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Deciphering simplified regional anticoagulation with citrate in intermittent hemodialysis: a clinical and computational study

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Regional citrate anticoagulation use in intermittent hemodialysis is limited by the increased risk of metabolic complications due to faster solute exchanges than with continuous renal replacement therapies. Several simplifications have been proposed. The objective of this study was to validate a mathematical model of hemodialysis anticoagulated with citrate that was then used to evaluate different prescription scenarios on anticoagulant effectiveness (free calcium concentration in dialysis filter) and calcium balance. A study was conducted in hemodialyzed patients with a citrate infusion into the arterial line and a 1.25 mmol/L calcium dialysate. Calcium and citrate concentrations were measured upstream and downstream of the citrate infusion site and in the venous line. The values measured in the venous lines were compared with those predicted by the model using Bland and Altman diagrams. The model was then used with 22 patients to make simulations. The model can predict the concentration of free calcium, bound to citrate or albumin, accurately. Irrespective of the prescription scenario a decrease in free calcium below 0.4 mmol/L was obtained only in a fraction of the dialysis filter. A zero or slightly negative calcium balance was observed, and should be taken into account in case of prolonged use.

Keywords Hemodialysis, Calcium, Citrate, Dialysate, Modeling, Anticoagulation

Abbreviation
CRRT Continuous renal replacement therapy

The European ERA-EDTA guidelines for chronic hemodialysis[1] and international KDIGO guidelines for hemodialysis during acute kidney injury[2] have made recommendations for anticoagulation of the blood circuit in intermittent hemodialysis to the effect that heparin or low molecular weight heparin should be used in the absence of hemorrhagic risk and no anticoagulation should be used in the case of hemorrhagic risk. The absence of anticoagulation entails the risk of impairing dialysis efficacy and premature discontinuation because of clotting. Alternatives have been proposed. It is now well established that repetitive blood circuit flushing or pre-dilution are ineffective. Coating membranes with heparin or vitamin E provides better results but is less effective than anticoagulation with heparin[3–5].

Citrate anticoagulation is a regional blood circuit anticoagulation technique currently used in routine practice for continuous renal replacement therapy (CRRT), where it is recommended regardless of the hemorrhagic

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risk[2]. It is based on the property of citrate molecules to chelate calcium ions in plasma. Calcium is necessary for the cascade activation of clotting factors. In addition to its anticoagulant properties, citrate helps to reduce the inflammatory response induced by hemodialysis[6]. A concentrated solution of sodium citrate is infused into the arterial line upstream of the dialysis membrane. A recent meta-analysis confirmed the advantage of citrate over heparin showing that citrate anticoagulation can reduce the incidence of bleeding and transfusion requirements during CRRT and increase the duration of use of the dialysis filter[7]. However, this technique has the drawback of requiring close biological monitoring, especially with intermittent hemodialysis, in which solute exchanges are faster. An alternative technique has been proposed involving use of a calcium-free dialysate with or without citrate and reinfusion of a calcium-containing solution into the venous line. The amount of calcium ions lost in the dialysate is estimated as being equal to the product of the dialysance and the patient's free calcium. The calcium infusion rate is readjusted regularly during the session according to dialysance value[8–11].

To simplify the procedure and guarantee a safe use in intermittent hemodialysis, a regional citrate anticoagulation technique using a dialysate containing calcium has been developed. It ensures that the patient's serum calcium level is maintained by establishing a balance between the calcium concentration of the dialysate and the patient's free serum calcium. In addition, the quantity of citrate returning to the patient and having to be metabolized in the liver is significantly reduced by elimination of the majority of citrate calcium complexes in the effluent dialysate. Evenopoel et al.[12] showed that this simplified technique was safe, effective and easier to implement than with a calcium-free dialysate. Fiaccadori et al.[13] showed that the use of a dialysate supplemented with calcium in patients treated with slow extended hemodialysis was an effective alternative to dialysate without calcium and compensation by post-filter calcium infusion. We recorded the results of its use in our department and found it was easy to implement, had stable dialysis efficacy and safety but could be associated with an increased risk of circuit clotting compared to heparin anticoagulation[14]. This manifest in premature stopping of dialysis and thus sub-optimal treatment to patients. Precise knowledge of the calcium and citrate concentrations in the dialyzer is essential to be able to optimize the technique. However, direct measurement is not possible in real practice.

