervised nonrandomized pilot study. Systemic electrolyte levels and acid–base parameters were stable and remained within physiologic levels. Ionized calcium levels declined slightly initially but stabilized at 1.1 mmol/L. Plasma citrate concentrations stabilized at approximately 0.6 mmol/L. All postfilter ionized calcium levels were <0.5 mmol/L, that is, an anticoagulation effect was reached. All filter pressures were normal indicating no clotting problems, and no visible clotting was observed. No calcium replacement was needed. This pilot study suggests that it is possible to perform regional citrate anticoagulation without the need for separate calcium infusion during CRRT. *ASAIO Journal* 2015; 61:437–442.

Key Words: citrate, calcium, anticoagulation, hemodialysis, CRRT

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The first two authors contributed equally to this work.

G.G., M.B., B.K., K.S., O.C., J.S., and A.W. conceived and designed the experiments; M.B. and B.K. performed the experiments; G.G., M.B., B.K., and O.C. analyzed the data; and G.G., M.B., B.K., K.S., and O.C. wrote the paper.

Disclosure: Drs. Sandin, Carlsson, Wieslander, and Sternby are employees at Gambro Lundia AB, Lund, Sweden. Dr. Godaly was an employee at Gambro Lundia AB, Lund, Sweden, when the clinical trial was performed.

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According to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, regional citrate anticoagulation (RCA) is the recommended anticoagulation method for patients, without contraindications for citrate, undergoing continuous renal replacement therapy (CRRT).[1] Citrate has many advantages compared with heparin regarding bleeding risk,[2] filter survival,[2] and patient survival.[3] It is also suitable for patients with heparin-induced thrombocytopenia. The anticoagulation effect is obtained by the ability of citrate to complex-bind calcium. The dialysis solution contains no calcium, and calcium is consequently lost over the dialysis membrane during dialysis. Thus, calcium needs to be replaced (see **Figure 1A** for a theoretically calculated concentration distribution of calcium along the filter). The therapy requires monitoring of calcium levels (ionized calcium [iCa] in the circuit and iCa and total calcium systemically) and handling of calcium replacement. Therefore, citrate increases the complexity of the treatment, and a strict individualized protocol is needed.[1]

Our hypothesis is that calcium infusions can be omitted in citrate CRRT by keeping the total calcium concentration constant in the whole extracorporeal circuit, whereas iCa is kept at low levels enough to avoid clotting (**Figure 1B**). This can be achieved by including both citrate and calcium in the anticoagulation solution, the dialysis solution, and the replacement solution.

A pilot clinical trial was performed to investigate the hypothesis. The primary aim of this study was to investigate whether we could achieve sufficient anticoagulation without supplementation of calcium other than that provided by the study solutions. Secondary aims were to evaluate the impact of this new method on vital physiologic parameters in critically ill patients and assess the effect on acid–base balance.

Materials and Methods

Ethical Statement and Trial Registration

The Regional Ethical Review Board, Lund University, Sweden, approved this study (2011/624). Patients or next-of-kin consented to participation. The trial received the EudraCT code number 2010-018553-35 and was registered at https://www.clinicaltrialsregister.eu/

Study Design

For safety reasons, this pilot clinical trial of a completely new concept included only a few patients (up to 10) treated for 5 hours. The patients were subjected to intense monitoring including blood sampling, and a physician present bedside. Patients undergoing CRRT at the general intensive care unit at Skane University Hospital in Lund, Sweden, with acute renal failure

