9. Shinohara K, Shoji T, Emoto M *et al.* Insulin resistance as an independent predictor of cardiovascular mortality in patients with end-stage renal disease. J Am Soc Nephrol 2002; 13: 1894–1900
10. Canbakan M, Sahin GM. Icodextrine and insulin resistance in continuous ambulatory peritoneal dialysis patients. Ren Fail 2007; 29: 289–293
11. Bonora E, Targher G, Alberiche M *et al.* Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000; 23: 57–63
12. Matthews DR, Hosker JP, Rudenski AS *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419
13. Szeto CC, Chow KM, Kwan BC *et al.* New-onset hyperglycemia in nondiabetic chinese patients started on peritoneal dialysis. Am J Kidney Dis. 2007; 49: 524–532
14. Duong U, Mehrotra R, Molnar MZ *et al.* Glycemic control and survival in peritoneal dialysis patients with diabetes mellitus. Clin J Am Soc Nephrol 2011; 6: 1041–1048
15. Yoo DE, Park JT, Oh HJ *et al.* Good glycemic control is associated with better survival in diabetic patients on peritoneal dialysis: a prospective observational study. PloS One. 2012; 7: e30072
16. Menon V, Greene T, Pereira AA *et al.* Glycosylated hemoglobin and mortality in patients with nondiabetic chronic kidney disease. J Am Soc Nephrol 2005; 16: 3411–3417
17. Babazono T, Nakamoto H, Kasai K *et al.* Effects of icodextrin on glycemic and lipid profiles in diabetic patients undergoing peritoneal dialysis. Am J Nephrol 2007; 27: 409–415
18. Sanchez-Villanueva R, Estrada P, del Peso G *et al.* Repeated analysis of estimated insulin resistance using the HOMAIR index in nondiabetic patients on peritoneal dialysis and its relationship with cardiovascular disease and mortality. Nefrologia 2013; 33: 85–92
19. Li Y, Zhang L, Gu Y *et al.* Insulin resistance as a predictor of cardiovascular disease in patients on peritoneal dialysis. Perit Dial Int 2013; 33: 411–418
20. Johnson DW, Arndt M, O'Shea A *et al.* Icodextrin as salvage therapy in peritoneal dialysis patients with refractory fluid overload. BMC Nephrol 2001; 2: 2
21. Amici G, Orrasch M, Da Rin G *et al.* Hyperinsulinism reduction associated with icodextrin treatment in continuous ambulatory peritoneal dialysis patients. Adv Perit Dial 2001; 17: 80–83
22. Reis da Silva D, Figueiredo AE, Antonelo IC *et al.* Solutes transport characteristics in peritoneal dialysis: variation in glucose and insulin serum levels. Ren Fail 2008; 30: 1–5
23. Twardowski Z, Nolph KO, Khann R *et al.* Peritoneal equilibration test. Perit Dial Int 1987; 7: 138–148
24. Cho KH, Do JY, Park JW *et al.* Effect of icodextrin dialysis solution on body weight and fat accumulation over time in CAPD patients. Nephrol Dial Transplant 2010; 25: 593–599
25. He Q, Zhang W, Chen J. A meta-analysis of icodextrin versus glucose containing peritoneal dialysis in metabolic management of peritoneal dialysis patients. Ren Fail 2011; 33: 943–948

Received for publication: 29.1.2015; Accepted in revised form: 16.5.2015

Nephrol Dial Transplant (2015) 30: 1911–1919
doi: 10.1093/ndt/gfv290
Advance Access publication 25 August 2015

ORIGINAL ARTICLE

Citrate pharmacokinetics at high levels of circuit citratemia during coupled plasma filtration adsorption

Filippo Mariano[1], Maurizio Morselli[2], Zsuzsanna Holló[1], Fulvio Agostini[3], Maurizio Stella[4] and Luigi Biancone[1,5]

[1]Nefrologia, Dialisi e Trapianto U, Dipartimento di Medicina Generale e Specialistica, Citta' della Salute e della Scienza di Torino, Ospedale CTO, Torino, Italy, [2]Biochimica Clinica, Dipartimento di Medicina di Laboratorio, Citta' della Salute e della Scienza di Torino, Ospedale CTO, Torino, Italy, [3]Anestesia e Rianimazione 5, Dipartimento di Anestesia e Rianimazione, Citta' della Salute e della Scienza di Torino, Ospedale CTO, Torino, Italy, [4]Chirurgia Plastica Grandi Ustionati, Dipartimento di Chirurgia Generale e Specialistica, Citta' della Salute e della Scienza di Torino, Ospedale CTO, Torino, Italy and [5]Nefrologia, Dialisi e Trapianto U, Dipartimento di Medicina Generale e Specialistica, Citta' della Salute e della Scienza di Torino, Universita' Degli Studi di Torino, Ospedale CTO, Torino, Italy

Correspondence and offprint requests to: Filippo Mariano; E-mail: filippo.mariano@hotmail.it

ABSTRACT

Background. The heparin requirement for coupled plasma filtration adsorption (CPFA) is usually high. Heparin administration often cannot be adherent to prescription, leading to a premature clotting of circuit and an insufficient volume of treated plasma. Regional citrate anticoagulation (RCA) could be an attractive alternative; however, no data are available on citrate pharmacokinetics at high levels of circuit citratemia.

Methods. Fifteen septic shock patients with acute kidney injury undergoing CPFA with RCA at target circuit citratemia of 6 mmol/L were treated with CPFA-haemofiltration in pure predilution (CPFA-HF predilution group, $n = 5$ patients), or predilution haemodiafiltration (CPFA-HDF predilution group, $n = 5$ patients) or pre- and postdilution haemofiltration (CPFA-HF pre/postdilution group, $n = 5$ patients). Citrate pharmacokinetics was carried out through its determination in systemic and circuit blood, and effluent at time 0, 0.2, 1, 3, 6 and 9 h.

Results. The systemic concentrations of citrate in the CPFA-HF predilution group significantly increased over the sessions (from basal level of 0.21 to 0.76 mmol/L at 3 h), whereas they did not change in CPFA-HDF predilution and CPFA-HF pre/postdilution groups. Circuit plasma citrate concentrations (from 3 to 8 mmol/L) correlated strongly with circuit iCa++ levels (Spearman $R = -0.7022$, $P < 0.01$). Sieving coefficients of citrate were near the unit in all three groups and unrelated to blood and infusion flow rates in predilution. However, the amount of citrate removed by effluent was ∼40% for the CPFA-HF predilution group and reached 60% for both the CPFA-HDF predilution and CPFA-HF pre/postdilution groups ($P < 0.05$). As for the efficiency of plasmafiltration, the plasmafiltrate volume (from 17 to 20 mL/kg/day) was not significantly different among the groups.

