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*Original Articles*

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# Blood and ultrafiltrate dosage of citrate as a useful and routine tool during continuous venovenous haemodiafiltration in septic shock patients

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## Abstract

**Background.** Citrate anticoagulation is gaining popularity in renal replacement therapies (RRT) for critically ill patients. In order to study whether citrate accumulates in septic shock patients, we determined citrate in plasma and dialysate during continuous venovenous haemodiafiltration (CVVHDF).

**Methods.** An automated routine determination of citrate was set up using a commercial kit (citrate lyase method). Twelve patients with septic shock on CVVHDF and citrate anticoagulation were studied *ex vivo* for citrate levels in systemic and circuit blood and in the ultrafiltrate (at 0, 0.5, 1, 3, 6, 9, 12, 24, 48 and 72 h).

**Results.** *In vitro* blood studies showed a near unit correlation between the plasma measured and predicted citrate concentrations for an exclusive extracellular distribution of citrate. Median systemic arterial citratemias were 0.09 (0.06–0.12) mmol/L (Time 0) and 0.23 (0.18–0.31) mmol/L during treatment; median sieving coefficient for citrate was 0.95 (0.88–1.02) and did not change with different volumes of CVVHDF effluent (from 1350 to 5100 mL/h). Net citrate and calcium removal by filter significantly correlated with effluent volume ($r = 0.85$ and 0.78, respectively). Median citrate load entering in the patients' bloodstream was 13.60 (9.1–19.6, $n = 68$) mmol/h. Although cost analysis of the citrate test demonstrated a minimally increased daily cost (from 2.96 to 3.51€), saving costs could be potentially relevant with more extended use of citrate anticoagulation.

**Conclusions.** In septic shock patients with liver dysfunction citratemia is useful in guiding clinical application of RRT, where the citrate losses in the ultrafiltrate can be efficiently modulated by increasing the effluent volume.

**Keywords:** continuous venovenous haemodiafiltration; regional citrate anticoagulation; septic shock; systemic citratemia; ultrafiltration volume

## Introduction

Citrate is an effective alternative anticoagulant to heparin in critically ill patients treated with renal replacement therapy (RRT) [1–4]. Citrate does not usually accumulate during haemodialfiltration or haemodialysis, since the amount of citrate infused into the prefilter blood circuit equals the extracorporeal loss through the filter and the intracorporeal metabolic elimination [4].

Citrate is primarily metabolized by the liver and, to a lesser extent, in the skeletal muscle and renal cortex. Complete citrate metabolism consumes hydrogen, leading to an increase in blood pH and generation of carbon dioxide and water [5]. The range of total body citrate clearance may be highly variable. Although healthy patients undergoing short-term infusion may have a total body clearance as high as 710 mL/min, the total clearance in cirrhotic patients is reduced to only 340 mL/min [6]. In these patients, decreased clearance can lead to citrate accumulation and to well-known complications associated with citrate toxicity such as a worsening of acidosis and alterations in calcium and sodium homeostasis [1–4, 7–11].

Septic shock patients with high doses of vasopressors can also be at risk of citrate toxicity [7]. Blood citrate can accumulate not only as a result of primary liver dysfunction but also due to failure of microcirculation and reduced oxidative liver and muscle uptake [5].

For this reason, the early and reliable assessment of citrate accumulation in critically ill septic patients appears to be of high relevance. This implies the availability of the direct dosage of blood citrate, in a simple, easy to perform and inexpensive way.

Few data are available in the literature regarding blood citrate dosage [4, 6, 7, 11–14]. Instead, several indirect markers of citrate accumulation have been studied, such as changes in pH, anion gap, total calcium or total/ionized

calcium ratio [1–3, 6, 8–10]. However, all these parameters correlate poorly with measured citrate levels with the exception of the total/ionized calcium ratio, which unfortunately in these studies [10, 13, 14] could not be performed at the bedside since it needs the determination of total calcium.

In our study, we first implemented the evaluation of citrate levels in plasma and dialysate as a routine laboratory test by adapting a commercial kit for citrate. Then, in septic patients with liver dysfunction on continuous venovenous haemodiafiltration (CVVHDF), we studied plasma and dialysate levels of citrate. Our results show that normal citratemia is associated to a marked loss of citrate through the filter and depends directly on the effluent volume.