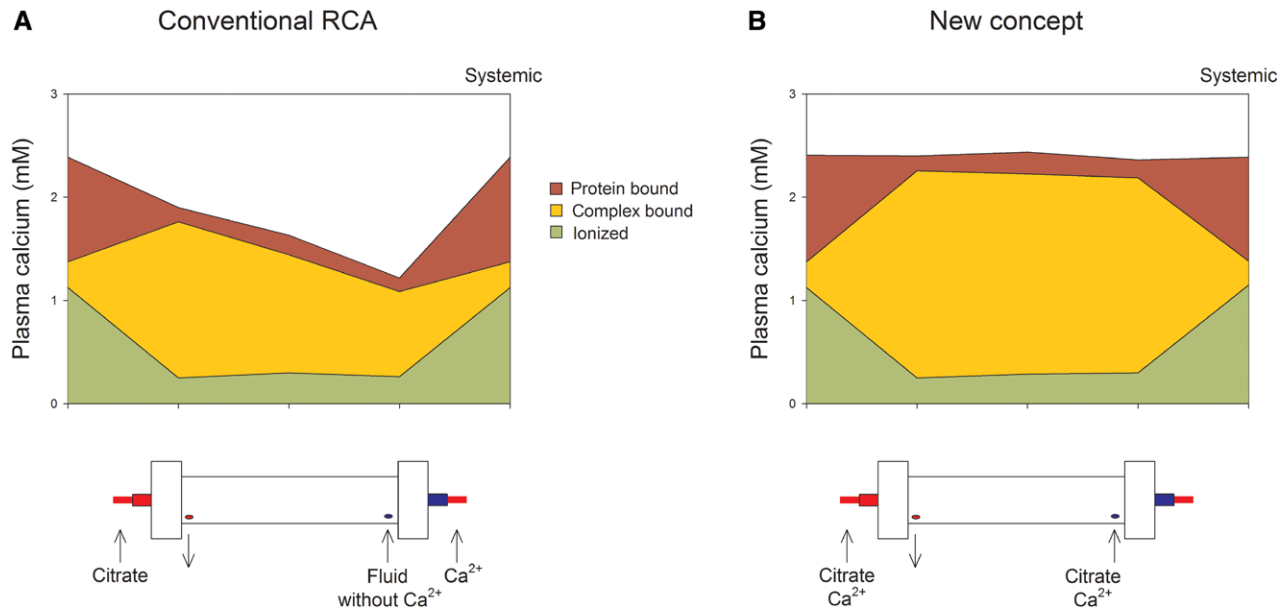

Figure 1. The distribution of the different forms of calcium in plasma for (**A**) conventional citrate anticoagulation and (**B**) the new concept. To the left in each panel is systemic distribution, in the middle the distribution in the dialysis membrane, and to the right the distribution in blood when the circuit blood is returned to the patient. For the new concept, the total calcium concentration is kept constant although the distribution is affected. Because no calcium is lost through the filter, there is no need for calcium replacement. RCA, regional citrate anticoagulation. full color online

of risk, injury, failure, loss, and end-stage kidney disease criteria class F were recruited to the study between February 29 and May 22, 2012. Patients were excluded if they were younger than 18 years, were under guardianship, had chronic kidney disease, had HIV and/or hepatitis B or C, and participated in other studies during the study period that could affect the study outcome. The patients were monitored during at least 10 hours of CRRT before inclusion to assure the stability of vital physiologic parameters and a well-functioning central dialysis catheter and were returned to conventional treatment after the study period (**Figure 2**). Nutrition was given enteral and/or parenteral to hemodynamically stable patients during the study period. Study dialysis details are shown in **Figure 3**. The prescribed dialysis doses per kilogram of body weight and hour (not corrected for predilution) were 27–53 mL/kg/hr (mean 35), depending on the patient weight and prescribed weight loss. The study products were produced and sterile filtrated at APL in Umeå, Sweden.

Clinical Parameters

During treatment with the study fluids, blood gases were monitored at 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 hours; basic chemistry and hematology were measured at 0, 2, and 5 hours of the study treatment; and plasma citrate was measured at 0, 1, 2, 3, and 4 hours. Blood samples were analyzed at the Laboratory for Clinical Chemistry (Skane University Hospital, Lund, Sweden). Plasma citrate levels were analyzed using spectrophotometry (Labor Limbach, Heidelberg, Germany). Stewart's equation developed by Fencl *et al.*[4] and verified by Schück and Matousovic[5] was used to calculate the strong ion difference.

Statistical Analysis

Data are presented as average ± standard deviation. Data not normally distributed are presented as median (range). SigmaPlot (Systat Software Inc., San Jose, California) for Windows version 11.0 was used for statistical analysis. The statistical difference between baseline and treatment periods was investigated using one-way repeated measures analysis of variance followed by Dunn's method for normally distributed data. For data not normally distributed, Friedman repeated measures analysis of variance on ranks followed by Dunnett's method was used. Differences were considered significant at $p < 0.05$ (***$p \leq 0.001$, **$p < 0.01$, *$p < 0.05$, ns = not significant).