In this context, the objectives of this study were to validate a mathematical model of intermittent hemodialysis using regional citrate anticoagulation and to use the model to study the impact of prescription parameters on anticoagulant effectiveness (reduction in free calcium in the blood circuit) and safety of use (patient calcium balance).

Material and methods

Population

A prospective study was conducted in the hemodialysis unit of the Clermont-Ferrand university hospital center, France, between January and December 2021. Patients aged over 18 years and treated with intermittent hemodialysis for renal failure were included. The non-inclusion criteria were severe hepatocellular insufficiency (prothrombin time < 50%, INR > 1.5 without anticoagulant), a hemoglobin level < 7 g/dL, and patients with psychiatric pathology or cognitive disorders. The study was carried out according to the Declaration of Helsinki, its protocol was approved by the Sud Ouest IV Research Ethics Committee (IORG009855) and registered at ClinicalTrials.gov (NCT04530175). All participants provided written informed consent.

Citrate anticoagulation protocol

Patients were treated with AK200 Gambro or Nikkiso DBB07 dialysis machines. Regional anticoagulation with citrate was carried out according to the protocol previously described[14]. Briefly, a 4% citrate solution (136 mmol/L) was infused into the arterial line of the blood circuit with a flow rate proportional to the blood flow (citrate 4% flow rate (mL/hour) = 1.75 × blood flow rate (mL/min)) in order to achieve a citrate concentration of 0.40 mmol per 100 mL of blood flow[14]. A dialysate with a calcium concentration of 1.25 mmol/L was used. The total ultrafiltration volume takes into account the infused volume of citrate solution (ultrafiltration volume = net ultrafiltration volume + infused volume of citrate solution). The blood and dialysate flow rates, the sodium, potassium and bicarbonate contents of the dialysate, and the net ultrafiltration volume were left to the discretion of the prescribing physician.

Measures

At H0, H1 and H4 a sample was taken in the arterial line upstream of the citrate injection site (patient), at H1 in the arterial line downstream of the citrate injection site (prefilter) and in the venous line (postfilter) (Supplementary Fig. S1). The samples were processed by a blood gas analyzer (GEM Premier 4000 Instrumental Laboratory®). Measurements were made of PO_2, SaO_2, PCO_2, pH and bicarbonate, hematocrit, sodium, potassium and free calcium. At H1, blood samples were taken from the patient and prefilter sites. The tubes were sent to the biochemistry laboratory of Clermont-Ferrand University Hospital for determination of total calcium, phosphate, magnesium, urea and albumin. Dry tubes with separator were centrifuged and the serum was collected (within 4 h after collection) and stored at − 20 °C. The frozen serum was sent to the biochemistry laboratory of Lille University Hospital for citrate measurement. Calcium balance ΔCa was calculated according to the following equations.

$$\Delta Ca = Ca_{totaloutlet} \times (1 - ht_{outlet}) \times Q_{bloodoutlet} - Ca_{totalintlet} \times (1 - ht_{inlet}) \times Q_{bloodinlet}$$

Calculations

The dissociation constants of calcium-citrate and calcium-albumin binding site complexes were estimated by nonlinear regression from measurements taken in patients at the prefilter and postfilter sites. The equation used gave the total serum calcium as the sum of the free calcium and calcium complexed with citrate or albumin.

$$c_{BCa_{tot}} = c_{BCa_i} + \frac{c_{BCa_i}}{c_{BCa_i} + K_{dCit}}.c_{BCit} + \frac{c_{BCa_i}}{c_{BCa_i} + K_{dalb}}.c_{Balb}$$

The same equation was used to calculate the concentrations of the five species in solution (free calcium, calcium-citrate, and calcium-albumin binding site, free albumin binding site and free citrate)[15] at the prefilter site. The rate of the association and dissociation reactions of the calcium-citrate and calcium-albumin binding site complexes were retrieved from literature data and adapted so that the ratio of the rate constants corresponded to the finding of dissociation at equilibrium[16].