Conclusions. These results demonstrated that in refractory septic shock patients on CPFA at circuit citratemia of 6 mmol/L both HDF predilution and HF pre/postdilution were the best dialysis modalities to maintain a normal systemic citratemia through a high rate of citrate loss in the effluent.

Keywords: citrate anticoagulation, citrate concentrations, coupled plasma filtration adsorption, haemodiafiltration, haemofiltration

ORIGINAL ARTICLE

INTRODUCTION

Coupled plasma filtration adsorption (CPFA) is a sorbent technology-based renal replacement therapy (RRT) for septic shock patients with acute kidney injury (AKI), aimed at the removal of endotoxin, bacterial products and both pro- and anti-inflammatory endogenous substances [1–4].

In clinical settings, the requirement of heparin, which is the common anticoagulant used during CPFA, is high up to 1000 U/h [2–6]. As a matter of fact, during CPFA, the plasmafiltrate is passed through a resin cartridge and reinfused into the blood line between the plasma filter and haemofilter. The contact of blood with foreign material triggers coagulation and complement cascades, as well as activating inflammatory cells and platelets [7]. An adequate anticoagulation of blood and plasma circuits with heparin is required to limit all these complex interactions between foreign materials and the patient. Besides that, in septic shock patients, heparin requirement is variable because of inconstant levels of ATIII and alterations in platelet count and functions. In addition, heparin is contraindicated in patients at high bleeding risk or with associated injuries such as head trauma [4–9].

For many reasons, circuit anticoagulation is still one of the most important challenges for the application of CPFA. A recent multicentre, randomized trial involving 192 critically ill patients with septic shock assessed the efficacy of CPFA in reducing mortality [10]. In this trial, a conservative heparin dosage and a high rate of premature circuit clotting were observed [10]. Nearly half of the patients randomized to CPFA were undertreated as per protocol stipulation. The main reason for not reaching the prescribed volume of treated plasma was in most cases (48%) the premature clotting of the circuit [10]. As a consequence, the next designed confirmatory trial on CPFA (COMPACT 2) will be planned with only citrate as the anticoagulant [10].

The use of citrate in septic shock patients is controversial since they are potentially at risk of citrate impaired metabolism and accumulation [11–16]. This risk increases when CPFA is carried out at levels of circuit citrate, such as 4–6 mmol/L capable of achieving a good patency of plasma circuit. However, as demonstrated in severe burns septic shock patients on continuous renal replacement therapy (CRRT) [13, 15], an optimization of citrate loss in the effluent can lead to a good tolerance and metabolic stability.

In 2004, preliminary data showed the safety and the efficacy of citrate anticoagulation in patients undergoing CPFA-haemofiltration in predilution [5]. However, there are no available data about pharmacokinetics of citrate during long-term CPFA in the different dialysis modalities, such as in pure convective or mixed diffusive/convective techniques.

In this study, we evaluated the citrate pharmacokinetics at high levels of circuit citrate on CPFA patients in a very practical way. We demonstrated that an optimization of citrate losses in the effluent could be reached through both predilution haemodiafiltration and pre/postdilution haemofiltration.

MATERIALS AND METHODS

Patients

Fifteen consecutive critically ill polytrauma/burns patients [aged 64 (39–78) years, median (intre-quartiles)] with septic shock refractory to conventional therapies were studied. All patients presented clinical and laboratory evidence of systemic infection. Eight patients out of 15 were severely burned. None of the patients had past history of impaired renal function. All of them suffered from AKI Stage I/F according to the RIFLE (Risk, Injury, Failure, Loss, End-stage kidney disease) classification [17] and had clinical indications for CPFA. The demographic characteristics of patients are shown in Table 1.

All patients received conventional therapy including crystalloid solutions, broad-spectrum antibiotics, diuretics and vasopressor agents.

The study was conducted according to the Helsinki Declaration, and it was approved by the Ethics Committee of our hospital. Informed consent was obtained from the patients or their caring relatives.

CPFA technique

In all patients, CPFA was performed with a five pump dedicated monitor (Lynda, Bellco, Mirandola, Italy) with regional

Table 1. Demographic characteristics and biochemical data in patients on CPFA for HF predilution ($n = 5$), HDF predilution ($n = 5$) and HF pre/postdilution ($n = 5$) groups

	All	HF predilution	HDF predilution	HF pre/postdilution	P[a]
Patients (*n* burned; session)	15 (8; 20)	5 (4; 5)	5 (1; 10)	5 (3; 5)	-
Age (years)	64 (39–78)	77 (65–78)	64 (48–78)	40 (39–50)	0.268
Mortality in ICU (%,dead/alive)[b]	53,3%, 8/7	80%, 4/1	60%, 3/2	25%, 1/4	-
Sex ratio (male/female)	10/5	3/2	2/3	5/0	-
Mechanical ventilation (%, *n*)	100%, 15	100%, 5	100%, 5	100%, 5	-
Septic shock (%, *n*)	100%, 15	100%, 5	100%, 5	100%, 5	-
Delay of CPFA occurrence (days)	19 (8–38)	21 (2–60)	24 (14–25)	16 (9–19)	0.148
SOFA score	12 (11–13)	11 (10–12)	13 (12–16)	11 (11–12)	0.101
Diuresis (mL/die)	2400 (1500–4800)	2400 (700–2500)	1500 (0–1800)	4800 (2400–6000)	0.063
Biochemical data					
MAP (mmHg)	95.3 (71.7–100)	80.0 (71.7–85.0)	96.7 (95.3–97.3)	96.7 (88.3–100.7)	0.429
Norepinephrine (μg/kg/min)	0.23 (0.0–0.50)	0.0 (0.0–0.30)	0.20 (0.15–0.23)	0.5 (0.3–0.7)	0.177
Dopamine (μg/kg/min)	6.0 (5.0–8.0)	3.3 (1.2–7.7)	6.0 (5.0–8.0)	6.0 (5.0–7.0)	0.434
Creatinine (mg/dL)	2.2 (1.3–3.0)	2.3 (1.9–5.0)	2.2 (2.0–2.2)	1.3 (1.2–2.2)	0.327
PO_2/FIO_2 ratio	2.0 (1.2–3.2)	2.5 (2.4–2.7)	1.3 (1.0–1.5)	1.5 (1.2–3.2)	0.312
Bilirubin (mg/dL)	2.5 (1.2–3.3)	2.0 (1.0–2.5)	3.0 (2.0–4.4)	2.9 (1.5–3.2)	0.455
WBC (1000/mm^3)	16.3 (8.0–24.4)	24.4 (16.0–25.0)	8.0 (7.3–8.4)	16.4 (16.3–20.0)	0.275
Htc (%)	30.8 (29.5–33.7)	31.2 (22.0–34.5)	31.0 (28.9–34.7)	30.4 (30.2–31.7)	0.955
Platelets (1000/mm^3)	97 (76–187)	144 (87–223)	91 (76–93)	144 (97–176)	0.617
Quick (%)	64 (56–72)	65 (57–74)	58 (54–72)	66 (62–70)	0.828
aPTT (s)	34 (27–36)	41 (36–47)	34 (30–35)	28 (26–34)	0.150
Lactate (mmol/L)	2.3 (1.5–2.6)	2.3 (1.8–2.6)	2.6 (1.9–4.0)	2.0 (1.5–2.5)	0.682
pH (units)	7.39 (7.34–7.47)	7.33 (7.30–7.35)	7.40 (7.35–7.47)	7.39 (7.35–7.45)	0.445
Bicarbonates (mmol/L)	27.4 (23.0–29.7)	23.0 (21.5–24.0)	26.6 (26.2–28.2)	29.2 (27.4–29.7)	0.441