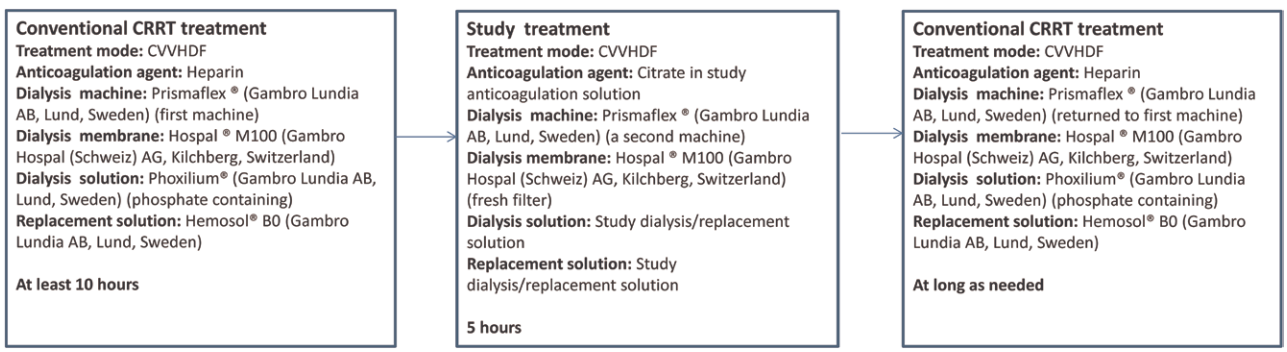

Figure 2. Flow chart of the study design. CRRT, continuous renal replacement therapy; CVVHDF, continuous venovenous hemodiafiltration.

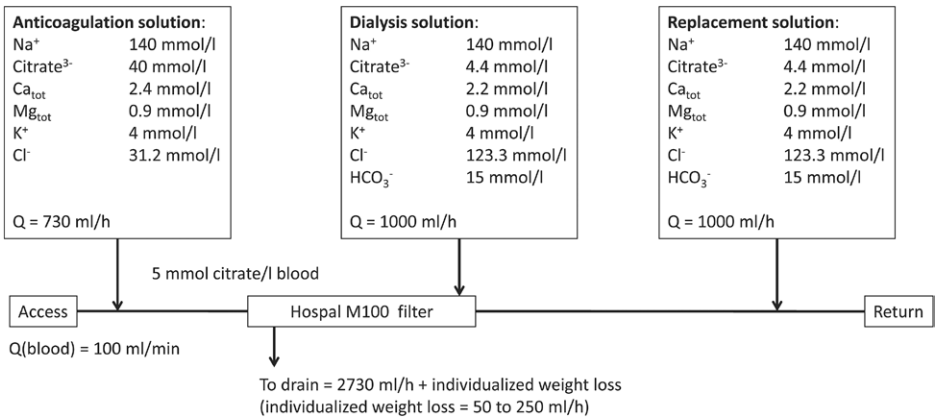

Figure 3. Schematic presentation of flow rates and compositions of the study solutions.

Results

Patient Clinical Characteristics

According to the protocol, up to 10 patients were planned to be included in the study. Thirteen patients undergoing CRRT were screened, and five were found eligible. All five patients completed the study. Demographic data of patients before treatment are shown in **Table 1**. The patients' medications remained unchanged during the study treatment. All patients survived the study treatment. One patient died 2 days after the study period at the general intensive care unit, and one patient died 1 month later at the ward without leaving the hospital; none of these related to the study.

Plasma Citrate, Calcium Homeostasis, and Clotting

As expected, plasma citrate levels increased during the first hour of treatment but remained stable at approximately 0.6 mmol/L thereafter, explaining the initial dip in iCa (**Figure 4**). The patients had low levels of total calcium concentration, initially and throughout the study (**Table 2**), probably because of low albumin values (**Table 3**). Postfilter iCa values were stable at approximately 0.37 mmol/L (**Table 2**). All filter pressures were normal, indicating no clotting problems. After the 5 hour study period, dialysis was stopped and blood returned to the patient. The patient was thereafter connected to another dialysis machine with a fresh filter. A visual inspection of the used filter was performed at this time, showing no visible clotting.

