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DISSERTATION

Incidence and outcome of citrate accumulation in critically ill patients undergoing continuous renal replacement therapy with regional citrate anticoagulation

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In memory of my beloved Father. όλα καλά, rest in peace Dad...

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1. **Abstract**

Background: Systemic citrate accumulation is a complication of regional citrate anticoagulation (RCA) during continuous renal replacement therapy (CRRT). Impaired liver function, shock, hypoxia and hypoperfusion were reported to be a risk factor. The objective of the present study was to determine incidence of clinical signs consistent with citrate accumulation in a large and representative cohort of ICU patients with acute renal injury undergoing RCA-CRRT.

Methods: Data from 2008 to 2010, taken from six intensive care units (ICU) of Charité Universitätsmedizin Berlin, were retrospectively analyzed. RCA was performed in conjunction with continuous veno-venous hemodialysis (RCA-CVVHD). We employed rapidly decreasing systemic ionized calcium (iCa) concentration along with increasing demand for systemic calcium substitution, development of an elevated total calcium/ionized calcium ratio (tCa/iCa), and metabolic acidosis with or without elevated anion gap as indicators for citrate accumulation.

Results: A total of 1070 patients were treated with RCA-CVVHD in the 3-year period. Metabolic signs of citrate accumulation occurred in 32 patients (2.99%, 64.5±14.0 years old, 65.6% male, APACHE score 34.2±9.7). In these patients systemic iCa concentration decreased to 1.01±0.10 mmol/l with a parallel need to increase the calcium substitution rate to 129±26%. The ratio of tCa/iCa went up to 2.51±0.54. At the time of clinical diagnosis of citrate accumulation, all 32 patients had therapy resistant shock with severe lactic acidosis (pH 7.20±0.11, lactate 136±61 mg/dl, bicarbonate 14.8±3.1 mmol/l) indicating severe cellular hypoxia. None of the patients survived. Eleven of the thirty-two patients (34.4%) had known pre-existing liver dysfunction. Median S-bilirubin before ICU admission was 1.4 mg/dl (0.3-24.6 mg/dl).

Conclusions: The incidence of citrate accumulation in ICU patients treated with RCA-CVVHD was, at 2,99%, rather low. Metabolic disarrangements consistent with citrate accumulation took place exclusively in patients with severe lactic acidosis due to multiorgan failure and having a poor outcome per se. All patients with signs of citrate accumulation died during their ICU stay. The results suggest that the appearance of citrate accumulation is secondary to a severe failure of cellular respiration.

Hintergrund: Die systemische Zitratakkumulation stellt eine Komplikation der regionalen Zitratantikoagulation bei kontinuierlicher Nierenersatztherapie dar. Als Risikofaktoren der Zitratakkumulation gelten Hypoxie, verminderte Gewebeperfusion, eingeschränkte Leberfunktion und Schock. Ziel der vorliegenden Arbeit war es, anhand einer repräsentativen Kohorte von kritisch kranken Patienten mit diaylsepflichtigem akutem Nierenversagen die Häufigkeit der Zitratakkumulation während der kontinuierlichen Nierenersatztherapie mit regionaler Zitratantikoagulation zu ermitteln.

Methoden: Die Daten aller Nierenersatzverfahren im Zeitraum von 2008 bis 2010 von den 6 Intensivstationen der Charité Campus Mitte, Universitätsmedizin Berlin wurden gesammelt und retrospektiv analysiert. Regionale Zitratantikoagulation wurde in Verbindung mit kontinuierlicher veno-venöser Hämodialyse (RCA-CVVHD) als eine Form der kontinuierlichen Nierenersatztherapie standardisiert durchgeführt. Eine rasch fallende Konzentration des ionisierten Kalziums bei gleichzeitig steigendem Kalziumsubstitutionsbedarf, ein erhöhter Quotient aus Gesamtkalcium zu ionisierten Kalzium, sowie das Auftreten einer metabolischen Azidose mit oder ohne erweiterter Anionenlücke wurden als Indikatoren der Zitratakkumulation gewertet.

Ergebnisse: Im analysierten Zeitraum von 3 Jahren wurden insgesamt 1070 Patienten mit CVVHD und regionaler Zitratantikoagulation auf den Intensivstationen der Charite Campus Mitte behandelt. Zeichen der Zitratakkumulation traten bei 32 Patienten (2.99%, 64.5±14.0 Jahre alt, 65.6% männlich, APACHE-Score 34.2±9.7) auf. Während der CVVHD-Behandlung ist neben des erhöhten Kalziumsubstitutionsbedarf (129 ± 26% im Vergleich zur Kalziumsubstitutionsrate bei CVVHD-Beginn) ein deutlicher Abfall des ionisierten Kalziums (auf 1,01 ± 0,10 mmol/L) sowie ein Anstieg des Quotient aus Gesamtkalzium zu ionisierten Kalzium (2,51 ± 0,54 mmol/L) zu beobachten. Zum Zeitpunkt der Diagnose der Zitratakkumulation befanden sich alle Patienten in einem therapierefraktärem Schock mit schwerer Laktatazidose (pH 7.20±0.11, Laktat 136±61 mg/dl, Bikarbonat 14.8±3.1 mmol/l), was auf eine schwerwiegende intrazelluläre Hypoxie hindeutete. Alle Patienten mit Zitratakkumulation sind in Folge des Schocks bzw. des Multiorganversagens verstorben. 11 von 32 Patienten (34.4%) hatten eine vorbestehende Leberinsuffizienz. Das mediane Serum-Bilirubin vor der Aufnahme auf die ITS betrug 1.4 mg/dl (0.3-24.6 mg/dl).

Schlussfolgerung: Die Inzidenz der Zitratakkumulation bei kritisch kranken

dialysepflichtigen Patienten während kontinuierlicher Nierenersatztherapie mit regionaler Zitratantikoagulation betrug 2.99%. Die metabolischen Veränderungen, die auf eine Zitratakkumulation hingewiesen haben, traten ausschließlich bei Patienten mit schwerer Laktatazidose aufgrund eines Multiorganversagens und folglich eingeschränkter Prognose auf. Alle Patienten mit Zeichen der Zitratakkumulation verstarben während des ITS-Aufenthalts. Die Ergebnisse sprechen für das Auftreten der Zitratakkumulation als Folge eines schwerwiegenden Versagens der Zellatmung.

2. Introduction

Continuous renal replacement therapy (CRRT) is increasingly applied in critically ill patients with acute kidney injury who requires renal support [1]. CRRT is often preferred to intermittent dialysis because of better hemodynamic stability and metabolic control [2]. A major drawback of CRRT is the need for continuous anticoagulation due to triggered hemostasis. Regional citrate anticoagulation (RCA) during CRRT (RCA-CRRT) is a common alternative to systemic heparin anticoagulation. Indeed, in recently published guidelines, RCA is now recommended as the anticoagulation strategy of choice in patients undergoing CRRT without contraindication to citrate and not already receiving effective systemic anticoagulation, regardless of their bleeding risk [3]. According to KDIGO guidelines, the major contraindications for RCA are: severely impaired liver function, or shock with muscle hypoperfusion - both representing a risk of citrate accumulation [3].