Modeling

The mathematical model used to calculate the calcium concentration during intermittent hemodialysis treatment has been described in detail elsewhere[16]. The model comprises two parts. The first describes the flow of blood and dialysate through a dialysis filter fiber and is made up of three coupled partial differential equations, two Navier–Stokes equations modeling the flow of blood and the flow of dialysate, combined with a Darcy law describing the porous flow through the membrane. The second part of the model is a reaction-convection–diffusion system that describes the spatial evolution of the concentrations of the five chemical species in a fiber from the dialysis filter. The fluid speeds calculated by the first part of the model intervene in the convective term of the reaction-convection–diffusion system. In order to take into account that the albumin do not cross the membrane we consider a Robin type boundary.

condition on the interface blood/porous membrane:

$$D_{alb}\frac{\partial c_{alb}}{\partial n} = v.n.c_{alb}$$

These conditions produce the increase of the albumin concentration in blood proportional to the ultrafiltration flow rate. We were particularly interested in the concentrations in the blood at the outlet of the dialysis filter and the values of these concentrations at the blood/membrane interface. To numerically simulate this model we developed a calculation code based on the finite element method using the FreeFEM Language[17].

Statistical analysis

To study the agreement between the measured value and the values calculated by the model for total and free calcium, the number of subjects was calculated from the hypothesis of nullity of the Lin concordance coefficient, which makes it possible to study the intensity of the concordance that might exist between quantitative parameters. Thus, to detect a concordance coefficient r statistically of at least 0.7, it was necessary to include 20 subjects for a bilateral risk of 5% and a power of 90%. In view of these elements, we decided to recruit 30 patients. Statistical analyses were performed with R (version 3.6.3) and Graphpad software (version 8.0.0). The qualitative variables were expressed as numbers and associated percentages and the quantitative variables by means (± standard deviation) and medians (with range). Values of quantitative variables were compared by Student's t test for paired data or repeated measures ANOVA followed by post-hoc Bonferroni test (normality of distribution assessed by the Shapiro–Wilk test). To study the agreement between the measured values and the values calculated by the model, the results were represented in the form of a Bland and Altman graph. Bias (average of differences) and dispersion index (95% confidence interval of difference between measured and calculated value) values were calculated.

Results

Patient characteristics

A total of 30 patients were included. One patient had zero dialysate flow at the time of sampling, and for 7 others laboratory analyses were missing. Hence, final analysis was made of 22 patients, of whom 5 had zero citrate flow at the time of sampling. Their data were not used to describe the variations in calcium and citrate concentrations according to the citrate anticoagulation protocol but retained in the evaluation of the model of exchanges in the dialysis filter. The characteristics of the study population are summarized in Table 1. For the 17 patients who received citrate infusion, citrate 4% flow was 452 ± 31.6 mL/hour.

Evolution of calcium and citrate concentrations in the blood circuit

The measurement of free calcium after one hour of citrate infusion at the different sampling sites of the circuit is shown in Fig. 1A. The free calcium at the patient sampling site was 1.052 ± 0.1159 mmol/L: it decreased after pre-dialysis filter citrate infusion to 0.1979 ± 0.05782 mmol/L and rose postfilter to 0.8895 ± 0.1137 mmol/L. For each patient, free serum calcium was measured at the patient sampling site at different times of the dialysis session (H0, H1 and H4). The results are shown in Fig. 1B with 1.038 ± 0.081 mmol/L at H0, 1.035 ± 0.110 mmol/L at H1, and 1.010 ± 0.079 mmol/L at H4. There was no clinically relevant variation in patient free serum calcium during a session. Measurements of citrate concentrations at H1 at the pre- and postfilter sites are shown in Fig. 1C. The prefilter citrate concentration was 6369 ± 1510 µmol/L and was only 596.6 ± 295.9 µmol/L postfilter. The relationship between free calcium and citrate concentration is shown in Fig. 1D. A decrease in free serum

Characteristics	n = 22
Men n (%)	17 (77.2)
Age (years)	62.9 ± 11,4
Dialysis vintage (months)	3.8 [0.5–45.7]
Comorbidities n (%)	
Cardiovascular disease	20 (90.9)
Respiratory disease	8 (36.4)
Hepatopathy	6 (27.3)
Cancer	7 (31.8)
Diabetes	9 (40.9)
Tobacco	4 (18.2)
Dyslipemia	10 (45.5)
Dialysis duration (min)	228/23.6
Vascular access n (%)	
Tunneled catheter	6 (27.3)
Non-tunneled cathéter	8 (36.4)
Arteriovenous fistula	8 (36.4)
Hémodialysis machine n (%)	
DBB07 Nikiso®	10 (45.5)
AK200 Gambro®	12 (55.5)
Blood flow (mL/min)	259 ± 19.7
Dialysate flow (mL/min)	500 ± 0
Ultrafiltration rate (mL/h)	706 ± 258
Filter surface (m^2)	2.05 ± 0.11

Table 1. Patients characteristics.

calcium was observed with increasing citrate concentration. A citrate concentration of at least 3000 µmol/L was required to achieve a free calcium level below 0.4 mmol/L. Total calcium balance was − 1.72 ± 1.15 mmol/hour.