Acid–Base Parameters and Plasma Electrolytes

No alkalosis or acidosis occurred during the study period; pH, carbon dioxide partial pressure (pCO_2), and bicarbonate (HCO_3^-) concentrations, as well as the calculated acid–base parameters, remained stable (**Table 3** and **Figure 5**). Plasma concentrations of Na^+, K^+, Mg^{2+}, and Cl^- remained stable (**Table 3**). Phosphate (P^-) concentrations decreased significantly at the start of the treatment but remained stable thereafter (**Table 3**).

Adverse Events

No adverse events or serious adverse events occurred.

Discussion

Since the first report in the early 90s, in patients undergoing CRRT, RCA has gained interest.[6] Citrate has been associated with longer circuit life, less bleeding, and possibly better patient and kidney survival compared with heparin.[7–9] Despite the beneficial effects of citrate, RCA is often perceived as complex and associated with high risk for metabolic derangements.[10] These concerns are based on the cumbersome protocols and laborious monitoring. For the first time, we have designed a new solution system for citrate anticoagulation, where calcium and citrate together with electrolytes at physiologic concentrations aim at maintaining the extracorporeal anticoagulation, as well as patient calcium homeostasis, and electrolyte balance.

Being a first clinical test of a new regime, this pilot study was performed on stabilized patients to reduce risk. Therefore, the

Table 1. Demographic Data

Patient	Diagnosis	Age	Sex	Weight (kg)	SAPS III*	APACHE II*	Diabetes
1	Cardiac insufficiency including multiorgan failure	60	F	110	74	16	No
2	Septic shock	67	M	96	69	27	Yes
3	Septic shock	80	M	92	90	25	No
4	Septic shock	61	M	83	64	16	No
5	Unspecified rheumatic disease exacerbation including multiorgan failure	37	F	55	61	22	Yes

*Simplified acute physiology score (SAPS) III and acute physiology and chronic health evaluation (APACHE) II scores at the admission to the general intensive care unit.

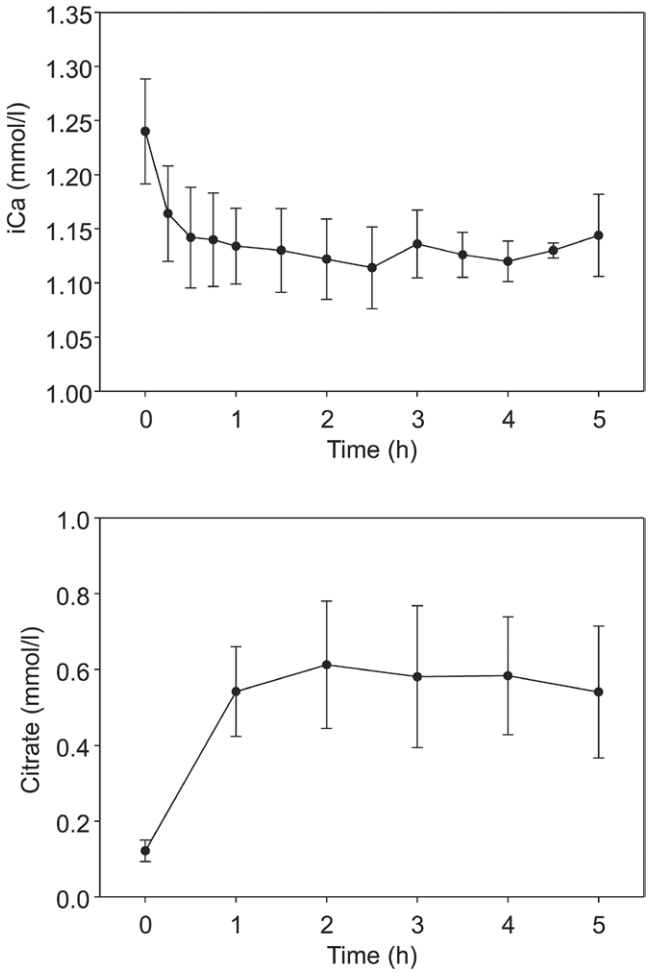

Figure 4. Plasma ionized calcium (iCa) and citrate concentrations (peripheral blood levels) during the study treatment.