There are numerous diverse protocols for RCA-CRRT with different efficacy published in literature. Most of them show the advantages of RCA over systemic heparin anticoagulation in metabolic control alone with less bleeding complications [4]. Nevertheless, citrate anticoagulation is not yet the standard anticoagulation mode for CRRT in most intensive care units (ICU) [5-7]. Due to the complex metabolism of citrate and highly diverse approaches regarding composition of dialysis fluids as well as the lack of standardization, RCA still remains a challenge for ICU-team, especially with limited experience and the absence of established protocol for CRRT. Unwillingness to switch to RCA is also explained by concerns about safety and possible metabolic complications of RCA in different types of protocols for RCA in CRRT.

Depending on the protocol used, reported complications of RCA are: metabolic alkalosis, hypernatremia, hypo-/ hypocalcaemia and citrate accumulation [4]. Recently published studies provides clear and easy-to-handle protocols for citrate anticoagulation using commercially available solution with precise recommendations in the case of metabolic disarrangements providing excellent acid-base and calcium homeostasis with no incidence of hypernatremia [8-11]. However, citrate accumulation remains a serious complication of CRRT with RCA. Citrate is an intermediate of energy metabolism and is not toxic itself. Its accumulation, however, could cause severe metabolic acidosis, decrease cardiac contractility or cause arrhythmias, as symptoms of systemic ionized hypocalcemia [12]. Acute or chronic impaired liver function, shock with arterial hypoxia

and reduced tissue perfusion are the major risk factors for citrate accumulation [3, 13, 14]. Unfortunately, measurement of citrate concentration in blood is not available on a daily routine basis, and, at least in Germany, available test kits are not approved for clinical use. However, there are commonly accepted markers for citrate accumulation such as: metabolic acidosis with or without increased anion gap, ionized hypocalcemia with simultaneous increased levels of total calcium and an increased total calcium to ionized calcium ratio (tCa/iCa) [13-15]. Those laboratory parameters do not confirm citrate accumulation in all cases, and often lead to false positive results, which makes the diagnosis of citrate accumulation a complex clinical issue. Moreover, information about the incidence of citrate accumulation in a general cohort of ICU patients undergoing RCA-CRRT is relatively limited due to the fact that patients with risk factors for citrate accumulation were either excluded from the prospective trials [8, 11, 16-19], or the observation of those patients was limited to the study period [20].

In this monocentric retrospective study we collected and analyzed all patients on RCA-CRRT over a three-year period (from 2008 to 2010) in order to identify risk factors for citrate accumulation. The primary objective was to reveal the incidence rate of citrate accumulation in a cohort of non-selected critically ill patients receiving RCA-CRRT. The secondary objective was to assess the clinical characteristics and outcome related to citrate accumulation based on a representative population of patients.

2.1 Acute renal injury and continuous renal replacement therapy

Acute kidney injury (AKI) is an abrupt and potentially reversible decrease of kidney function [3]. It is clinically hallmarked with the accumulation of urea along with other uremic toxins, affected fluid balance and electrolyte homeostasis. The definition of acute kidney injury was made further distinct with the implication of the RIFLE and Acute Kidney Injury Network (AKIN) criteria (Table 1) [21, 22].

Table 1: RIFLE and AKIN criteria. *Adopted from [3]*

RIFLE			AKIN	
Class	**Serum creatinine or GFR**	**Urine output** (common for both)	**Serum creatinine**	**Stage**
Risk	Increase in serum creatinine x 1.5 or GFR decrease >25%	<0.5 ml/kg/h for 6-12 hours	Increase of ≥ 0.3 mg/dL (≥26.5 µmol/L) or 1.5- to 2-fold increase from baseline	1
Injury	Serum creatinine x 2 or GFR decreased >50%	<0.5 ml/kg/h for ≥ 12 hours	> 2- to 3-fold increase from baseline	2
Failure	Serum creatinine x 3, or serum creatinine >4 mg/dl with an acute rise >0.5 mg/dl) or GFR decreased >75%	<0.3 ml/kg/h for ≥ 24 hours or anuria for ≥ 12 hours	Increased to > 3-fold from baseline, or more than or equal to 4.0 mg/dl with an acute increase of at least 0.5 mg/dl or on RRT	3
Loss	Persistent acute renal failure=complete loss of kidney function >4 weeks			
End-Stage	ESRD >3 months			

AKI is a common complication in critically ill patients in the intensive care unit (ICU), with the reported incidence from 1 to 25% [1, 23]. Approximately 4% of such patients require renal replacement therapy (RRT) [1]. Although there is no agreement and available evidence on the best therapy mode for RRT in critically ill patients [6, 24], continuous renal replacement therapy (CRRT) is frequently preferred to intermittent dialysis especially in hemodynamic instable patients due to better fluid balance, less need for inotrops, advanced metabolic control, improved control of solute concentration, without rapid fluid shifts and the greater flexibility of the therapy modalities [2, 25, 26]. Thus, in a multinational, multicenter, prospective, epidemiological survey of acute renal injury at ICU, 80% of patients requiring RRT were treated with CRRT [7]. Indeed, according to the recent recommendations, CRRT should be considered as a first choice RRT-modality at hemodynamically unstable patients [3].

Despite obvious advantages of CRRT over standard intermittent RRT, there are some drawbacks as well; need for immobilization, high costs and necessity for

continuous anticoagulation are all major drawbacks of CRRT.

2.2 Anticoagulation for continuous renal replacement therapy

In patients with AKI undergoing RRT, the blood is conducted through the extracorporeal circuit and dialysis filter. The contact of blood with the alien surface and air, as well as non-laminar flow in the extracorporeal tubing system, results in activation of plasmatic coagulation, tissue factors, leucocytes and platelets, which initiate a clotting. Premature filter clotting reduces circuit lifetime and treatment efficacy, and increases blood loss, workload and costs. Therefore, improving circuit life is clinically relevant, especially in terms of CRRT. Protocols involving CRRT with no anticoagulation were studied in small trials where adequate CRRT-circuits survival was described only in patients with some degree of coagulopathy [27-29]. Thus anticoagulation is generally required, representing a great challenge for critically ill patients. Until recently, systemic anticoagulation with heparins was a common choice.

2.2.1 Heparin anticoagulation

Since its first implementation at the beginning of the 20th century, unfractionated heparin (UFH), and, much later, low-molecular-weight heparin (LMWH) were considered standard anticoagulation modalities and, thus, were the most commonly prescribed anticoagulant agents used during continuous renal replacement therapy (CRRT) [7]. The overview of advantages and disadvantages of each type of heparin is presented in Table 2.

Table 2.: Overview of each heparin type. Adopted from [3].

Anticoagulant	Advantage	Disadvantage
Unfractionated heparin (UFH)	Wide availability Large experience avialable Short half-life Available antagonisation Monitoring with routine tests (aPTT, ACT) Low costs	Narrow therapeutic index, and thus elevated risk of bleeding Unpredictable kinetics with necessary monitoring Possible development of HIT Heparin resistance

Low-molecular-weight heparin (LMWH) *	More predictable kinetics with possible weight-based dosing More reliable anticoagulant response with no required monitoring Reduced risk of HIT	Risk of accumulation in kidney failure Monitoring requires non-routine test (anti–Factor Xa) Different drugs not interchangeable Incomplete reversal by protamine In most countries more expensive than unfractionated heparin

* mostly applicable for the intermittent RRT

Nevertheless, anticoagulation with any kind of heparins has a systemic impact, and thus, their major drawback is bleeding. This is especially the case in CRRT, where continuous anticoagulation is administered. Besides critically ill patients are, *per se,* predisposed to bleeding complications due to endothelial disruption, coagulopathy, recent surgery, trauma or mucosal lesions. Taking into account each of the application methods and heparin agents, the incidence of bleeding varies from 10% to 50%, with a bleeding mortality of 15% [30-32]. Thus, heparin in high doses is at least an important challenge in critically ill patients with active bleeding or at high risk of bleeding [3].