## Material and methods

### *Analytical methods*

Blood and dialysate samples were drawn in sterile Li-Heparin syringes (Vacutainer System; Becton Dickinson, Rutherford, NJ) for determination of ionized calcium and Li-Heparin tubes (Becton Dickinson) for determination of total calcium and citrate (see below).

Li-Heparin tubes were immediately centrifuged at high speed, and plasma total calcium was measured in a modular analytical system with an online sample preparation unit (Architect c8000; Abbott Italia, Milan, Italy).

Ionized calcium was measured bedside in systemic and venous circuit blood and dialysate samples by an ion-selective electrode method using an Instrumentation Laboratory haemogas analyser (GEM 3000; IL-Italia, Milan, Italy).

Haemochromocytometer counts were determined by standard laboratory methods.

*Citrate determinations in plasma and ultrafiltrate.* Citrate was measured in fresh plasma and ultrafiltrate within 6 h after collection using a modular analyser (Architect c8000, Abbott Italia) and a citrate lyase method (Citric acid UV; R-Biopharm AG, Darmstadt, Germany). This method, intended for analysis of citrate in foodstuffs and other materials, was adapted for use in plasma by lowering the sample volume [4, 14, 15]. This adaptation allowed the measurement of citrate concentrations without additional dilution in systemic plasma (range 0.1–4 mmol/L) and with 1:2 sample dilution in circuit plasma or ultrafiltrate (range 0.2–8 mmol/L).

As an external quality control, we used sequential dilutions of the ACD-A infusion solution at the stated level of 112.88 mmol/L, i.e. the citrate formulation used as anticoagulant in patients on CVVHDF.

*Linearity.* We constructed a calibration curve by assaying in duplicate six citrate standards, ranging from 0.1 to 8 mmol/L. Samples containing >4000 mmol/L were diluted 1:2 with de-ionized water and re-assayed. Repeated analysis ($n = 4$) showed the curve to be linear up to 4000 mmo1/L.

*Precision.* In water solutions, analytical recovery was 99.2% ($n = 60$, range 92.4–102.8 with a coefficient of variation (CV) of 2.85%) at concentration of 0.25 mmol/L, 99.5% ($n = 60$, range 98.8–100.5 with a CV of 0.4%) at concentration of 2.00 mmol/L and 100.5% ($n = 60$, range 97.6–102.2 with a CV of 1.0%) at concentration of 4.00 mmol/L.

The plasma supplemented with different concentration of citrate (taking into account the basal concentration of citrate) gave an analytical recovery of 102.1% ($n = 36$, range 94.9–105.7) with a CV of 2.2%. Considering that measuring citrate in ACD required a 100-fold dilution, we concluded that our method was able to measure the citrate concentration of the infusion solution.

*Distribution of citrate in whole blood.*

Citrate does not penetrate red blood cells and has only an extracellular distribution [16]. In order to study extracellular distribution of citrate, heparinized blood from six healthy volunteers was supplemented with citrate by serial dilution. Taking into account the haematocrit value and the plasma basal citrate concentration already present in a single sample, the final plasma citrate concentrations reached after addition of exogenous citrate (ranging from 0.15 to 4.8 mmol/L) were calculated and correlated with the measured plasma citrate concentration.

### *Patients*

Twelve critically ill patients with septic shock [17] and at high bleeding risk were studied. They were treated with citrate anticoagulation for CVVHDF [30 sessions, total 37 days of CVVHDF for a cumulative time of 824 h, median duration of sessions 20.0 h (interquartiles 11–43)] (Table 1). This study was part of a citrate anticoagulation protocol for CVVHDF applied in high bleeding risk patients as an alternative anticoagulant [4, 18].

The study protocol was in accordance with the Helsinki Declaration. Informed written consent was obtained by either close relative or a legal representative.

*Citrate anticoagulation protocol in CVVHDF patients.* CVVHDF was performed with a dedicated monitor (Multifiltrate; Fresenius Medical Care, Bad Homburg, Germany) with a replacement fluid infusion set in predilution (filter AV1000, surface area of 1.8 mq; Fresenius Medical Care).