patients were well heparinized when introduced to the study products although this effect declined during the study (**Table 2**). The iCa concentration was kept less than 0.5 mmol/L in the extracorporeal circuit during the entire study period, indicating that sufficient anticoagulation would have been achieved irrespective of the presence of heparin.[11]

Systemic iCa levels declined initially but stabilized after approximately 1 hour and ended up at 1.14 ± 0.04 mmol/L after 5 hours of treatment. There were no instances of clinically

Table 2. Calcium Homeostasis and aPTT

	Time (hr)			
	0	2	5	*p**
Ca_{tot} (mmol/L)	2.15 ± 0.15	2.22 ± 0.11	2.28 ± 0.09	0.171
iCa (mmol/L)	1.24 ± 0.05	1.12 ± 0.04	1.14 ± 0.04	0.022
Ca_{tot}/iCa ratio	1.74 ± 0.13	1.97 ± 0.11	1.96 ± 0.06	0.017
$iCa_{extracorporeal}$ (mmol/L)†	0.37 ± 0.04	0.37 ± 0.03	0.38 ± 0.04	0.409
aPTT‡	60.2 ± 28.2	40.0 ± 4.74	38.8 ± 3.96	0.179

*Significance of differences between values at the beginning and at the end of the treatment.
†Measured postfilter.
‡Normal reference value from local laboratory is 22–44.
aPTT, activated partial thromboplastin time; iCa, ionized calcium.

Table 3. Concentrations of Plasma Electrolytes and Acid–Base Parameters

	Normal Values*	Start	End	*p*†
Na^+ (mmol/L)	137–145	137 ± 3	136 ± 3	0.667
K^+ (mmol/L)	3.5–4.4	4.3 ± 0.5	4.6 ± 0.5	0.358
Mg^{2+} (mmol/L)	0.70–0.95	0.9 ± 0.1	0.9 ± 0.1	0.421
Cl^- (mmol/L)	98–110	107 ± 3	106 ± 2	0.379
P^- (mmol/L)	0.7–1.5	0.9 ± 0.03	0.7 ± 0.1	0.008
Alb (g/L)	36–48	27 ± 5	27 ± 3	0.874
Lactate (mmol/L)	0.5–1.6	1.3 (0.9–1.7)	1.1 (0.5–1.7)	0.500
pH	7.35–7.45	7.42 ± 0.04	7.43 ± 0.05	0.709
pCO_2 (mm Hg)	34.6–45.1	37 ± 3	36 ± 3	0.646
CO_{3-} (mmol/L)	22–27	24.1 ± 2.6	24.6 ± 1.8	0.773
BE (mmol/L)	−3.0 ± 3.0	−0.3 ± 3	−0.2 ± 2	0.980
$AG_{measured}$ (mmol/L)	16 ± 2	10 ± 4	10 ± 2	0.813
AG_{corr}‡ (mmol/L)	16 ± 2	14 ± 3	15 ± 1	0.794
SID (mmol/L)	39 ± 1	35 ± 4	36 ± 2	0.607
Cl^-_{corr}‡ (mmol/L)	98–110	111 ± 4	110 ± 2	0.665
UA^- (mmol/L)	8 ± 2	3 ± 3	3 ± 1	0.702
$UA-_{corr‡}$ (mmol/L)	8 ± 2	−1 ± 5	−1 ± 3	0.991
A^-_{tot} (mmol/L)	15	9 ± 1	9 ± 1	0.841

*Normal values from healthy controls[4] and reference values at Clinical Chemistry Laboratory, Skane University Hospital, Lund, Sweden.
†Significance of differences between values at the beginning and at the end of the treatment.
‡Corrected for water excess/deficit.
AG, anion gap; SID, strong ion difference; UA, undetermined anions.

significant hypocalcemia or hypercalcemia, and no adjustment of the flow rates of the study solutions was needed.