Modeling

The dissociation constants of calcium-citrate (K_d calcium-citrate) and the calcium-albumin binding site complex (K_d calcium-albumin) were estimated to be $4.034 \times 10^{-4} \pm 2.301 \times 10^{-5}$ and $7.073 \times 10^{-3} \pm 6.156 \times 10^{-4}$, respectively. The rate constants of the association or dissociation reactions of the calcium-citrate and calcium-albumin binding site complexes chosen are provided in supplemental data. The values of the concentrations of free calcium, free citrate, calcium-citrate, free albumin binding site and calcium-albumin binding site calculated from the measurements at the filter outlet and the values calculated by the model from the data at the dialysis filter inlet are shown in Table 2. The Bland and Altman diagrams comparing the concentration values measured and calculated by the model are presented in Fig. 2. The bias values and the corresponding 95% agreement interval are shown in Table 3.

Simulations

The model was used to conduct several simulations for a putative patient (supplemental data), with varying blood, dialysate, and citrate infusion flow rates. The proportion of fiber length for which the ionized calcium is < 0.4 mmol/L reflects the effectiveness of blood anticoagulation in the dialysis filter[18]. This proportion increases when blood flow increases or dialysate flow decreases. The results are reported in Fig. 3A. Total, free serum calcium, and calcium balance are given in Figs. 3B,C and D. All three decrease as blood flow increases, dialysate flow decreases or citrate infusion increases. Total calcium balance was − 1.37 mmol/h with a 250 mL/min blood flow, a 500 mL/min dialysate flow and a 5 mmol/L blood citrate concentration.

Our model was also used to compute a dialysis session using a citrate dialysate without calcium, and then a dialysate with neither citrate nor calcium. The results are given in Supplementary Figs. S2A–D and S3A-D. Of note, free calcium was below 0.4 mmol/L only in the last part of the filter. The losses of calcium ions in the dialysate are usually estimated to be equal to the product of dialysance (ionic[8] or dialysance measured by UV absorptiometry on the effluent dialysate[9] depending on the dialysis machine used) and the patient's free calcium. This estimation is based on two hypotheses. First, only free calcium and calcium complexed (mostly) with citrate can cross the membrane i.e. the release of calcium from albumin is negligible because it is thought to be too slow. This is equivalent to the situation of blood without albumin and a total serum calcium equal to the free calcium and calcium citrate. Secondly calcium citrate and free calcium have dialysance equal to that of small solutes like sodium or urea. However, literature data indicate a diffusibility of calcium citrate about twofold lower than that of calcium[19]. Using the model, we calculated the calcium balance for different blood and dialysate flow rates. For example, with a blood flow rate of 250 mL/min and a dialysate flow rate of 500 mL/min free calcium is