Anticoagulation with heparins can also cause bleeding-independent complications, such as: heparin-induced thrombocytopenia (HIT), with incidence between 1% and 5% [33]; heparin resistance due to reduced antithrombin concentration [34, 35] and heparin-binding proteins [36]; activation of pro-inflamative processes [35, 37, 38]; and impaired microcirculation in sepsis [39, 40].

Hence, there is increasing evidence questioning the safety of heparin anticoagulation during CRRT, particularly in critically ill patients. Several methods of regional anticoagulation, as an alternative to systemic heparin use, have been proposed over the past fifty years. The regional citrate anticoagulation demonstrated most promising results over time. Several randomized clinical trials showed the superiority of RCA over heparin anticoagulation regarding bleeding incidence and need of blood transfusion (Table 3).

Table 3. Comparison of citrate and heparin anticoagulation for CRRT in RCT in respect of bleeding and transfusion. *Adopted from [4].*

Reference	Design	Bleeding		Transfusion (RBC)	
		Citrate	Heparin	Citrate	Heparin
Monchi et al [19]	RCOT, *n = 20*	**n = 0**	**n = 1**	**0.2** (RBC/day*) *P < 0.001*	**1.0** (RBC/day*)
Kutsogiannis et al [16]	RCT, *n = 30*	**n = 1** (RR 0.17) *P = 0.06*	**n = 8**	**n = 15** (RR 0.53) *P = 0.13*	**n = 20**
Betjes et al [41]	RCT, *n = 48*	**0%** *P < 0.01*	**33%**	**0.43** (RBC/day*) *P = 0.01*	**0.88** (RBC/day*)
Oudemans-Van Straaten et al [11]	RCT, *n = 200*	**6%** *P = 0.08*	**16%**	**0.27** (RBC/day*) *P = 0.31*	**0.36** (RBC/day*)
Hetzel et al [8]	RCT, *n = 170*	**14.5%** *P = 0.06*	**5.7%**		

RCOT, randomized cross-over trial; RCT, randomized controlled trial; RR, relative risk. *Number of red cell units per day of continuous venovenous hemofiltration.

Hence, according to the latest guidelines, regional citrate anticoagulation during CRRT should be used independently of the bleeding risk [3].

2.2.2 Regional citrate anticoagulation

Citrate was first applied as a regional anticoagulant for intermittent hemodialysis in the 1960s-1980s [42, 43]. Since the development of the renal replacement therapy, this method has received a lot of implementation. Especially encouraging results were achieved in CRRT, where citrate anticoagulation was first applied in 1990 by Mehta et al [44]. Calcium is an important cofactor required at different steps of the coagulation cascade for proper function. Being infused pre-filter in appropriate doses, citrate acts by chelating the ionized calcium in the extracorporeal circuit, consequently blocking the coagulation cascade (Figure 1).
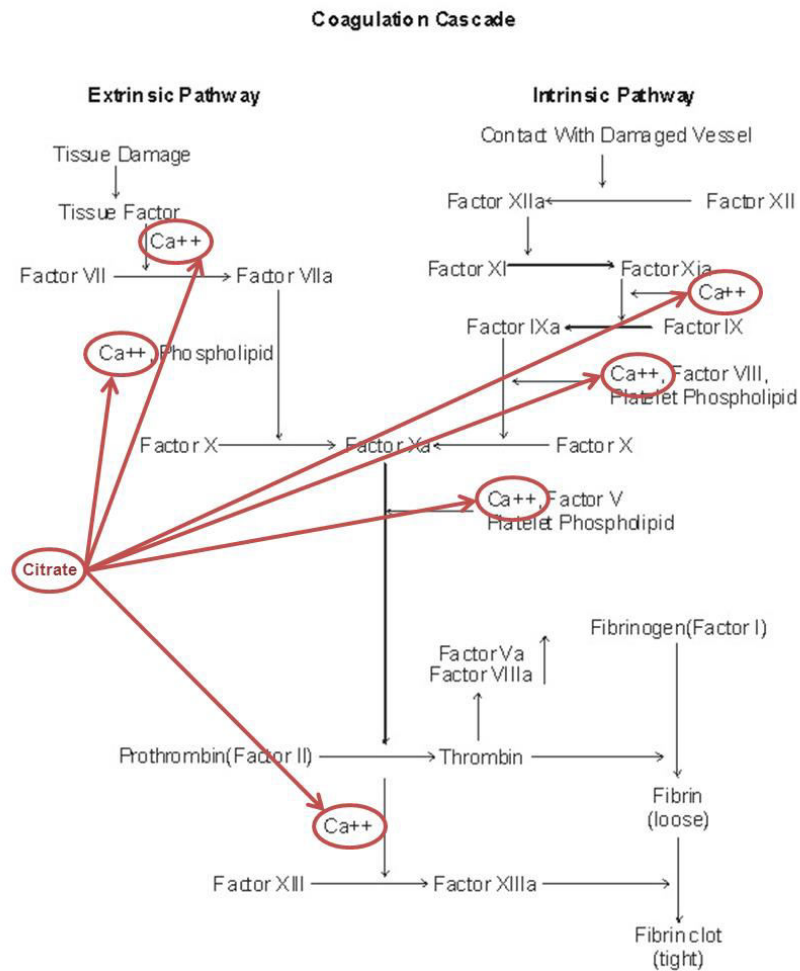
Figure 1. Calcium as a cofactor in the coagulation cascade. *Adopted from [45]*.

A large amount of calcium-citrate complexes are removed by a dialyser, which generally requires use of a calcium-free dialysate, and partially enters the systemic circulation. Having a sieving coefficient of about one, citrate is partially removed by dialysis as iCa-citrate complexes. Depending on the CRRT modality and blood-to-effluent ratio, the removed fraction of citrate varies between 20 and 50%[46]. By entering a patient's blood circulation, unfiltered calcium-citrate complexes are diluted by the total blood volume and are rapidly metabolized in the citric acid cycle, as it is generally supposed, mainly in the liver, kidney and skeletal muscle tissue. Moreover, the metabolism of citrate is necessary to avoid relevant systemic accumulation of citrate and calcium-citrate complexes. The concentration of systemic iCa is partially restored