Briefly, the blood circuit was anticoagulated by an ACD-A-containing replacement solution infused in predilution, and a commercial 10% calcium chloride solution was infused in a separate line at the end of the venous circuit [4, 11]. CVVHDF was performed using a blood flow rate varying from 100 to 150 mL/min, and a predilution infusion rate from 800 to 1050 mL/h [4, 11]. The predilution infusion rate was titrated by 50 mL/h increments for 10 mL/h increment of blood flow (starting with 800 mL/h of infusion at blood flow of 100 mL/min). Administration of calcium chloride 10% solution was titrated (by 0.5 mL/h increments for each increment of 500 mL of ultrafiltrate) in order to maintain systemic ionized calcium levels between 0.9 and 1.4 mmol/L [11].

Calcium-free fluid containing bags (CiCa; Fresenius Medical Care) were used for the dialysate. Dialysate (from 750 to 4200 mL/h) rate was modified according to the metabolic and fluid balance requirements of the patient.

**Table 1.** Clinical data of patients at beginning of CVVHDF[a]

| Patient no. | Age/sex, years | Diagnosis | CVVHDF days/filters | NA/DA[a] ug/Kg/min | AST/ALT UI/L | Bilirubin mg/dL | Exitus |
|---|---|---|---|---|---|---|---|
| 1 | 65/M | Burns 25% | 1/1 | 0.60/3.0 | 85/48 | 3.8 | Yes |
| 2 | 77/F | Burns 35% | 2/3 | 0.30/3.0 | 69/52 | 4.0 | Yes |
| 3 | 78/F | Burns 18% | 4/2 | 0.00/5.0 | 24/14 | 1.3 | Yes |
| 4 | 62/M | Burns 18% | 5/4 | 0.00/8.0 | 11/11 | 2.1 | No |
| 5 | 78/F | Burns 15% | 1/1 | 0.50/10 | 29/13 | 2.6 | Yes |
| 6 | 81/M | Polytrauma | 10/ | 0.20/6.0 | 31/41 | 7.8 | Yes |
| 7 | 49/M | Polytrauma | 7/3 | 0.25/5.0 | 239/37 | 35.0 | Yes |
| 8 | 67/M | Fasciitis | 3/5 | 0.00/5.0 | 120/78 | 1.6 | No |
| 9 | 53/M | Peritonitis | 2/1 | 0.00/5.0 | 35/41 | 2.1 | No |
| 10 | 28/M | Polytrauma | 3/1 | 0.00/7.0 | 1443/295 | 2.2 | No |
| 11 | 67/M | Burns 35% | 3/1 | 0.10/7.0 | 13/14 | 2.5 | Yes |
| 12 | 46/M | Burns 90% | 5/2 | 0.70/8.0 | 46/25 | 2.1 | Yes |

[a]ALT, alanine aminotransferase; AST, aspartate aminotransferase; F, female; M, male; NA/DA, norepinephrine/dopamine.

*Sampling protocol.* Systemic blood samples were always drawn directly from systemic arterial blood. Venous blood samples were taken from circuit venous line after the filter and before the venous air bubble trap. Ultrafiltrate samples were taken from the ultrafiltrate line. Since no blood-taking site was available in circuit between the citrate infusion site (in postpump just before air bubble trap) and the filter, prefilter citrataemia were calculated and not directly measured.

Calculated prefilter circuit citratemia (citrate concentration entering the filter) was done according to the following formula: [(Infusion flow rate (mL/min) * $Ccit_{inf}$ (mmol/mL))] / [(blood flow rate (mL/min) + Infusion flow rate (mL/min)) * ((100-haematocrit) / 100)], where $Ccit_{inf}$ = citrate concentration in infusion fluid.

### *Cost analysis*

The average daily costs of citrate dosage in Euro were calculated from the following values: (i) costs for citrate testing (systemic blood citratemia determined once daily) and (ii) costs for determination of total calcium and haemogas analyser (ratio total/ionized ratio determined twice daily). Cost of labor and equipment were not included.

### *Statistical methods*

Descriptive statistics and linear regression analysis were performed with software Statistica (Statistica 6.1; StaSoft Inc., Tulsa, OK). Values were expressed as median (interquartiles range).

P-values $<0.05$ were considered statistically significant.

## Results

### *Distribution of citrate in whole blood*

By studying *in vitro* the blood distribution of citrate between intra- and extracellular compartments, we found the significant correlation ($r = 0.9997$, $y = 0.00066 + 1.020x$, $n = 36$) between the plasma measured and the predicted citrate concentrations for an exclusive extracellular distribution (taking into account the haematocrit value).