Data were recorded at start of CPFA.
Data are given as median (quartile 1–quartile 3).
[a]P-value with Kruskal–Wallis test.
[b]Intention-to-treat analysis.

citrate anticoagulation. CPFA was carried out by using a polyethersulfone plasma filter (0.5 m^2, MPS 05, Bellco) placed in series with a highly permeable polyethersulfone haemodialyser (1.4 m^2, BLS814G, Bellco)(see Figure 1). Plasmafiltrate was adsorbed on an unselective hydrophobic resin cartridge (140 mL for 70 g, with a surface of ∼700 m^2/g) [18].

According to our CPFA protocol, blood flow rate ranged from 100 to 180 mL/min. The blood flow and effluent rates were set on the basis of the target of dialysis adequacy. In effect, the dedicated monitor for CPFA displayed the set blood flow and the effective blood flow provided by the pump. The difference between the set and provided blood flows ranged from 5 to 10% of set blood flow.

We set an exchange of 3–4 L/h of effluent at start to get an amount of 28–38 L/session, accomplishing the dialysis target of 20–25 mL/kg/die [19]. This target was irrespective of patients' residual renal function and dialysate/infusion proportion. Moreover, as these rates were occasionally limited by the efficiency and patency of the vascular access, a dedicated nurse recorded the effective flow rates hour by hour.

According to manufacturer's protocol, plasma filtration rate was maintained between 15 and 25% of blood flow rate.

The vascular access of RRT was a 12 F double lumen venous catheter inserted in the jugular or femoral vein.

Anticoagulation protocol

In all patients, blood circuit was anticoagulated by ACD-A dispersed in a solution infused in predilution. The study was carried out when no citrate-based CRRT treatment was available on the Italian market. Therefore, commercial ACD-A was used as off-label citrate source. The circuits, functional parameters and sampling points for the different groups are depicted in Figure 1A, B and C.

According to planned protocol, 5 consecutive patients out of 15 were treated with CPFA-haemofiltration in pure predilution (CPFA-HF predilution group, $n = 5$ sessions in predilution with citrate solution) (Figure 1A), 5 patients with predilution haemodiafiltration (CPFA-HDF predilution group, $n = 10$ sessions in predilution with citrate solution + dialysate solution) (Figure 1B) and 5 patients with pre- and postdilution haemofiltration (CPFA-HF pre/postdilution group, $n = 5$ sessions in predilution with citrate solution + infusion in postdilution solution) (see Figure 1C). All the solutions (predilution, dialysate and postdilution) were sterile and pyrogen-free with bicarbonate buffer.

The ACD-containing solution was prepared by nurses immediately before CPFA sessions. To obtain a high volume of effluent, for CPFA-HF predilution group, the predilution solution was made by adding 500 mL of ACD-A solution [ACD-A solution 500 mL, Fresenius Kabi Italia Srl, Isola della Scala (VR), Italy; composition: citrate 112.9 mmol/L, sodium 224 mmol/L] to a bicarbonate-containing solution bag (5 L calcium-free bag, HDF/501, Pierrel Medical Care SpA, Milan, Italy; composition: sodium 143 mmol/L, chloride 103 mmol/L, bicarbonates 40 m/Eq/L). In predilution solution, the final concentration of citrate was 10.2 mmol/L. For CPFA-HF predilution group, the target circuit citrate was 3–4 mmol/L.

For CPFA-HDF predilution and CPFA-HF pre/postdilution groups, the predilution solution was made by adding 1000 mL of ACD-A solution (see above) to a bicarbonate-containing solution bag (see above). In the predilution solution, the final concentration of citrate was 18.8 mmol/L. For the CPFA-HDF predilution and CPFA-HF pre/postdilution groups, the target circuit citrate was 6 mmol/L.

For the CPFA-HDF pre/postdilution group, the dialysate solution was Ca++-free (bag Ci-Ca, Fresenius Medical Care, Bad Homburg, Germany; composition: sodium 133 mmol/L, chloride 116.5 mmol/L, bicarbonate 20 mmol/L, magnesium 0.75 mmol/L, potassium 2 mmol/L, zero calcium and glucose 1 g/L). For the CPFA-HF pre/postdilution group, the postdilution solution contained Ca++ [bag BI32, Hospal, Mirandola, MO, Italy; composition (mmol/L): Na+, 140; K+ 2.0; Ca++ 2; Mg++ 0.75; Cl−: 108; bicarbonate 32; acetate 4; glucose 5.5].

In all patients, a commercial 10% calcium chloride solution was infused by the monitor heparin pump in a separate line at the end of the venous circuit [13, 15]. The infusion rate started at 8.0 mL/h for CPFA-HF predilution group, and at 6 mL/h for CPFA-HDF predilution and CPFA-HF pre/postdilution groups. To maintain systemic iCa++ levels between 0.9 and 1.4 mmol/L, the amount of 10% calcium chloride solution was titrated by 0.5 mL/h increase for each increment of 500 mL of effluent volume [15].

The postdilution infusion and dialysate rates were modified according to the required effluent for the patients.

Monitoring and sampling protocol of CPFA patients

Standard laboratory samples were collected at the beginning of the session every day. Biochemical data including serum creatinine, electrolytes, clotting parameter and haemochromocytometer were determined by standard laboratory methods (Architect c8000, Abbott Italia, Milan, Italy; ACL Top 500, IL Italia, Milan, Italy; XE 2100, Dasit, Milan, Italy).

iCa++, pH and bicarbonates in both blood and effluent samples were sequentially determined during sessions at the beginning, after 10 min, 1, 3, 6 and 9 h. All determinations were done by an emogasanalyzer (Stat Profile M, Nova Biomedical, GEPA s.r.l., Milan, Italy). Systemic blood samples and effluent samples were collected from systemic arterial access and the circuit ultrafiltrate line, respectively. Venous blood samples were taken from the circuit venous line, after the haemofilter and before the venous air bubble trap (see Figure 1).