In this study, a fixed dose of citrate (5 mmol/L) in relation to blood flow was used. This is higher than the recommended target at 3 mmol/L for conventional RCA,[12] albeit no significant systemic effect was noticed. When calcium is included in the dialysis solution, a higher concentration of citrate is needed in the circuit to maintain the iCa on a level where clotting is prevented.

Citrate accumulation can occur if the citrate metabolism is insufficient,[13,14] indicated by a total Ca/iCa ratio more than 2.25.[15] The ratio stabilized at 1.97 after 2 hours, that is, well less than 2.25. Plasma citrate reached a stable concentration of 0.4–0.7 mmol/L, confirming no accumulation in the patients. The citrate concentrations are comparable with the previously published data on patients treated with RCA; a plasma citrate concentration of 1.04 ± 0.46 mmol/L in 23 patients[16] and a citrate concentration of 0.69 ± 0.28 in 5 children receiving continuous venovenous hemofiltration (CVVH) or continuous venovenous hemodiafiltration (CVVHDF).[17]

The amount of bicarbonate in the fluids needs to be adjusted to compensate for bicarbonate generated in the metabolism of citrate received from the anticoagulation solution. Both measured and calculated acid–base parameters remained stable during the study, indicating that the study solutions were well balanced regarding citrate and bicarbonate. In case the citrate solution does not contain a physiologic concentration of other ions, for example, sodium, this need to be adjusted in the dialysis and/or replacement solution.

The patients received phosphate-containing solution (Phoxilium, Gambro Lundia AB, Lund, Sweden) before and after the study period. As no substitution of phosphate was carried out during the study period, plasma phosphate levels were significantly decreased although the mean value was not less than the normal reference value after the study period. Because the study was performed for only 5 hours, the decrease in

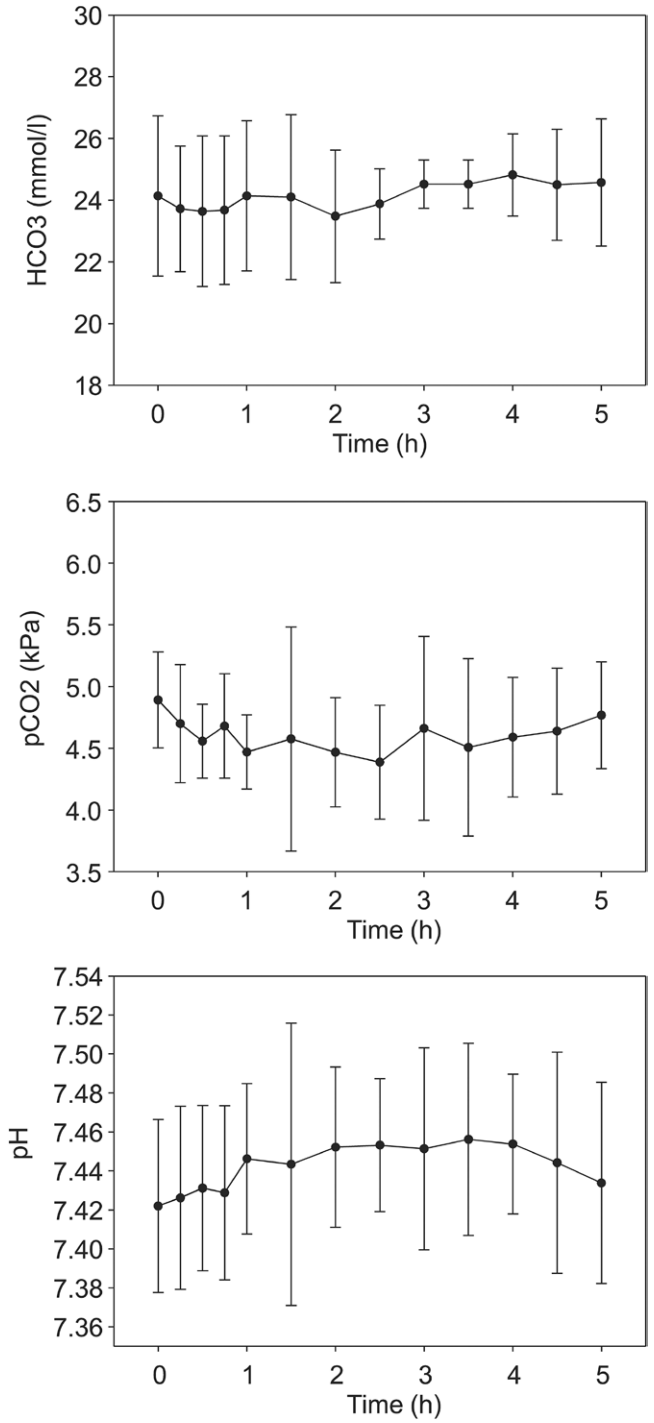

Figure 5.. Values of HCO_{3-}, pCO_2, and pH during study treatment.