due to liberation of chelated calcium when citrate is metabolized. In RCA protocol using calcium-free dialysates an additional calcium substitution is needed to compensate for the loss of calcium into the dialysate. The calcium solution is generally infused into the venous line (post-filter) of the extracorporeal circuit. As a result, calcium balance remains in equilibrium. The schematic representation of general principles of regional citrate anticoagulation is demonstrated in Figure 2.
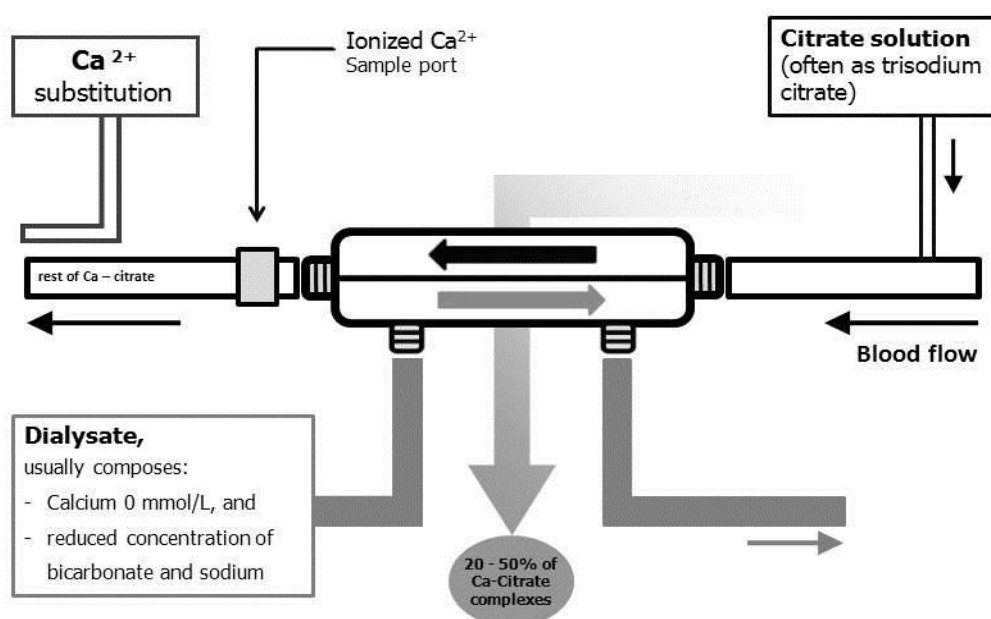
Figure 2. Principles of regional citrate accumulation

Citrate infusion decreases the concentration of iCa in a dose-dependent manner, however the relationship between iCa - concentration and the anticoagulation effect is not in a linear proportion [47].

Anticoagulation is unaffected when iCa concentration is more then 0,50 mmol/L, whereas concentrations of iCa less then 0,25 – 0,35 mmol/L allow near total inhibition of anticoagulation [48, 49]. Thus, the anticoagulation effect can and should be monitored by measuring the concentration of iCa in the extracorporeal circuit (post-filter) with subsequent adjustment of delivered citrate dose according to the post-filter iCa target (0,25 – 0,35 mmol/L) [8, 10]. In a situation with normal concentration of iCa in the

patient's peripheral blood, one would require a citrate dose of about 4 mmol per liter blood in the extracorporeal circuit to reach the mentioned post-filter iCa concentration [8, 10]. Some other protocols propose a less complex approach using a fixed citrate dose proportional to blood flow, with a citrate concentration about 3 mmol per one liter blood. Those protocols, however, did not show the superiority over heparin anticoagulation in terms of circuit lifetime [11].

2.3 Complications of regional citrate anticoagulation

Being consequent with the basic principles of this anticoagulation modality, the most common complications of RCA are:

2.3.1 Hypernatremia

Due to the fact that citrate solution is generally applied as a trisodium citrate, there is a certain risk of accompanied hypernatremia. However, this complication was often a problem at the beginning of clinical implementation of RCA, and with the application of dialysis solutions with reduced sodium concentration for RCA, has become negligible [11, 41, 50].

2.3.2 Metabolic alkalosis

Additionally to its anticoagulation effects, citrate possesses profound effects on acid-base homeostasis as well. Being infused in systemic circulation, in a normal physiological state, citrate is rapidly metabolized to bicarbonate via the Krebs cycle in liver, kidney and skeletal muscle. Trisodium citrate is converted into citric acid, and after metabolism 1 mmol of citrate produces 3 mmol of bicarbonate. Thus, citrate also contributes to the bicarbonate buffer system, and an excessive citrate load leads to metabolic alkalosis. This complication was relatively common in the beginning of clinical implementations of RCA [44]. Nevertheless, the metabolic alkalosis could be easily avoided by using a dialysate solution with reduced bicarbonate concentration [50]. Using such protocols, in case of metabolic alkalosis, bicarbonate overload could be compensated by elevated dialysate flow rate or reduced citrate load [8, 10, 50].

Moreover, in recently published meta-analysis, it has been shown that RCA has no

significant increase in the incidence of metabolic alkalosis in comparison with heparin anticoagulation [51] and, if the RCA protocol is strictly followed, metabolic disarrangements are easy to identify and control [52].

2.3.3 Hyper- or hypocalcemia

The induction of severe systemic hypocalcemia was the most threatening complication of RCA in the early years of implementation [12]. Severe hypocalcemia leads to weakness, muscle cramps, myocardial dysfunction, and, in the most severe cases, death [53-58]. Systemic hypocalcemia during RCA can occur due to an imbalance between the elimination of the calcium through the dialysis filter as part of calcium-citrate complexes and the systemic calcium substitution rate, or technical failure of the calcium replacement system. Currently, the bedside monitoring of the ionized calcium has become a standard method available in almost every intensive care unit and calcium replacement systems have been integrated into the CRRT device. Since then, disarrangements of calcium homeostasis can be avoided and easily detected and corrected. Application of the commercially available dialysis solutions together with CRRT devices designed for RCA-based CRRT minimized the incidence of hypocalcemia to negligible levels [8, 10, 50].

Hypercalcemia is a well-known problem during citrate anticoagulation [59, 60]. However, it could be easily corrected by adjusting the calcium substitution rate, as described elsewhere [10].

In some patients undergoing RCA-CRRT, mild to severe ionized hypocalcemia is accompanied by elevated concentration of total calcium. It is particularly distinct in patients with slowed down or disturbed citrate metabolism for whatever reasons [60]. This condition is often a hallmark of a possible citrate accumulation and would be discussed further.

2.3.4 Citrate accumulation

In contrast to other metabolic disarrangements during RCA-CRRT, citrate accumulation remains a serious complication with often severe consequences. In case of slowed down or disturbed citrate metabolism, concentration of citrate continuously rises during the ongoing RCA-CRRT. Inability to metabolize citrate, and subsequent

citrate accumulation during RCA-CRRT seems to worsen the prognosis of the patients and indicate a high risk of death [61].

Citrate is not toxic itself, but in the case of impaired citrate metabolism, the essential part of the physiologically active ionized calcium remains chelated in the calcium-citrate complexes. Hence, severe ionized hypocalcemia may appear and lead to life-threatening complications (decreased cardiac contractility, arrhythmias, etc.). Thus, ionized hypocalcemia is one of the most sensitive indicator and/or most severe complications of citrate accumulation [62]. In contrast to decreased concentration of active ionized calcium, the concentrations of physiologically inactive total calcium are continuously increasing during the citrate accumulation, owing to a growing fraction of calcium-citrate complexes. Thus the total to ionized calcium ratio is a useful marker to detect citrate accumulation [60]. The ionized hypocalcaemia is often masked due to continuously elevated calcium substitution. That is why continuously increasing calcium demand itself is a possible clinical marker for citrate accumulation. That explains why total-to-ionized calcium ratio is probably the most specific marker for citrate accumulation [60, 62, 63].

Besides decreased levels of ionized calcium and elevated concentration of total calcium, citrate accumulation is accompanied by metabolic acidosis [46]. This occurs because of a negative bicarbonate balance: on the one hand, missing metabolism of citrate to bicarbonate; on the other hand, continuous application of the dialysis solution with reduced bicarbonate concentration applied during RCA-CRRT. Additionally, citrate accumulation goes together with elevation of anion gap, due to an increased concentration of citrate in the blood.