Citrate determinations in venous plasma and effluent

The measurement of citrate was done 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). The method was validated as previously described [15]. Briefly, the method, which is intended for analysis of citrate in foodstuffs and other materials, was adapted for use in plasma by lowering the sample volume. By this adaptation, we measured

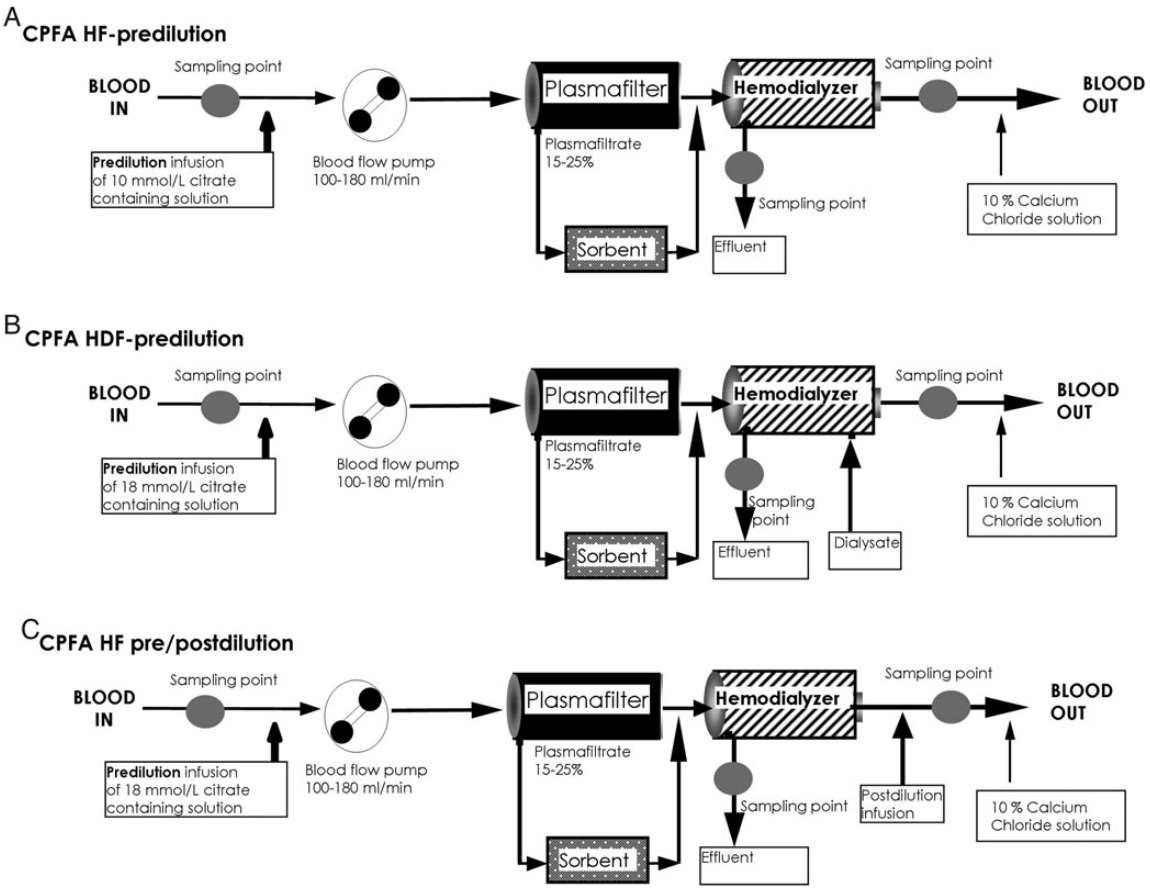

FIGURE 1: Circuits, functional parameters and sampling points of CPFA-HF predilution (**A**), CPFA-HDF predilution (**B**) and CPFA-HF Pre/postdilution (**C**).

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) [15].

Since a sampling point was not available after the citrate infusion point and before the haemofilter (see Figure 1), the prefilter citrate values were calculated according to the following formula:

$$\text{Prefilter citrate (mmol/L)} = \frac{\text{Qcit}_{\text{inf}} * \text{Ccit}_{\text{inf}}}{(\text{QB} * ((100\text{-Htc})/100)) + \text{Qcit}_{\text{inf}}}$$

where
Qcit_{inf} = citrate infusion flow rate (mL/min)
Ccit_{inf} = citrate concentration (mmol/mL)
QB = blood flow rate (mL/min).

Statistical analysis

Values were expressed as median (quartile 1–quartile 3). Data were evaluated by descriptive statistics and non-parametric tests (Mann–Whitney test, Kruskas–Wallis ANOVA test, Spearman *R* test). All statistical tests were done on Statistica spreadsheet (Statistica 6.1, StaSoft Inc., Tulsa, OK). P values <0.05 were considered significant.

RESULTS

Demographic characteristics and biochemical data of the patients are given in Table 1. Of these 15 patients (8 severely burned and 7 polytrauma), 8 died (5 burned and 3 polytrauma patients).

All patients developed a late form of AKI associated with septic shock (Table 1). At the CPFA occurrence (at median delay of 19 days from initial injury, see Table 1), all patients did not present any clinical evidence of muscle injury. All patients were mechanically ventilated and were supported by catecholamine infusion. MAP, catecholamine dosage and routine biochemistry were not significantly different among the groups, whereas a higher SOFA score in CPFA-HDF group predilution tended to be significant (Table 1). Norepinephrine (see Figure 2, lower right panel) and dopamine requirement (data not depicted) did not show any significant change over 9 h CPFA sessions.

Systemic arterial Na+, total Ca++/iCa++ ratio and bicarbonates were stable during the 9-h CPFA sessions (Figure 2). In addition, we did not find any noticeable changes in body temperature due to the cooling effects of the three dialysis modalities.

As shown in Figure 3, in systemic plasma of the CPFA-HF predilution group, the citrate concentrations increased at a maximum of 3-fold (from basal level of 0.23 to 0.86 mmol/L at 3 h). These levels were significantly higher than those observed in