### *Systemic plasma citrate and calcium concentrations*

Median basal citrate value at Time 0 was 0.09 (0.06–0.12) mmol/L. It increased to 0.21 (0.20–0.50) mmol/L at 30 min and peaked to 0.26 (0.22–0.62) at 9 h (Figure 1a). With exclusion of basal data at Time 0, median systemic citrate level in patients during treatment was 0.23 (0.18–0.31) mmol/L. After the end of CVVHDF, systemic citratemia returned to a lower basal value of 0.16 (0.11–0.18) mmol/L at 60 min (five experiments, data not shown).

No significant correlation was found between systemic plasma citrate and total calcium levels, ionized calcium or total/ionized calcium ratio (Table 2).

### *Filter handling of citrate and calcium*

Figure 1a shows circuit prefilter citrate value and Figure 1b circuit venous and effluent citrate values. Venous and effluent citrate concentrations were data measured in plasmas, whereas prefilter values were data calculated taking into account haematocrit, infusion and blood flow rates. The overall median citrate concentrations were 4.02 (3.62–4.89) mmol/L in prefilter plasma (Figure 1a), 2.51 (1.76–3.40) mmol/L in venous plasma and 3.69 (3.29–4.21) mmol/L in effluent (Figure 1b). Median sieving coefficient for citrate calculated with different volumes of CVVHDF ultrafiltrate from 1350 to 5100 mL/h was 0.95 (0.88–1.02, $n = 43$).

The absolute amount of citrate in ultrafiltrate (citrate removal by filter, millimoles per hour) was directly correlated to the effluent volume (Figure 2; $P < 0.01$).

The median load of citrate entering in the patient bloodstream, obtained by taking the amount of citrate infused minus the measured amount lost in ultrafiltrate, was 13.60 (9.1–19.6, $n = 68$) mmol/h.

As for citrate, the amount of total calcium lost in the ultrafiltrate (millimoles per hour) was directly related to effluent volume (Figure 3).

### *Cost analysis*

By implementing the citrate test on Architect c8000, the volume of testing solution decreased from 2 mL

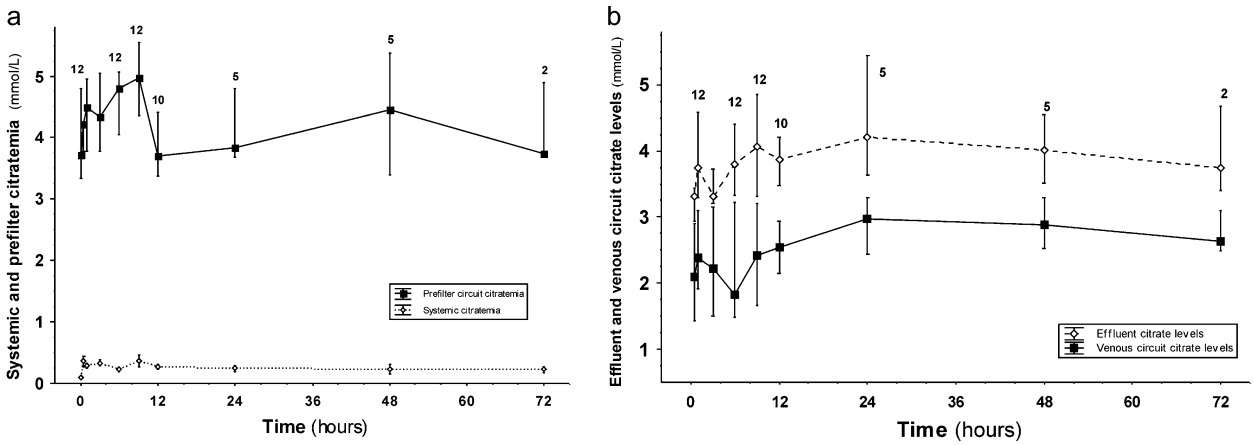


**Fig. 1.** Citrate levels in systemic, prefilter and venous plasma and in effluent during CVVHDF (12 patients, 30 sessions). Panel (**a**) shows citrate levels in systemic plasma and in prefilter circuit plasma. Prefilter circuit plasma citrate concentration was calculated according to the following: Calculated prefilter plasma citrate = [((Infusion flow rate (mL/min) × $Ccit_{inf}$ (mmol/mL))/(blood flow rate (mL/min) + Infusion flow rate (mL/min))] × [(100 − haematocrit)/100] [(Infusion flow rate (ml/min) * $Ccit_{inf}$ (mmol/ml))] / [(blood flow rate (ml/min) + Infusion flow rate (ml/min)) * ((100-haematocrit) / 100)], where $Ccit_{inf}$ = citrate concentration in infusion fluid. Panel (**b**) shows citrate levels in venous circuit plasma and effluent. Venous circuit plasma (blood entering to patient, after filter handling) and effluent citrate concentrations were measured data (see Materials and methods). Numbers at different time point specify the number of patients. Values are given as median (interquartiles).