Methods for measurement of citrate concentration in blood are available, but unfortunately, at least in Germany, still not accessible on a daily routine basis. Consequently, one can only speculate the citrate accumulation according to metabolic disarrangements. Taken together, commonly accepted clinical markers for citrate accumulation are:

1) increased ratio of total calcium – to – ionized calcium (tCa/iCa) due to

2) ionized hypocalcemia with simultaneous increased levels of total calcium,

3) metabolic acidosis with or without increased anion gap and

3) elevated demand in calcium substitution.

However, those laboratory parameters do not confirm citrate accumulation in all cases, and often lead to false positive results [14, 15, 60, 64]. That makes the diagnosis of citrate accumulation a complex clinical issue.

Impaired liver function, arterial hypoxia and reduced tissue perfusion are described in literature as risk factors for citrate accumulation. Recently published clinical practice guidelines for acute kidney injury postulates a severely impaired liver function or shock with muscle hypoperfusion as a major contra-indication for the use of RCA [3]. Some other authors report that high concentration of lactate and severe heart failure should also be suggested as risk factors for citrate accumulation [65, 66], as Krebs cycle only works under aerobic conditions.

Nevertheless, there is enough evidence that at least impaired liver function doesn't have to be seen as an absolute contraindication for RCA [20, 64, 67]. Taking into consideration that CRRT is often preferred in hemodynamically unstable patients, recommendation of KDIGO 2012 not to apply RCA-CRRT in patients with septic shock automatically excludes a significant portion of those ICU-patients, who would supposedly benefit from CRRT [11]. As a result, some authors are concerned that a considerable proportion of patients with shock do tolerate citrate anticoagulation, especially those with septic shock and high lactate levels if circulation improves and lactate concentration decreases [46].

Taken together, citrate accumulation, a potentially life-threatening complication of RCA-CRRT, is probably the most important issue that holds back wide implementation of RCA during CRRT. The frequency of this complication in clinical daily routine is of great importance and at the same time remains controversial. For instance, patients with acute liver failure or severe liver cirrhosis were excluded from all until now published randomized trials [3]. Moreover, until recently there were few reports on the incidence and outcome of citrate accumulation in critically ill patients treated with RCA-CRRT. They were based on occasional clinical cases or on the prospective trials what representing a selected cohort of patients observed over a limited timeframe, and which may bring a certain bias leading to underestimation of the incidence rate.

2.4 Aim of the study

In the present single-center retrospective study, all cases with clinical diagnosis of citrate accumulation in critically ill patients undergoing RCA-CRRT over a three-year period were collected and analyzed. Due to the fact that RCA-CVVHD as a modality of CRRT is the modality of choice in all ICUs of our university hospital, the weight-adapted protocol for RCA-CVVHD according to Morgera et al [10], was conducted in all patients requiring CRRT, independent of the patients' liver function status, shock status or risk of bleeding, without any contraindication. This scenario provides a unique opportunity to deal with an unselected representative cohort of patients with diverse morbidity having AKI and undergoing RCA-CRRT.

Thus, the **primary objective** of our study was:

- to reveal the incidence of metabolic disarrangements consistent with citrate accumulation in a cohort of unselected critically ill patients receiving RCA-CRRT.

The **secondary objectives** of our study were:

- **(1)** to assess the common clinical characteristics of patients with citrate accumulation, and

- **(2)** to evaluate the outcome of citrate accumulation based on a representative patient population.

3. Methods and Materials

3.1 Study design and study population

The retrospective single-center study, evaluating the incidence of metabolic disorders consistent with citrate accumulation during RCA-CRRT, was performed in six intensive care units (one general, two surgical, two medical, and one neurological ICU) all having a total of 72 beds at the University Hospital Charité Campus Mitte, Berlin, Germany.

The study was approved by the local ethical review committee (Ethikkommission der Charité - Universitätsmedizin Berlin; EA1/035/12). Upon approval by the local ethical review committee, the need for patients' informed consent was waived due to the observational character of study combined with the absence of any intervention and the anonymisation of all data sets used for analysis.

All patients between January 1, 2008 and December 31, 2010 with kidney injury and treated with the RCA-CRRT, were included in this retrospective analysis. The interdisciplinary team of nephrologists and the ICU staff determined the diagnosis of acute kidney injury and indication for CRRT-treatment.

Patients were identified and data were collected from three different sources:

- a computerized billing database (SAP, Germany)

- the patient data management system used in the ICUs (Computer Organized Patient Report Assistant (COPRA), COPRA System GmbH, Sasbachwalden, Germany), and

- records of the daily prescriptions of renal replacement procedures used by the nursing staff at the nephrology department.

3.2 Continuous renal replacement therapy with RCA

Being the modality of choice in all intensive care units of this hospital, the weight-adapted protocol for continuous veno-venous hemodialysis (CVVHD) with citrate anticoagulation according to Morgera et al [10] was conducted in all patients requiring CRRT, independent of the patients' liver function status or risk of bleeding (Figure 3). Suspected or clinically obvious citrate accumulation was the only indication for termination of RCA-CRRT.
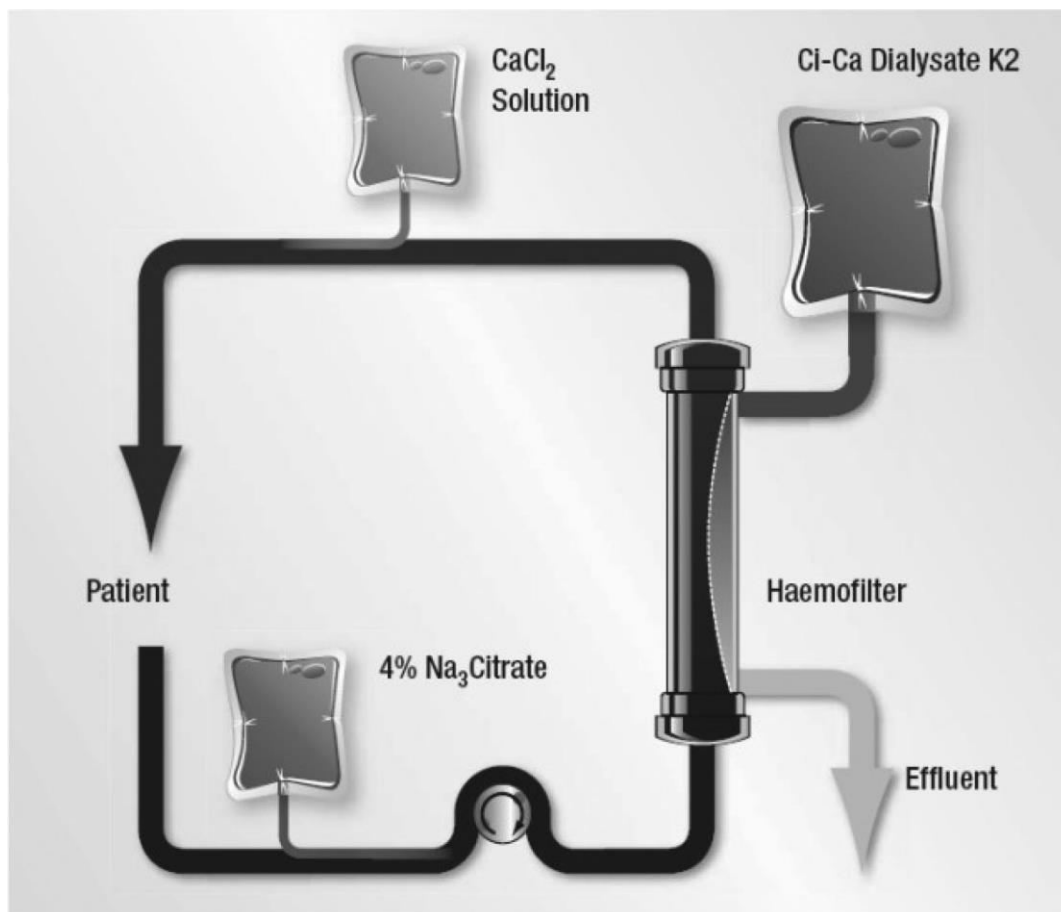
Figure 3. Scheme of the RCA-CVVHD. *From [10].*