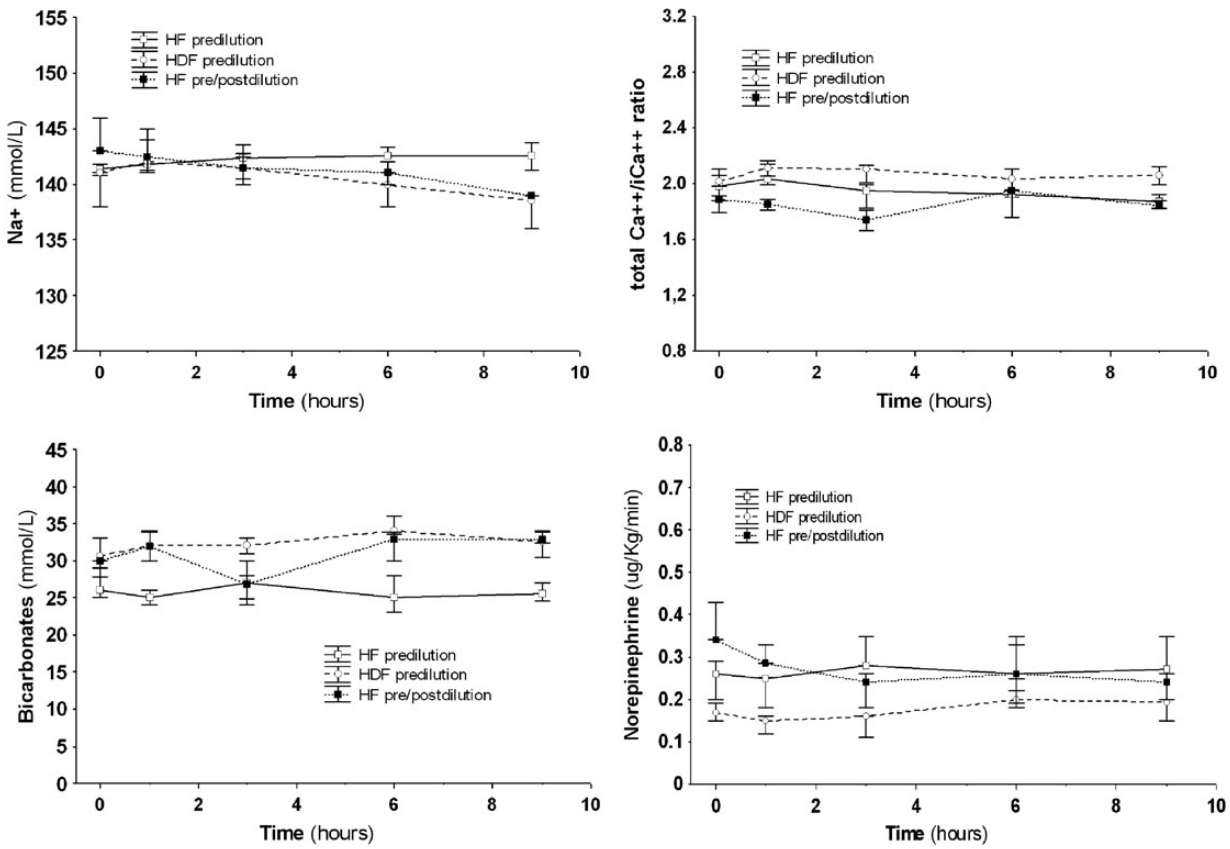

FIGURE 2: Systemic arterial Na+ (upper left panel), bicarbonates (lower left panel), systemic total Ca++/iCa++ ratio (upper right panel) and norepinephrine requirement (lower right panel) over CPFA sessions for groups HF predilution, HDF predilution and HF pre/postdilution. Values are given as median (inter-quartiles).

CPFA-HDF predilution and CPFA-HF pre/postdilution groups (Figure 3).

Figure 4 shows the circuit citrate levels in plasma before the haemofilter (prefilter plasma), after the haemofilter (venous plasma) and in effluent (effluent) for the three groups. In plasmas and effluents, the citrate levels tended to overlap, except for the venous plasmas of the CPFA-HDF predilution group (Figure 4, right panel).

Sieving coefficients for citrate were near the unit in all groups (Figure 5), even if both blood and infusion flow rates in predilution were different among groups (see Table 2). The effluent volumes were overlapping (Table 2), whereas the losses of citrate were significantly different (Figure 6). The percentage of citrate losses varied from 40% for CPFA-HF predilution group to near 60% for both CPFA-HDF predilution and CPFA-HF pre/postdilution group (Figure 6).

Among groups, the absolute values of blood flow rate were different. Besides that, as suggested by the inter-quartiles, the variability of median blood flows measured during each 9-h study period was low (Table 2). As the amount of citrate required during each 9-h study period was related to blood flow rate, citrate showed the same pattern of variability. The plasma

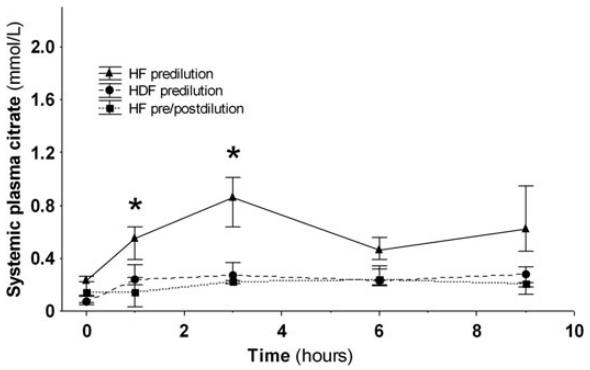

FIGURE 3: Systemic citrate concentrations (mmol/L) in plasmas over CPFA sessions for groups HF predilution, HDF predilution and HF pre/postdilution. Values are given as median (inter-quartiles). *P-value <0.05 between groups (Kruskal–Wallis ANOVA).

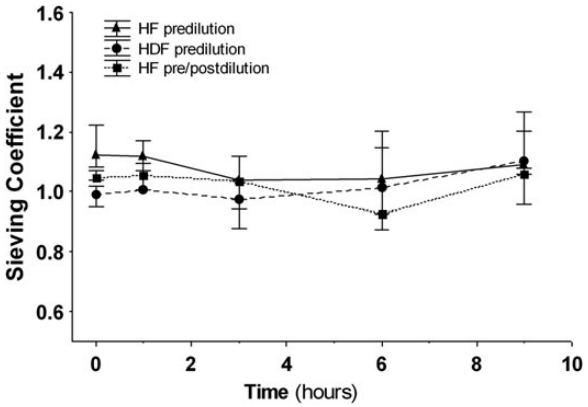

FIGURE 5: Citrate sieving coefficient over CPFA sessions for groups HF predilution, HDF predilution and HF pre/postdilution. P-values >0.05 between groups (Kruskal–Wallis ANOVA).