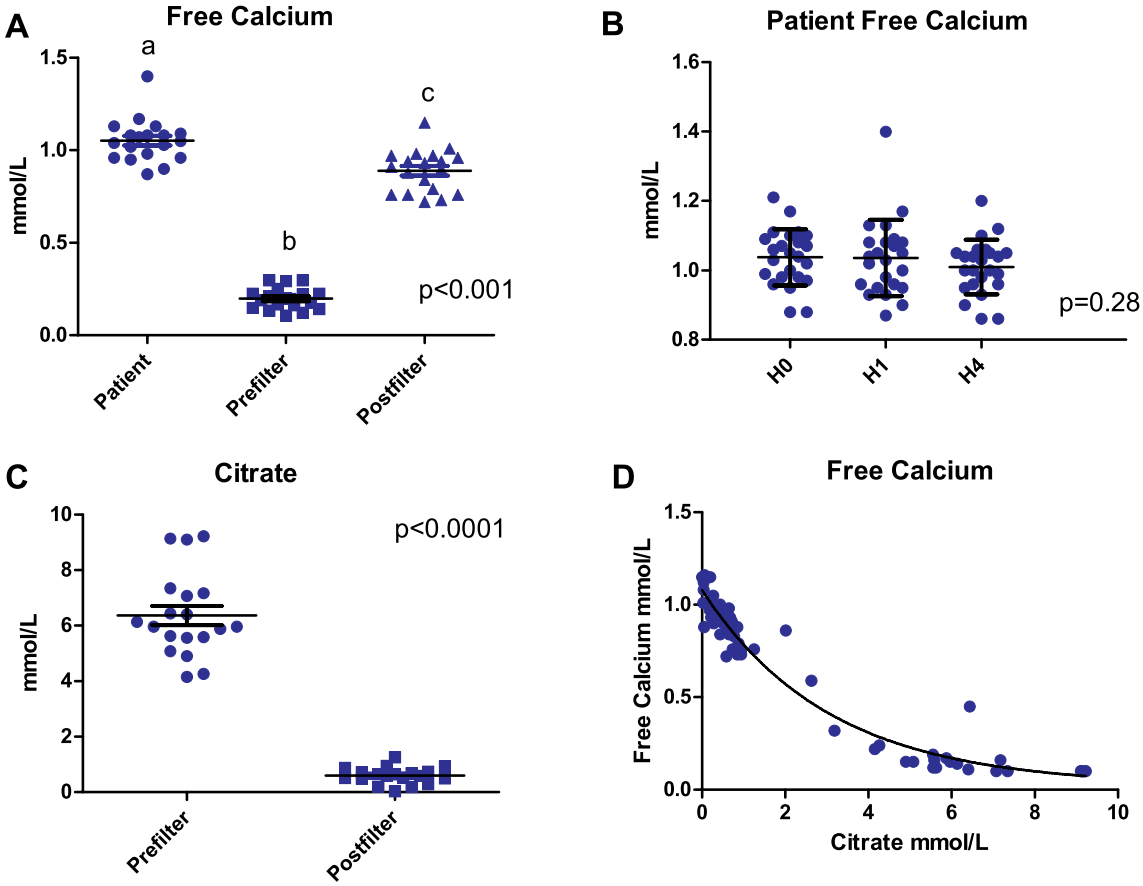

Fig. 1. (**A**) Free calcium measured in blood at H1 on the arterial line upstream of the citrate injection site (patient), on the arterial line downstream of the citrate injection site (prefilter) and on the venous line (postfilter). P-value of ANOVA for repeated measures. Values with different letters are significantly different with post-hoc Bonferroni test. (**B**). Patient free calcium measured at H0, H1 and H4. P-value of ANOVA for repeated measures. (**C**). Serum citrate concentration pre- and postfilter. P-value of paired T test. (**D**). Free calcium vs citrate concentration. Black line represents polynomial regression.

	Measured	Predicted
Free calcium (mmol/L)	0.923 ± 0.141	0.979 ± 0,160
Calcium-citrate (mmol/L)	0.344 ± 0.228	0.282 ± 0,167
Citrate (mmol/L)	0.165 ± 0.121	0.130 ± 0,090
Calcium-albumin binding site(mmol/L)	0.619 ± 0.143	0.640 ± 0,145
Albumin binding site (mmol/L)	4.76 ± 0.974	4.682 ± 1,016
Total calcium (mmol/L)	1.89 ± 0.210	1.97 ± 0.108

Table 2. Measured and predicted blood concentrations post-filter.

– 10.74 mmol/hour whereas it is – 19.9 mmol/hour when albumin and calcium-albumin are added to the model with a calcium-free dialysate containing 0.8 mmol/L citrate, and – 18.9 mmol/hour with a dialysate containing neither calcium nor citrate.

Discussion

In this study we evaluated the impact on calcium exchanges of simplified regional citrate anticoagulation techniques in hemodialysis. The results obtained made it possible to validate modeling of calcium exchanges in the dialysis filter. The model was then used to determine the effects of the dialysis prescription parameters (i.e. dialysate, blood and citrate infusion flows) on anticoagulant effectiveness and calcium balance.