Briefly, CVVHD was conducted using multiFiltrate dialysis devices (Fresenius Medical Care AG) or BM25 RRT-devices (Baxter, Germany) and, if not otherwise required, a 1.4 m^2 high-flux polysulfone dialyzer PF140H (Gambro Hospal GmbH, Germany). Conventional hemodialysis catheters, usually placed in the internal jugular or femoral vein, were used for vascular access. The Ci-Ca $^®$ Dialysate K2 solution (Fresenius Medical Care AG, Bad Homburg, Germany) containing 2 mmol/l potassium, 133 mmol/l sodium, 116.5 mmol/l chloride, 20 mmol/l bicarbonate, 0.75 mmol/l

magnesium and zero calcium was used. Blood flow was set accordingly to dialysate flow to maintain a ratio of 3 to 1. Regional citrate anticoagulation was performed by infusion of 4% trisodium citrate solution (136 mmol/l; Fresenius Kabi, Bad Homburg, Germany) in the "arterial" line of the extracorporeal circuit with a starting dose of 4 mmol/l blood. Further, the citrate infusion rate was adjusted to reach post-filter ionized calcium (iCa) levels of 0.25-0.35 mmol/l. Calcium substitution flow ($CaCl_2$ solution, 91 mmol/l) was initiated with 1.7 mmol calcium per liter total effluent flow and adjusted accordingly to maintain patients' ionized calcium in the physiological range of 1.1-1.2 mmol/l. The correction of acid-base status was performed, changing the ratio of blood/dialysate flow: increasing the dialysate flow was used to correct the metabolic alkalosis, whereas decreasing the dialysate flow led to correction of metabolic acidosis. Alternatively, correction of acidosis was managed by increasing the citrate infusion with a parallel increase of blood flow while keeping the dialysate flow constant. Citrate and calcium infusions were performed through integrated citrate and calcium pumps while using multiFiltrate Ci-Ca. The dialysis filters were changed routinely after 72 hours with an allowed tolerance of ±12 hours.

The patients were initially categorized by body weight into three groups (<60kg, 60-90kg and >90kg) and each group was treated at a matching efficacy level by adaptation of dialysate flow to maintain an effective dose of 25-30 ml/kg/h.

3.3 Metabolic disorders consistent with citrate accumulation

As the measurement of citrate blood concentration, at least in Germany, is not clinically approved for clinical use and, so far, not available for routine bedside testing, the diagnosis of citrate accumulation could be suspected according to clinical criteria which have been previously described and are generally accepted, as follows:

1) Decrease of systemic ionized calcium (iCa) (<1.1 mmol/l), despite increasing calcium replacement;

2) Concomitant increase of total calcium concentration and, thus, increase of total to ionized calcium ratio (tCa/iCa > 2.1);

3) Relevant metabolic acidosis (pH < 7.2 and/or BE < -5 mmol/l) without, or

4) with an increase of anion gap (> 11 mmol/l).

The ionized hypocalcemia, a sensitive indicator of citrate accumulation, is not adequately specific for diagnosis of citrate accumulation, and could be related to other causes. Increase of total-to-ionized calcium ratio, correlating best with citrate plasma levels, may still not predict citrate accumulation in all cases. Thus, the citrate accumulation was clinically suspected when the systemic ionized hypocalcemia was accompanied by at least two additional factors taken from the clinical criteria described.

Moreover, citrate accumulation was also suspected when an unusually high calcium substitution rate, judged as >3 mmol of calcium substitution per liter of effluent, was needed [10]. With sufficient evidence of citrate accumulation, the regional citrate anticoagulation was immediately stopped and, if the clinical situation required further RRT, CVVHD was performed without anticoagulation, or it was altered to conventional systemic anticoagulation. CVVHD without RCA was performed using the same CRRT devices, dialyzers and multiBic® dialysate (Fresenius Medical Care AG, Bad Homburg, Germany) containing 2 or 4 mmol/l potassium, 140 mmol/l sodium, 111 mmol/l chloride, 35 mmol/l bicarbonate, 0.5 mmol/l magnesium, 1.5 mmol/l calcium, 5.55 mmol/l glucose. Standard flows with heparin anticoagulated CVVHD were: blood flow of 150 ml/hour, dialysate flow of 2 l/hour, adjusted according to clinical needs.

3.4 Data collection

Patient demographics: age, gender, reason for admission, APACHE II score, SOFA score, length of ICU stay, and ICU mortality. CRRT parameters: duration of RCA-CRRT, reason for circuit discontinuation, filter life-time and post-filter iCa; blood, citrate, dialysate and calcium flows; net ultrafiltration; and, if applicable, duration of further CRRT with other anticoagulation mode when the clinical diagnosis of citrate accumulation was made.

Biochemical data (creatinine, urea, phosphorus, magnesium and total calcium), total blood cell count, international normalized ratio (INR) and activated partial thromboplastin time (aPTT) were measured once daily in the central laboratory, whereas blood gas analyses (electrolytes, pH, base excess, standard bicarbonate, ionized calcium and lactate) were measured bed-side (ABL, Radiometer Medical ApS, Denmark) on a 6-hour basis or more often if there was clinical need. Post filter ionized calcium for anticoagulation monitoring was measured immediately after initiation of CRRT and then every 12 hours. Prior to the time of citrate-accumulation diagnosis, metabolic data were collected during the last hours of treatment with RCA-CVVHD (up to 48 hours). After the CVVHD anticoagulation mode was switched to heparin, metabolic data were recorded also for up to 48 hours.

3.5 Statistical analysis

All data are expressed as mean ± standard deviation (SD). According to the characteristics of the data, group comparisons were carried out by ANOVA and Chi-Square test. A p value below 0.05 was considered significant. For analysis IBM SPSS Statistis Version 19 (IBM Corporation, USA) was used.

4. Results

4.1 Study population and incidence of metabolic signs consistent citrate accumulation

A total of 1070 critically ill patients with acute kidney injury received RCA-CRRT according to the protocol described. Among those patients, 32 were identified as having signs of citrate accumulation, corresponding to an incidence rate of 2.99%. Among those 32 patients, 3 patients experienced citrate accumulation twice during their ICU stay, giving a total of 35 episodes detected. Demographic characteristics of patients with suspected citrate accumulation, as well as the demographic characteristics of the total cohort of critical ill patients undergoing RCA-CRRT are shown in Table 4.