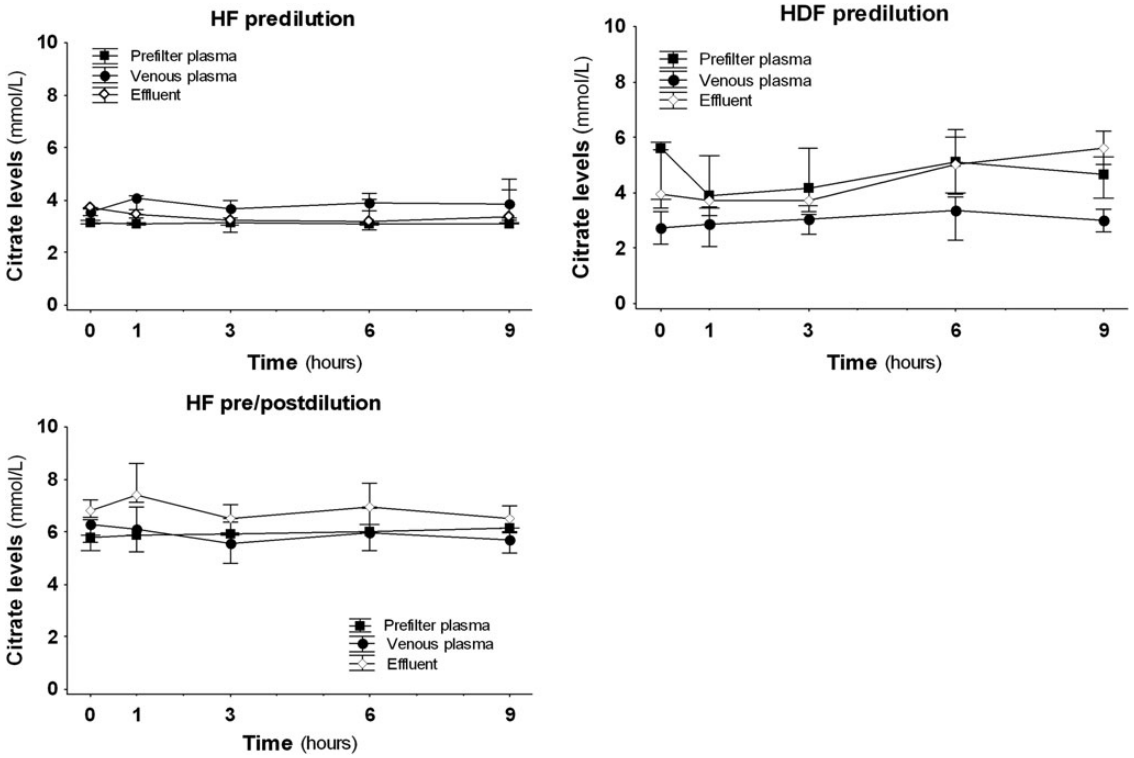

FIGURE 4: Citrate concentrations in extracorporeal circuit plasmas over CPFA sessions. Figure shows the citrate determinations in pre-plasma filter (prefilter plasma citrate), post-haemofilter (venous plasma citrate) and effluent for group HF predilution (upper panel), HDF predilution (right panel) and HF pre/postdilution (lower panel).

Table 2. Dialysis flow rates for patients of groups HF predilution, HDF predilution and HF pre/postdilution

	HF predilution	HDF predilution	HF pre/postdilution	P[a]
Patients (*n*)	5	5	5	-
Blood flow rate (mL/min)	150 (150–165)	110 (100–120)	140 (140–150)	<0.05
Infusion flow rate pre (mL/h)	3000 (3000–3300)	1600 (900–2400)	2000 (2000–2300)	<0.05
Dialysate flow rate (mL/h)	-	2000 (2000–2450)	-	-
Infusion flow rate post (mL/h)	-	-	1300 (1000–1500)	-
Effluent flow rate (mL/h)	3900 (3750–4200)	4100 (3400–4400)	3500 (3000–4200)	>0.05
Calcium chloride (mmol/L)[b]	1.83 (1.81–1.85)	1.10 (1.20–1.00)	1.23 (1.17–1.42)	>0.05
Plasma flow rate (mL/min)	32.0 (30.0–33.0)	24 (24–25)	28 (28–30)	<0.05
Plasmafiltration rate (L/kg/day)	0.19 (0.16–0.22)	0.20 (0.13–0.22)	0.17 (0.17–0.23)	>0.05

Data are expressed as median (quartile 1–quartile 3).
[a]P-value with Kruskal–Wallis test.
[b]Calcium chloride infusion is expressed as mmol/litre of effluent.

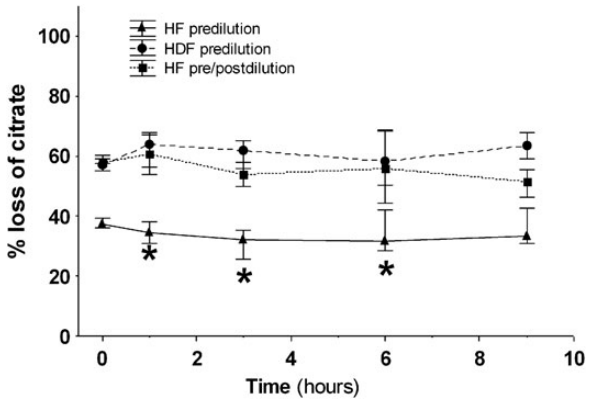

FIGURE 6: Losses of citrate in effluent during CPFA sessions. Figure shows the percentage of infused citrate lost in effluent for groups HF predilution, HDF predilution and HF pre/postdilution. *P-value <0.05 between groups (Kruskal–Wallis ANOVA).

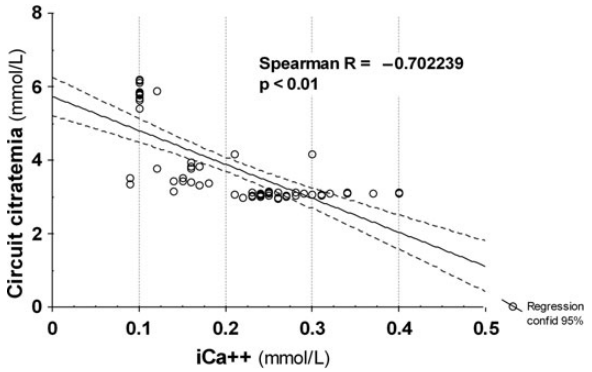

FIGURE 7: Citrate relationship between iCa++ and citrate in blood circuit. Figure shows the significant direct inverse relationship between prefilter plasma citrate concentrations and venous iCa++ levels (Spearman $R = -0.7022$, $P < 0.01$). The relationship was evaluated for citrate concentrations from 2 to 8 mmol/L.

filtration was maintained around 20% of blood flow rate, and the efficiency of plasmafiltration, expressed as mL/kg/die of treated plasma, was similar in the groups (Table 2).

Mean iCa++ concentrations in venous plasma at prefilter citrate levels of <4 and >4 mmol/L were 0.27 (0.17–0.28) and 0.10 (0.10–0.12) mmol/L, respectively ($P < 0.05$). In addition, we found the citrate dependence of iCa++ concentrations in circuit plasma patients. As shown in Figure 7, prefilter plasma citrate concentrations (from 3 to 8 mmol/L) strongly correlated with venous iCa++ levels (Spearman $R = -0.7023$, $P < 0.01$).

DISCUSSION

The results of the present study demonstrated that during CPFA with regional citrate anticoagulation, the citrate losses in effluent could be as high as 60% in predilution haemodiafiltration and pre/postdilution haemofiltration. Therefore, at high levels of circuit citrate, both these modalities of dialysis are suitable for an optimization of citrate losses.