phosphate concentration did not constitute a problem. However, this indicates that phosphate should be included in future solutions.

Limitations of our study include the safety precautions; a small number of patients, the short study period, and that the patients were stabilized before inclusion. Despite this, verification of our hypothesis was possible.

Regional citrate anticoagulation dialysis is normally performed with calcium-free dialysis solutions, and the calcium that is lost into the effluent is replaced by a separate calcium infusion. In this pilot study, we investigated a novel concept for citrate anticoagulation with the aim of eliminating the need for calcium replacement. Not properly performed, calcium infusion represents an increased risk for the patient; furthermore, calcium supplementation increases the complexity and the cost of the treatment.

Some authors have previously used calcium-containing solutions in RCA,[16–20] in some cases with the aim to reduce or omit calcium infusions. In the study performed by Gupta *et al*.,[18] it was reported that the need for calcium replacement was reduced in comparison with the studies using calcium-free dialysis solution,[21] although the systemic iCa was significantly decreased during the treatment and the mean iCa after 48 hours of treatment was 0.87 mmol/L. This can be compared with 1.14 mmol/L noted in our study, which is a physiologically normal value. In the study performed by Mitchell *et al*.,[20] continuous calcium supplementation could be avoided in 14 of 19 patients. However, filter survival was impaired compared with filter survival reported in other studies,[6,21] maybe because of the calcium-containing dialysis fluid.[20] Studies on chronic hemodialysis patients using calcium-containing dialysis solutions report a reduced need of calcium replacement, although with a high incidence of venous bubble trap clotting.[22,23] A new protocol for RCA in CRRT was recently developed by Ong *et al*.[24] to omit calcium infusions. A few important differences can be noted between our concept and the concept of Ong *et al*. The flexibility when it comes to treatment modalities is higher in our system because it is possible to run CVVH, continuous venovenous hemodialysis (CVVHD), and CVVHDF, whereas the protocol of Ong *et al*. concerns only CVVH. Also, the concept from Ong *et al*. shows inflexibility in flow rates except for adjustment of calcium balance. Besides, there may also be a risk for clotting in the system after the infusion of calcium because iCa concentration is brought back to normal level in the blood returned to the patient.

Our protocol aims to achieve control of the calcium balance by maintaining the total calcium concentration constant through the extracorporeal system. Szamosfalvi *et al*.[25] have developed a protocol for 24 hour sustained low-efficiency dialysis–RCA, where they use a diametrically different approach to achieve control of the calcium balance; a high dose of citrate is infused in the blood, all calcium and citrate are removed through the filter, and an accurate calcium compensation is infused before blood return. The concentration of iCa is always <0.25 mmol/L; hence, no monitoring of postfilter iCa is performed. Because citrate concentration is low in the blood returned to the patient, the protocol is feasible also for patients with impaired citrate metabolism. One advantage with our system is that iCa is low through the whole circuit, whereas in the protocol of Szamosfalvi, iCa is increased to normal levels before return to the patient, with a subsequent risk of clotting. All our fluids are in balance regarding calcium and acid–base status; hence, nothing fatal will happen in our system if any solution flow rate would accidentally be incorrect, for example, due to empty containers, whereas the Szamosfalvi system is probably more sensitive to incorrect flow rates.

In the concept presented here, only two different solutions are needed compared with normal RCA, which requires up to three different fluids plus a separate calcium infusion. The need for monitoring calcium levels could possibly be significantly

reduced with our concept although this needs to be evaluated further. In summary, the presented novel concept succeeded in omitting continuous infusion of calcium, greatly simplifying citrate anticoagulation during CRRT.

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