When using citrate infusion and a 1.25 mmol/L calcium-containing dialysate, the post-filter free calcium level does not decrease significantly compared to that measured pre-filter before citrate infusion. The diffusive exchange of calcium from the dialysate is sufficient to restore the patient's free calcium. Free calcium into the

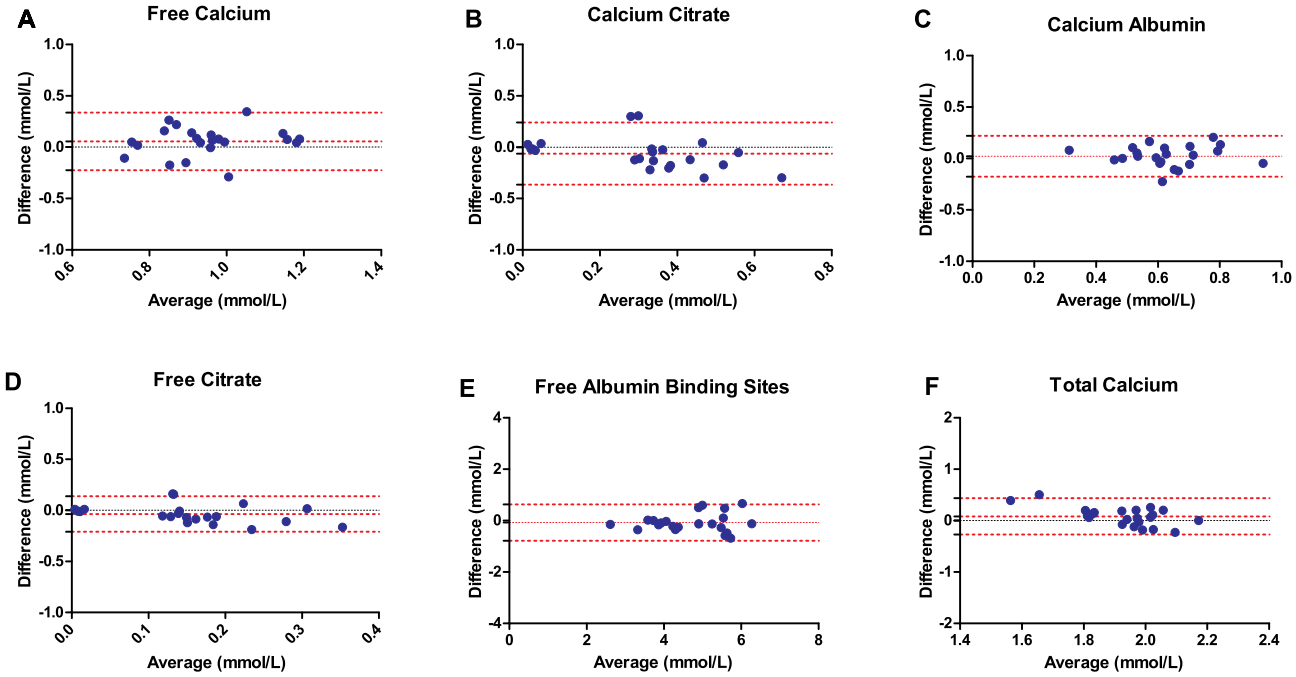

Fig. 2. Bland Altman diagram of postfilter values calculated with modeling, and measured values.

	Bias	95% Limits of agreement
Free calcium (mmol/L)	0.056 ± 0.143	−0.224–0.337
Calcium-citrate (mmol/L)	−0.062 ± 0.154	−0.364–0.241
Citrate (mmol/L)	−0.035 ± 0.089	−0.210–0.139
Calcium-albumin binding site (mmol/L)	0.021 ± 0.101	−0.178–0.220
Albumin binding site (mmol/L)	−0.078 ± 0.362	−0.788–0.631
Total calcium (mmol/L)	0.082 ± 0.181	−0.273–0.437

Table 3. Bias and limits of agreement.

arterial line after citrate infusion drops below 0.4 mmol/L. Thereafter no significant variation in the patient's free calcium level is observed. In addition, the total calcium balance is close to 0 mmol/min. The risk of hypocalcemia therefore seems to be low, as we showed in a previous study[14]. These results are comparable to those obtained by Evenopoel et al.,[12] and Fiaccadori et al.,[13]. However, they provide no conclusive evidence on the long-term effects on mineral and bone disorders notably parathyroid hormone and bone turnover.

Pre- and post-filter citrate dosages indicated that more than 90% of the citrate is eliminated by diffusion and convection in the dialysate. This is a guarantee of patient safety since less than 10% of the infused citrate solution returns to the general blood circulation and is then metabolized by the liver. In order to obtain effective anticoagulation in the circuit (i.e. a free calcium level lower than 0.4 mmol/L), a pre-filter citrate concentration of at least 3000 µmol/L is necessary, a figure that is consistent with the scarce data from the literature on the subject[18].