Table 4. Baseline demographic characteristic

Parameter	Patients with citrate accumulation mean (95% CI)	Patients without citrate accumulation [‡], mean (95% CI)	P-Value
n	32	1038	
Age, *years*	64.5 (59.4-69.6)	68.2 (67.5-68.9)	0.096
Gender, *male* (%)	65.6%	65.4%	0.572
Bodyweight, *kg*	80.7 (70.2 -91.2)	81.2 (79.8-82.6)	0.893
APACHE II Score	34.2 (30.7-37.7)	25.6 (25.1-26.2)	<0.001
SAPS II Score	64.4 (56.5-72.3)	50.5 (49.5-51.5)	<0.001
SOFA Score (range)	11.0 (9.9-12.2)	8.2 (7.9-8.4)	<0.001
S-creatinine before RRT, *mg/dl*	2.4 (2.1-2.8)	3.0 (2.9-3.1)	0.076
Duration of CVVHD with RCA, *hours*	124 (45– 203)	208 (190– 225)	0.101
ICU stay, *days*	16.6 (1-52)	25.6 (1-296)	0.112
Reasons for ICU admission, %:			
Postsurgical	62.5%	53.2%	0.558
Medical	37.5%	46.8%	

Unless otherwise stated, data are present as mean and 95% confidential interval (CI). ‡ Data from a total of 1038 patients are gathered in the time period of three years: 2008-2010.

As shown in Table 4, there was no significant difference in age, gender, bodyweight, S-creatinin at initiation of RRT, duration of CRRT, ICU stay, or reasons for ICU-admission between the patients with citrate accumulation, and other patients treated with RCA-CVVHD. Remarkably, patients with citrate accumulation had significantly higher severity of disease, presented in APACHE, SAPS II and SOFA scores, as the group of patients without signs of citrate accumulation.

4.2 CRRT treatment before and at the time of diagnosis of citrate accumulation

When citrate accumulation was clinically suspected, patients were immediately switched to heparin anticoagulation with standard dialysis solution, or they received no anticoagulation at all. Due to a very limited prognosis, treatment of four patients with suspected citrate accumulation with any further RRT was not applied.

There was no significant difference in the CVVHD flows at the beginning of RCA-CRRT compared with those immediately before RCA was stopped - blood flow was 101 ± 8 versus 104 ± 13 mL/hour ($p = 0.206$) (Figure 4 - A); citrate infusion per liter of blood flow was 3.9 ± 0.15 versus 3.8 ± 0.40 mmol ($p = 0.786$) (Figure 4 - B) and dialysate flow was 2.0 ± 0.2 versus 2.0 ± 0.3 L/hour ($p = 0.929$) (Figure 4 - C). Remarkably, calcium substitution per liter effluent flow was significantly elevated at the time when anticoagulation was switched to heparin compared with that at the beginning of CRRT with RCA (Figure 4 - D): 2.87 ± 0.66 versus 2.23 ± 0.35 mmol/L effluent ($p < 0.001$), respectively.