**Table 2.** Correlation between systemic plasma citrate and total, ionized or ratio total/ionized calcium[a]

| | Median (interquartiles) | *n* | *r* | P |
|---|---|---|---|---|
| Plasma citratemia (mmol/L) | 0.19 (0.10–024) | 64 | no data | no data |
| Plasma citratemia versus total calcium (mg/dL) | 8.75 (8.20–9.45) | 64 | 0.1156 | 0.3629 |
| Plasma citratemia versus ionized calcium (mmol/L) | 1.04 (0.98–1.15) | 64 | 0.0262 | 0.8368 |
| Plasma citratemia versus total/ionized ratio | 2.10 (2.01–2.15) | 64 | 0.1311 | 0.3019 |

[a]No significant correlation was found between all studied parameters. Values are given as median (interquartiles).

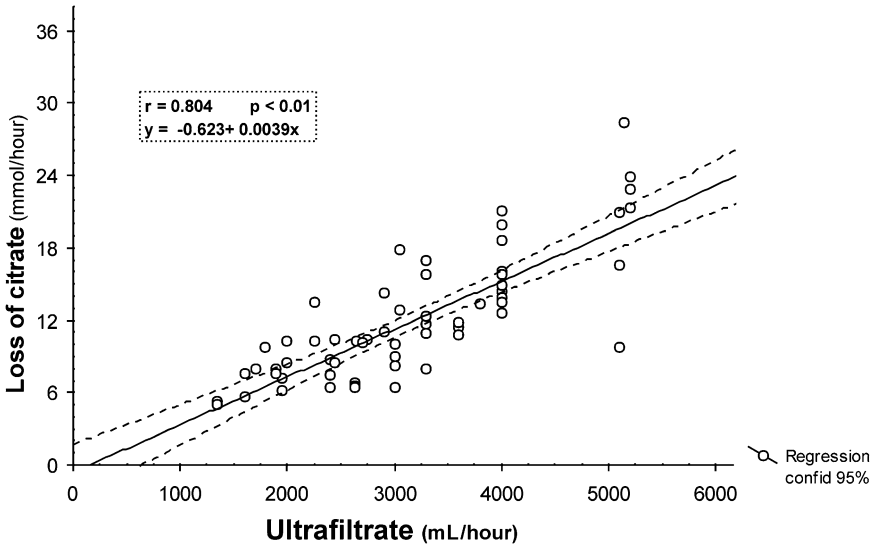


**Fig. 2.** Correlation between loss of citrate and ultrafiltrate volume. Graph shows a significant linear correlation between loss of citrate (millimoles per hour) and ultrafiltrate volume (milliliters per hour) ($n = 50$). Loss of citrate was calculated according to: Loss of citrate (mmol/h) = UF citrate concentration (mmol/mL) × UF volume (mL/h).

(manual test) to 0.2 mL (automated test). Assay number of each kit increased from 36 to 360, with a final cost for citrate test of 0.75€ (including planned calibration controls). The cost of lab total calcium determination and bedside haemogas analyser analysis were 0.1 and 1.38€, respectively.

Therefore, the total daily costs during citrate dialysis were 2.96€ with total/ionized calcium monitoring and 3.51€ with systemic citrate monitoring (based on per-protocol analysis, see Material and methods).

## Discussion

In septic shock patients with liver dysfunction, the routine determination of citrate may be a useful tool in guiding the clinical application of citrate anticoagulation in RRT. In our study, we observed that by increasing the dialysate volume, we could obtain a lower total citrate patient load and thus were able to maintain blood citrate within a safe range.

The advantage of citrate anticoagulation in septic shock patients with active bleeding or at high bleeding risk still remains to be determined since to date no clear data support citrate clearance. Hepatic dysfunction and high amine requirement can restrain metabolic liver and muscle aerobic metabolism of citrate, exposing these patients to risk of citrate accumulation. In these clinical settings, blood citrate monitoring would be advocated.