During CPFA, it is mandatory to obtain adequate anticoagulation to prevent not only the coagulation of blood lines, but also the formation of fibrin fragments in the plasma circuit. The requirement of heparin could be as high as 800–1000 U/h [2–6, 8, 10]. The importance of this observation was strengthened by a recent multicentre trial showing that heparin was not an adequate anticoagulant for CPFA [10]. For enrolled patients at high bleeding risk, the circuit anticoagulation was limited by a conservative heparin dosage. This led to a frequent premature clotting of the circuit and a low volume of plasma treated per day. As a result, the study terminated early, and the next randomized trial on CPFA (COMPACT 2) is going to be carried out only with citrate anticoagulation [10].

As a matter of fact, over the last 15 years, citrate has emerged as an effective alternative anticoagulant in patients needing CRRT [7, 20, 21]. Advances in citrate anticoagulation have automatically implemented it into dialysis machine, with clinically efficient and low cost circuit design. Nowadays, nursing staff can set the desired circuit citrate concentration, up to 5–6 mmol/L. In 2012, Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommended the use of citrate for patients on CRRT who do not have contraindications. Among contraindications, KDIGO guidelines include septic shock, the usual clinical condition in patients undergoing CPFA [19]. Actually, CPFA was usually carried out with heparin as anticoagulant on patients with haemodynamic instability, multiorgan failure and alterations of coagulation and platelet count [2–6, 8, 10, 22].

However, the KDIGO position about citrate and septic shock is not accepted unanimously by the nephrology community [23, 24]. Concerning CPFA and regional citrate

anticoagulation, reported experience is limited. More than 10 years ago, a pilot study showed that citrate was a good alternative to conventional heparin as for circuit patency and metabolic tolerance [5]. A recent report confirmed the feasibility, efficacy and safety of citrate for CPFA patients [22]. In addition, in patients with severe liver failure, it has been described an adapted regional citrate anticoagulation safely performed in conventional CRRT and extracorporeal liver assist devices [25–27]. However, so far there are no available data on citrate pharmacokinetics at high circuit citrate concentrations with either diffusive or convective dialysis modalities.

In our study, we assessed the capacity of three different dialysis protocols of removing citrate in effluent during CPFA. We focused on trying to obtain both a high level of citrate in the extracorporeal circuit and a normal citrate concentration in systemic blood by reducing the citrate load given to the patient. It is well known that at plasma citrate concentrations of 3–4 mmol/L blood is largely anticoagulated. However, since previous experiences demonstrated a linear inverse relationship between citrate and iCa++ levels up to a citrate concentration of 8 mmol/L [28], we chose to work at a circuit citrate target of 3–8 mmol/L. As a matter of fact, we found a significant difference in median iCa++ levels when circuit citrate was below 4 mmol/L or above 4 mmol/L. In addition, there was a significant inverse relationship between circuit citrate and iCa++ concentrations (Figure 7). Therefore, high citrate levels up to 6–8 mmol/L could be advantageous to obtain an iCa++ level below 0.1 mmol/L and a more complete blood anticoagulation.

All treated patients showed a high vasopressor requirement due to refractory septic shock, and they suffered from hepatic dysfunction and coagulation disorders (Table 1). Even if the severity of liver disease and the impaired muscle microcirculation are often difficult to quantify, these are all well-known factors for the risk of citrate accumulation [16]. As shown in Figure 4, in prefilter plasma (before the plasma filter), the levels of citrate reached 5–6 mmol/L during both HDF predilution and HF pre/postdilution. During HF predilution, circuit citratemia was only 4 mmol/L, a lower value due to the effect of a higher predilution volume. As previously reported [5], the same citrate levels of prefilter plasma were also present in plasmafiltrate before and after the cartridge. These citrate concentrations were high enough to ensure the patency of cartridge over the whole length of session and to avoid a premature circuit clotting. And as shown in the COMPACT trial [10], the premature circuit clotting was critical to the inadequate generation of treated plasma volume per day and to the trial failure.

In our septic shock patients, the median basal level of systemic citratemia was 0.10 mmol/L (Figure 3), similar to reference value <0.140 mmol/L reported in normal subjects [13, 14]. As shown in Figure 3, during both HDF predilution and HF pre/postdilution modalities, the systemic citrate concentrations slightly increased to 0.40 mmol/L, whereas during HF predilution at 3 h, they significantly reached the values of 0.8 mmol/L. These systemic citrate concentrations were not high enough to change total Ca++/iCa++ ratio, as it is just an indirect parameter of citrate accumulation [15, 29]. However, the lowest concentrations of systemic citrate (see Figure 3) were found in the same patients with the highest concentrations of circuit citrate (Figure 4, left lower panel and right panel). These data suggest that both HDF predilution and HF pre/postdilution are the best modalities for citrate pharmacokinetics, because they can ensure a high efficiency of plasma generation and high levels of safety.

As shown in the curves of prefilter, venous plasma and effluent citrate (Figure 4), a large part of infused citrate amount was lost in the effluent. In the lower panel of Figure 4, the effluent citrate concentrations were higher than prefilter plasma concentrations. As matter of fact, like all other anions (for example HCO3 has a sieving coefficient near 1.1), citrate has a sieving coefficient just above 1 due to Gibbs–Donnan effects.

In addition, we found some other differences among the tested dialysis modalities. As expected by its molecular weight of 192 Daltons, citrate was free filtered in both diffusion and convection modalities [30]. Therefore, without distinction in haemodiafiltration and haemofiltration, the amount of citrate removal was proportional to the effluent volume. However, data showed a high rate of citrate removal (at ∼60%, Figure 6) in HDF predilution. This large loss of citrate was obtained at a high dialysate (and effluent) volume and at a relative low blood flow of 110–120 mL/min (Table 2). This observation was consistent with operative conditions of haemodiafiltration at dialysate-limited flux, in which any increased solute removal followed a proportional increment of effluent [31].

In both HF predilution and HF pre/postdilution, we had to set the blood flow at 140–150 mL/min to obtain a high volume of effluent and simultaneously a high plasma volume generation (at ∼30 mL/min) (Table 2). However, the citrate removal was significantly lower on HF predilution modality (Figure 6), as the level of citrate in the effluent (and in circuit plasma, see upper panel of Figure 3) was only 4 mmol/L. In fact, in this modality, the high rate of predilution infusion diluted the circuit plasma >30%, as citrate does not enter in red cells [15]. At blood flow rate of 150 mL/min and circuit citrate concentration of 6 mmol/L, the potential citrate load was up to 50 mmol/h. In these operative conditions, the loss of citrate by effluent was mandatory, as it was the unique factor able to restrain the citrate load and the risk of its accumulation. By an efficient citrate loss in effluent, the HDF predilution and HF pre/postdilution modalities were able to assure a good metabolic stability. This was not entirely true for HF predilution. As shown by the slightly increased citratemia (Figure 3), in group HF predilution the citrate load exceeded the metabolic capacity of patients.