Modeling makes it possible to predict fairly accurately the variations in concentration during the passage of blood through the dialysis filter. It enabled us to precisely study the impact of dialysis parameters (blood, dialysate and citrate infusion flow) on anticoagulant effectiveness and calcium balance. Thus, it appears that only the first half of the dialysis filter is effectively anticoagulated. In the second half and the venous bubble trap, the free calcium level is greater than 0.4 mmol/L. This could explain the occurrence of blood circuit coagulation episodes with this technique, which varies from 0.5 to 12%[12–14]. Calculations by modeling show that it is possible to increase the length of the anticoagulated dialysis filter effectively by increasing the blood flow or decreasing the dialysate flow (Fig. 3A). However, this leads to a decrease in free calcium (Fig. 3C) and an increase in citrate-calcium complexes in the blood at the outlet of the dialysis filter (as evidenced by a decrease in the free calcium/total calcium ratio, Fig. 3B,C). This can lead to a risk of metabolic alkalosis if the increased amount of citrate is normally metabolised in the liver into bicarbonate. On the contrary, in case of liver failure, citrate accumulation can cause hypocalcemia.

An alternative technique has been developed that involves use of a calcium-free dialysate with or without citrate and reinfusion of a calcium-containing solution into the venous line. The amount of calcium ions lost in the dialysate is estimated to be equal to the product of the dialysance and the patient's free calcium. The calcium infusion rate is readjusted regularly during the session according to dialysance value[8–11]. The model shows that: (i)