However, citrate dosage is not routinely available, and several indirect parameters of citrate accumulation such as pH, anion gap, total calcium, ionized calcium or total/ionized calcium ratio have been suggested. Among all these parameters, only total/ionized calcium ratio demonstrated a correlation with citratemia, although for values of citratemia [10, 13, 14].

In fact, when citrate accumulates, systemic iCa++ decreases, whereas total Ca++ concentration remains constant. Therefore, total/ionized calcium ratio can predict

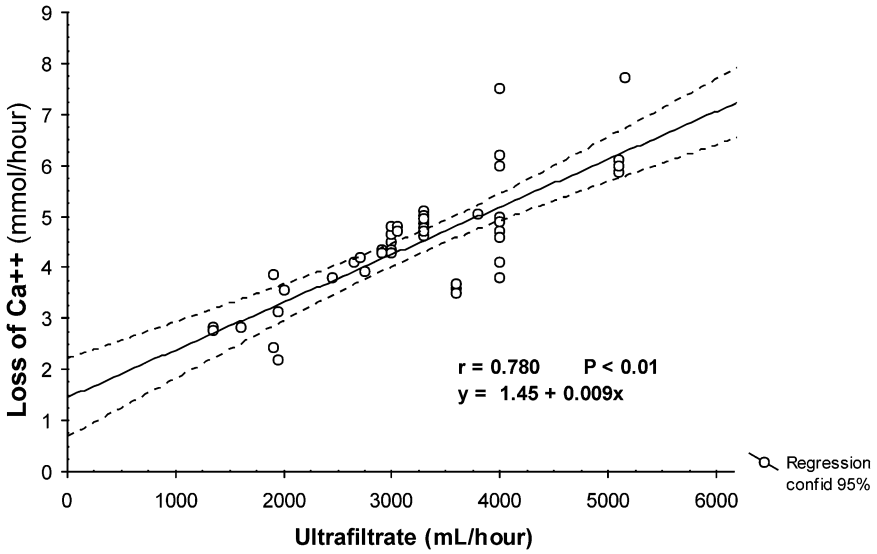


**Fig. 3.** Correlation between loss of total calcium and ultrafiltrate volume. Correlation was significant and linear, with $r = 0.780$. Loss of total calcium was calculated as: Loss of calcium (mmol/h) = UF total calcium concentration (mmol/mL) × UF volume (mL/h).

an increased risk of subsequent hypocalcaemia [10]. In our septic shock patients, we did not find any significant correlation between systemic citratemia and different calcium values (ionized, total and total/ionized calcium ratio). Our data could simply have reflected the absence of accumulation during CVVHDF, as demonstrated by normal systemic citratemia. However, we cannot rule out other confounding factors for calcium concentration, such as calcium kinetics and plasma protein concentration. As to kinetics, calcium is continuously lost by the filter and replaced by an external infusion at the end of the circuit [11]. Therefore, plasmatic total calcium first reflects the instantaneous balance between removal and infusion. In addition, total calcium is largely determined by albumin concentration, and plasma albumin is often very low in septic shock critically ill patients [19]. For all these confounding factors, in septic shock patients, calcium levels or total/ionized calcium ratio may not be as reliable as a direct measurement of citrate in patients with septic shock.

In searching for a reliable dosage for citrate in plasma and ultrafiltrate, first, we automated a commercially available citrate assay currently used for foodstuff and urine [17]. Adapting the test in commonly automated equipment allowed us to avoid manual errors, save time and money by reducing the volume solutions, bring uniformity to the procedure and evaluate the test in routine clinical practice. Second, the validation phase demonstrated that the citrate test had reliable linearity, precision and recovery in ultrafiltrate and plasma.

Interestingly, data obtained with our automated commercial citrate assay confirm an old observation about the distribution of radioactive citrate in plasma and erythrocyte phases [18]. Citrate added to the blood does not cross the cell membrane and it has an exclusive extracellular distribution. When concentrations of citrate–blood mixture are calculated for whole blood (and not taking into account the haematocrit value), the level of plasma for citrate could be underestimated and the sieving coefficient could be overestimated. Concerning the costs of direct automated citrate determinations instead of total/ionized calcium ratio for monitoring citrate dialysis, they increased from 2.96 to 3.51€. These costs are irrelevant and do not take into account the costs saved due to potential extended use of citrate anticoagulation for the direct monitoring of early citrate accumulation. Because of longer filter survival and reduced haemorrhagic complications [1–4], lower costs of anticoagulation with citrate occurred (reduced consumption of disposable, nursing staff workload, shorter ICU stay), despite the repeated determinations of systemic blood citrate.