In conclusion, the present study demonstrated that during CPFA with circuit citratemia of 6 mmol/L either HDF predilution or HF pre/postdilution dialysis modalities can sustain a normal systemic citratemia by a high rate of citrate losses in effluent. Therefore, in refractory septic shock patients with AKI, these dialysis modalities are the most suitable for decreasing the hazard of citrate accumulation.

CONFLICT OF INTEREST STATEMENT

None declared. We state that the results presented in this paper have not been published previously in whole or part, except in abstract format.

REFERENCES

1. Tetta C, Gianotti L, Cavaillon JM *et al.* Continuous plasmafiltration coupled with sorbent adsorption in a rabbit model of endotoxic shock. Crit Care Med 2000; 28: 1526–1533
2. Ronco C, Brendolan A, Lonnemann G *et al.* A pilot study of coupled plasma filtration with adsorption in septic shock. Crit Care Med 2002; 30: 1250–1255
3. Formica M, Olivieri C, Livigni S *et al.* Hemodynamic response to coupled plasmafiltration-adsorption in human septic shock. Intensive Care Med 2003; 29: 703–708
4. Bellomo R, Tetta C, Ronco C. Coupled plasma filtration adsorption. Intensive Care Med 2003; 29: 1222–1228
5. Mariano F, Tetta C, Stella M *et al.* Regional citrate anticoagulation in critically ill patients treated with plasma filtration and adsorption. Blood Purif 2004; 22: 313–319
6. Mao HJ, Yu S, Yu XB *et al.* Effects of coupled plasma filtration adsorption on immune function of patients with multiple organ dysfunction syndrome. Int J Artif Organs 2009; 32: 31–38
7. Oudemans-van Straaten HM. Hemostasis and thrombosis in continuous renal replacement treatment. Semin Thromb Hemost 2015; 41: 91–98
8. Abdul Cader R, Abdul Gafor H, Mohd R *et al.* Coupled Plasma Filtration and Adsorption (CPFA): a single center experience. Nephrourol Mon 2013; 5: 891–896
9. du Cheyron D, Bouchet B, Bruel C *et al.* Antithrombin supplementation for anticoagulation during continuous hemofiltration in critically ill patients with septic shock: a case-control study. Crit Care 2006; 10: R45
10. Livigni S, Bertolini G, Rossi C *et al.* Efficacy of coupled plasma filtration adsorption (CPFA) in patients with septic shock: a multicenter randomised controlled clinical trial. BMJ Open 2014; 4: e003536
11. Palsson R, Niles JL. Regional citrate anticoagulation in continuous venovenous hemofiltration in critically ill patients with high risk of bleeding. Kidney Int 1999; 55: 1991–1997
12. Oudemans van Straaten H, Bosman RJ, Koopmans M *et al.* Citrate anticoagulation for continuous venovenous hemofiltration. Crit Care Med 2009; 37: 232–239
13. Mariano F, Tedeschi L, Morselli M *et al.* Normal citratemia and metabolic tolerance of citrate anticoagulation for hemodiafiltration in severe septic shock burn patients. Intensive Care Med 2010; 36: 1735–1743
14. Hetzel GR, Schmitz M, Wissing H *et al.* Regional citrate versus systemic heparin for anticoagulation in critically ill patients on continuous venovenous haemofiltration: a prospective randomized multicentre trial. Nephrol Dial Transplant 2011; 26: 232–239
15. Mariano F, Morselli M, Bergamo D *et al.* Blood and ultrafiltrate dosage of citrate as a useful and routine tool during continuous venovenous haemodiafiltration in septic shock patients. Nephrol Dial Transplant 2011; 26: 3882–3888
16. Kramer L, Bauer E, Joukhadar C *et al.* Citrate pharmacokinetics and metabolism in cirrhotic and noncirrhotic critically ill patients. Crit Care Med 2003; 31: 2450–2455
17. Bellomo R, Ronco C, Kellum JA *et al.* Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004; 8: R204–R212
18. Ronco C, Brendolan A, d'Intini V *et al.* Coupled plasma filtration adsorption: rationale, technical development and early clinical experience. Blood Purif 2003; 21: 409–416
19. Kidney Disease: Improving Global Outcomes (KDIGO)Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl 2012; 2: 95–100
20. Wu MY, Hsu YH, Bai CH *et al.* Regional citrate versus heparin anticoagulation for continuous renal replacement therapy: a meta-analysis of randomized controlled trials. Am J Kidney Dis 2012; 59: 810–818
21. Zhang Z, Hongying N. Efficacy and safety of regional citrate anticoagulation in critically ill patients undergoing continuous renal replacement therapy. Intensive Care Med 2012; 38: 20–28
22. Pozzato M. Citrate: an additional resource for anticoagulation in continuous replacement therapy. G Ital Nefrol 2012; 29: 20–26
23. James M, Bouchard J, Ho J *et al.* Canadian Society of Nephrology commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. Am J Kidney Dis 2013; 61: 673–685
24. Fiaccadori E, Pistolesi V, Mariano F *et al.* Regional citrate anticoagulation for renal replacement therapies in patients with acute kidney injury: a position statement of the Work Group "Renal Replacement Therapies in Critically Ill Patients" of the Italian Society of Nephrology. J Nephrol 2015; 28: 151–164
25. Morath C, Miftari N, Dikow R *et al.* Sodium citrate anticoagulation during sustained low efficiency dialysis (SLED) in patients with acute renal failure and severely impaired liver function. Nephrol Dial Transplant 2008; 23: 421–422
26. Faybik P, Hertz H, Mitterer G *et al.* Regional citrate anticoagulation in patients with liver failure supported by a molecular adsorbent recirculating system. Crit Care Med 2011; 39: 273–279
27. Balogun RA, Turgut F, Caldwell S *et al.* Regional citrate anticoagulation in critically ill patients with liver and kidney failure. J Nephrol 2012; 25: 113–119
28. Bakker AJ, Boerma EC, Keidel H *et al.* Detection of citrate overdose in critically ill patients on citrate-anticoagulated venovenous haemofiltration: use of ionised and total/ionised calcium. Clin Chem Lab Med 2006; 44: 962–966
29. Hetzel GR, Taskaya G, Sucker C *et al.* Citrate plasma levels in patients under regional anticoagulation in continuous venovenous hemofiltration. Am J Kidney Dis 2006; 48: 806–811
30. Ahmad S, Yeo K, Jensen WM *et al.* Citrate anticoagulation during in vivo simulation of slow hemofiltration. Blood Purif 1990; 8: 177–182
31. Fiaccadori E, Regolisti G, Cademartiri C *et al.* Efficacy and safety of a citrate-based protocol for sustained low-efficiency dialysis in AKI using standard dialysis equipment. Clin J Am Soc Nephrol 2013; 8: 1670–1678

Received for publication: 5.2.2015; Accepted in revised form: 6.7.2015