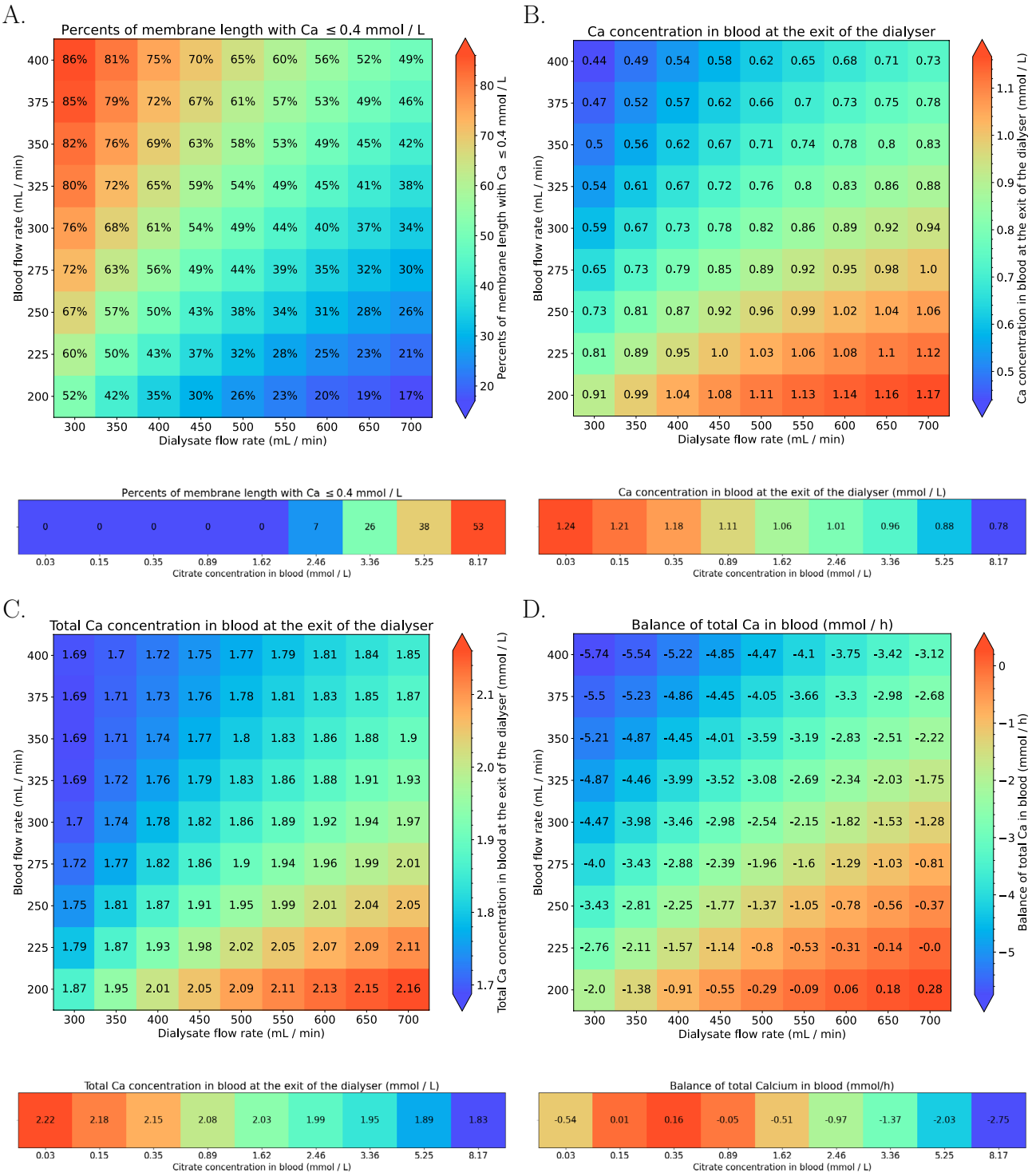

Fig. 3. Simulations of hemodialysis with blood citrate infusion and a dialysate containing calcium (1.25 mmol/L). Percentage of membrane length with free calcium concentration < 0.4 mmol/L (**A**). Postfilter free calcium (**B**) and total calcium concentration (**C**) values. Calcium balance (**D**). Values are calculated for different dialysate and blood flows (prefilter citrate concentration 5 mmol/L) or different prefilter citrate concentrations (dialysate and blood flows set at 500 and 300 mL/min, respectively).

these techniques allow effective anticoagulation of the venous trap but do not ensure anticoagulation of the first half of the dialysis filter and as much as the first two thirds with a citrate-free dialysate; (ii) the addition of citrate to the dialysate reduces the calcium balance (it becomes more negative); and (iii) using the product of dialysance by free calcium concentration to estimate the calcium exchanges in the dialysis filter is an oversimplification that

leads to an underestimation of the quantity of calcium to infuse into the venous line (even more with a citrate dialysate). Taking into consideration blood calcium-albumin and albumin in the model increases calcium transfer to the dialysate. Thus, the amount of calcium delivered from albumin during the passage of blood through the dialysis filter should not be overlooked. These findings are in agreement with the results of Kozik-Jaromin et al. [15], who showed that diffusible calcium accounts for 80% of total calcium and that total calcium is a better predictor of calcium balance than ionized calcium.

Our work has certain limitations. It was a single-center study with a small sample size, and certain patients had to be excluded from the analysis owing to errors or the absence of samples. However, the number of patients analyzed was greater than the miniminum sample size calculated and sufficient to draw conclusions regarding the validity of the proposed model. Some patients were not administered citrate but we were able to use their measurements to validate the model in different situations. Finally, our model does not take into account the Gibbs-Donnan effect on calcium exchange through the dialysis membrane. A solution proposed by Muhawari et al[20] is to use a 1-dimensional model and assume Gibbs-Donnan equilibrium is reached. In this case the concentration ratio of the ions on either side of the membrane can be provided as a function of the protein level according to an empirical relationship provided by Gotch et al[21]. Unfortunately, this simplification is not applicable to our model. To take into account the Gibbs-Donnan effect while retaining the ability to compute solutes concentration distribution inside the dialysis filter it would be necessary to add other charged solutes, introduce electric migration to the advection–diffusion-reaction system and Poisson equation to calculate the electric field. This adds a level of complexity which does not seem necessary because the predicted results are sufficiently close to those observed in patients.

Conclusion

Anticoagulation of extracorporeal blood circulation in hemodialysis is a significant concern, particularly in patients at high risk of bleeding. Simplified alternatives to conventional regional citrate anticoagulation have been proposed. In this study we show that it was possible to model the exchange of calcium and citrate in the dialysis filter quite accurately. Whatever the alternative techniques used (infusion of citrate and dialysate containing calcium, citrate dialysate without calcium, or dialysate without calcium) only part of the blood is effectively anticoagulated. Whether this provides clinical benefit compared to complete absence of anticoagulation remains unclear. In addition, the calcium balance is modified compared to standard hemodialysis. It is important to take these findings into account if we wish to use the techniques for patients undergoing chronic hemodialysis.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 25 April 2024; Accepted: 20 August 2024
Published online: 26 August 2024

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Acknowledgements
We are indebted to Mr. Jeffrey Watts for assistance in the preparation of the manuscript.

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Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-70708-9.

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