However, plasma levels mainly depend on liver metabolic capacity for citrate [6, 20]. Studying citrate accumulation in cirrhotic patients, Kramer *et al.* [6] found that a total Ca++/iCa++ ratio >2.5 identified only 3 of the 15 samples with citrate concentration >1.5 mmol/L and that systemic clearance of citrate reduced at ~50% was neither predictable by renal function nor APACHE II score [6]. Similarly, Hetzel *et al.* [13] found that in acute kidney injury patients treated with CVVHF, systemic citrate clearance was not related to altered liver function tests [13]. As previously described in patients with liver failure by Meier-Kriesche *et al.* [10], Hetzel *et al.* [13] found that citratemia was correlated to total Ca++/iCa++ ratio but at steady state citrate levels on CVVHF Day 3 of 0.85 mmol/L (range 0.13–3.8 mmol/L). This value is three to four times higher than normal and just below the considered upper limit of safety in critically ill patients without liver dysfunction [14].

However, our study suffers from some limitations. First, in the present study, none of the patients developed a relevant citrate accumulation, and the utility of total/ionized calcium ratio could not be estimated by the protocol

applied. Second, the number of patients included may be considered relatively low; however, comprising 12 patients of a population at risk of citrate accumulation (e.g. septic shock patients), the present study dealt with clinical settings so far not well defined by demonstrating only minimal variations on systemic citratemia and ruling out any metabolic interference [6, 7, 11–14]. Basal systemic plasma citrate was 0.09 mmol/L, similar to the reference value of <0.140 mmol/L reported in normal subjects [17]. During CVVHDF, systemic citrate slightly increased (at ~0.25 mmol/L) and maintained this relatively stable concentration over the whole session.

The observed minimal plasma increment of citrate on CVVHDF was probably physiologically necessary to induce and sustain citrate uptake and metabolism by liver and muscle. Of interest, citrate metabolism could be induced very quickly (no peak concentrations after the starting of infusion) and rapidly recovered 30 min after the end of CVVHDF session to basal values of 0.10 mmol/L.

The detected values for citrate in systemic plasma were within the normal range and lower than those reported in similar studies [12–14]. These data suggest that in our septic patients, the clearance of endogenous citrate (infused citrate minus citrate loss by filter) was adequate and complete. The calculated citrate load returning to the patient was always <15 mmol/h over the entire range of used blood flow (ranging from 100 to 150 mL/min) and dialysate (up to 5100 mL/h). The value of 15 mmol/h is among the lowest described [4, 7, 11–13], and it may be one of the main reasons for the normal citrate levels in septic shock patients.

A marked amount of citrate was lost in CVVHDF by the filter with a sieving coefficient near unit. We could not directly measure prefilter citratemia because of sampling point lack. Though a significant accumulation of citrate was not found, higher systemic plasma levels should have influenced the prefilter values to a certain extent and have led to the calculation of lower sieving coefficients. Nevertheless, we found a closed directs positive correlation between ultrafiltrate volume and amount of lost citrate. All these data are consistent with CVVHDF operative conditions of a citrate removal which dialysate flow rate is the limiting factor for. As demonstrated in intermittent haemodialysis for urea [21] and citrate [22–24], we found that diffusive clearance of citrate in CVVHDF markedly restrained citrate load entering into the patient and played a pivotal role in increasing its feasibility and tolerance in septic shock patients.

Similarly, a calcium loss proportional to effluent volume was also observed. However, neutral calcium balance was achieved by increasing infusion of calcium, which was easily titrated on ultrafiltrate volume.

In conclusion, we set a rapid and automated method to measure citrate levels in plasma and effluent. Routine citrate dosage could increase the safety and confidence in the use of citrate in these complex patients, where the citrate losses in the effluent can be efficiently increased by increasing the effluent volume.

*Conflict of interest statement*. C.T. is a full-time employee of Fresenius Medical Care. All the other authors had no conflict of interest.

The results presented in this paper have not been published previously in whole or part, except in abstract format.

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