Figure 4 (A, B). Time course of RCA-CVVHD flows (**A** – blood flow; **B** – citrate flow) of 32 patients with signs of citrate accumulation over a 48-hour observation period (last 48 hours of CVVHD with RCA). Data are present as mean ± SD. At time point 0 the citrate accumulation was diagnosed and CVVHD with RCA was switched to conventional CRRT. Number of patients at the time point -48, -36, -24, -12, 0, was 12, 16, 21, 34, 35 patients, respectively.
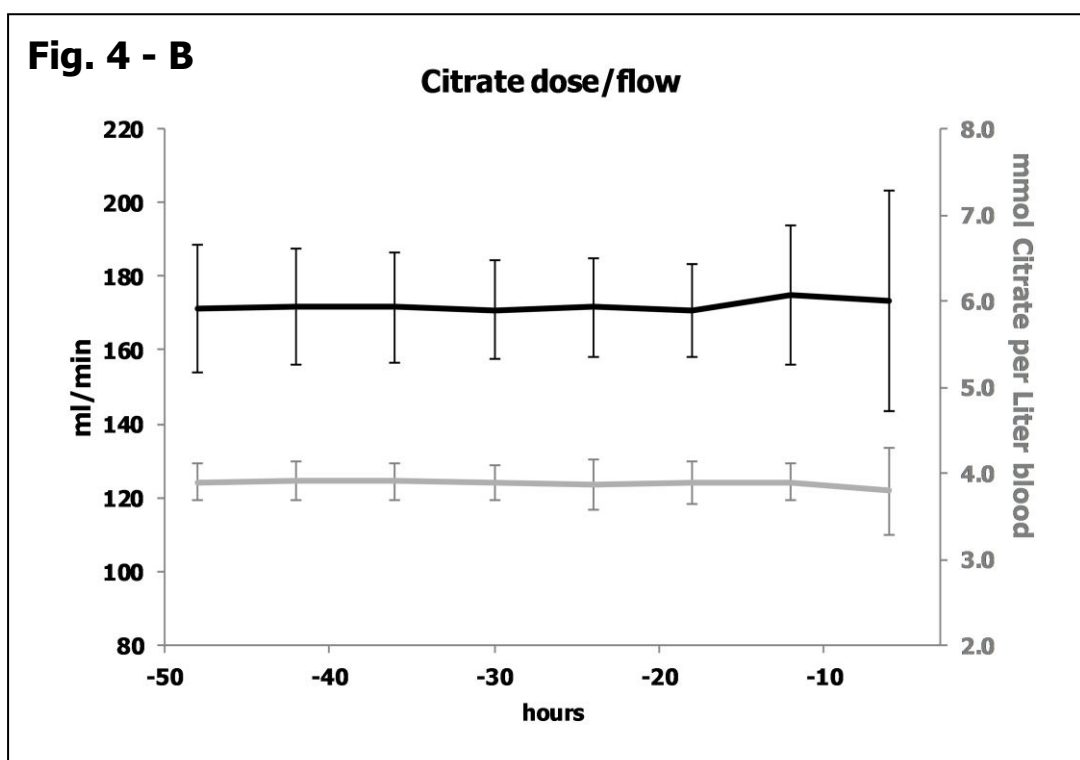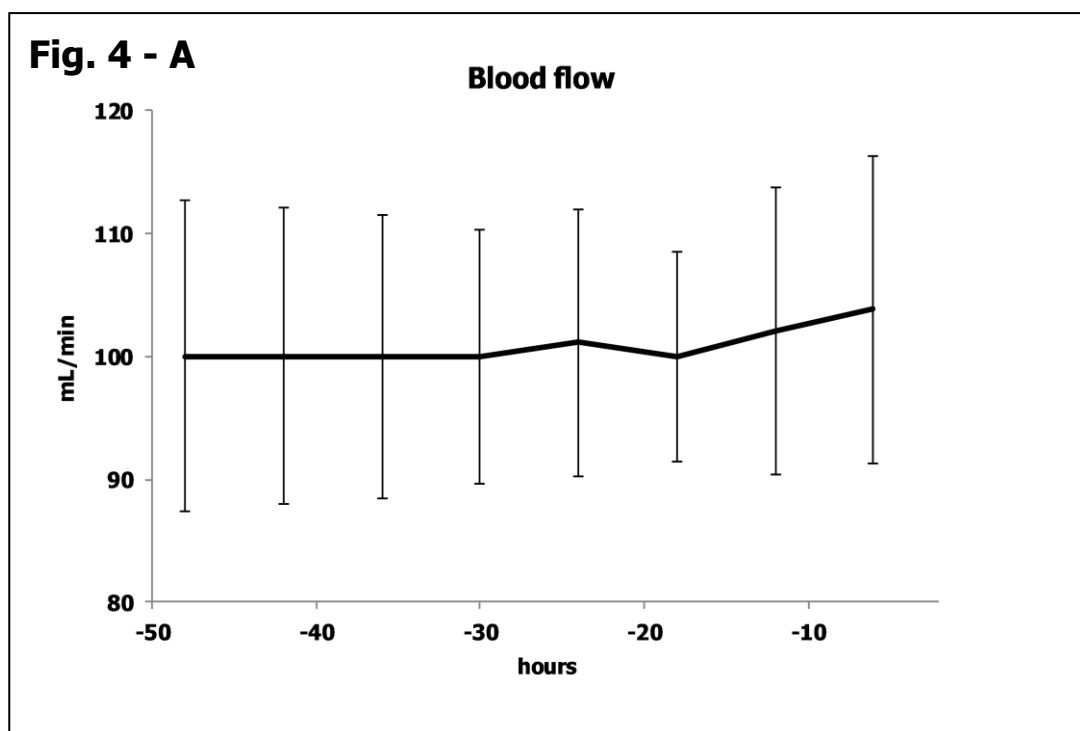
Figure 4 (C, D). Time course of RCA-CVVHD flows (**C** – dialysate flow; **D** – calcium flow) of 32 patients with signs of citrate accumulation over a 48-hour observation period (last 48 hours of CVVHD with RCA). Data are present as mean ± SD. ** $p<0.01$, ***$p<0.001$ versus time point -48 hours. At time point 0, the citrate accumulation was diagnosed and CVVHD with RCA was switched to conventional CRRT. Number of patients at the time point -48, -36, -24, -12, 0, was 12, 16, 21, 34, 35 patients, respectively.
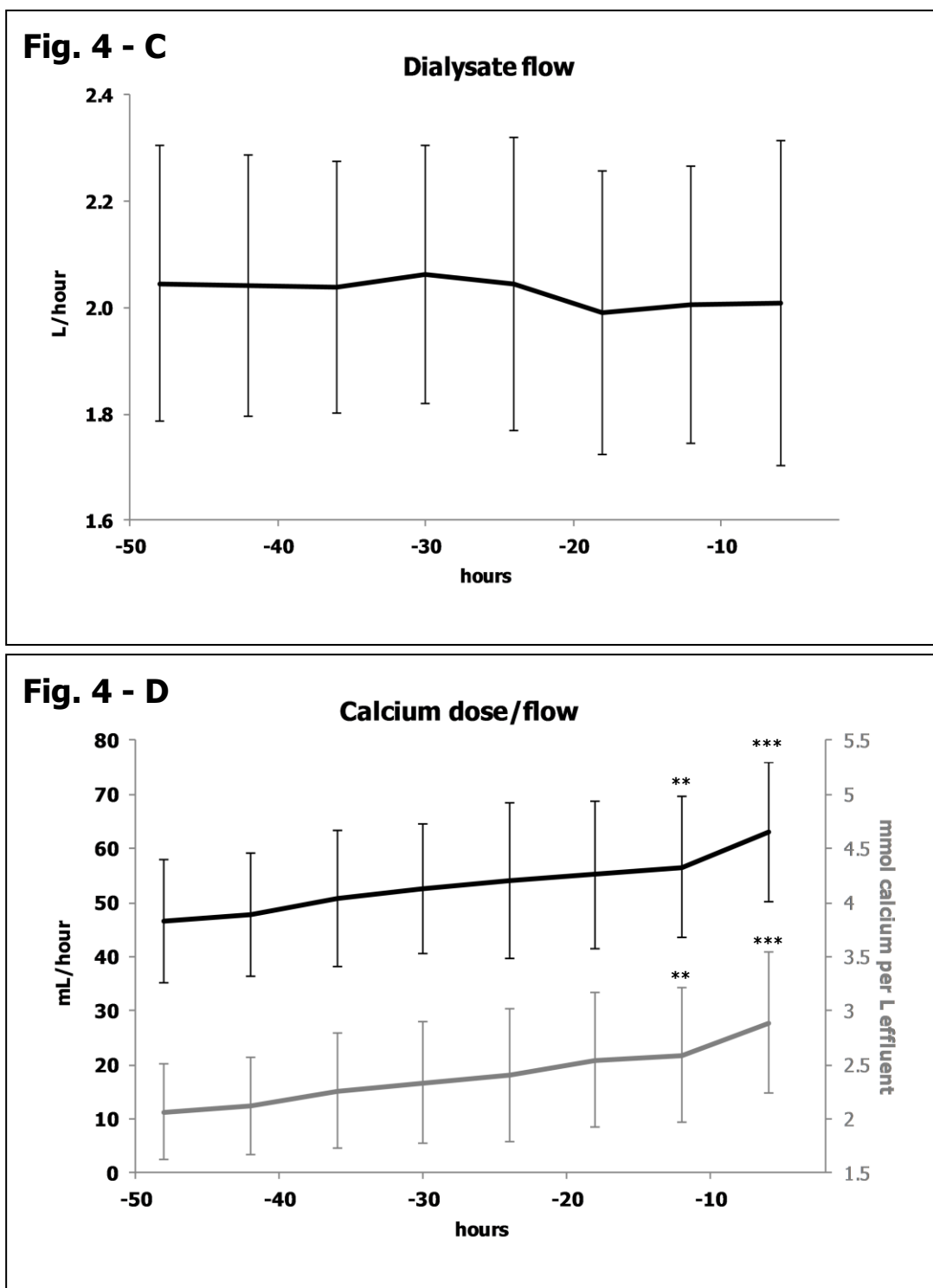
4.3 Common metabolic characteristics of citrate accumulation

At the time of developing the metabolic signs of citrate accumulation all of these patients had clinical diagnosis of severe shock with multiorgan dysfunction syndrome (based on International Guidelines for Management of Severe Sepsis and Septic Shock: 2012). All 32 patients required mechanical ventilation and vasopressor support. Mean S-bilirubin before ICU admission was 2.39 mg/dl (95%CI; 0.92-3.86 mg/dl). Only 11 of 32 patients (34.4%) with suspected citrate accumulation had known pre-existing liver dysfunction. At the time when CVVHD treatment with RCA was started, mean S-bilirubin increased to 4.65 mg/dl (95%CI; 2.0-7.29 mg/dl), indicating progression of acute liver failure in multi organ dysfunction syndrome. Laboratory parameters of the patients before initiation of RCA-CRRT, and at the moment when citrate accumulation was diagnosed, are presented in Table 5.

Table 5. Metabolic characteristic of patients with citrate accumulation

	before initiation of RCA-CVVHD, mean *(95% CI)*	at time of diagnosis of citrate accumulation, mean *(95% CI)*	*P-Value*
pH	7.31 *(7.27-7.35)*	7.20 *(7.16-7.24)*	<0.001
S-bicarbonate, mmol/l	20.2 *(18.5-22.0)*	14.8 *(13.7-15.8)*	<0.001
Ionized calcium, mmol/l	1.14 *(1.10-1.18)*	1.01 *(0.97-1.05)*	<0.001
Total calcium, mmol/l	2.13 *(1.99-2.27)*	2.50 *(2.29-2.72)*	<0.001
Total/ionized calcium ratio	1.87 *(1.79-1.94)*	2.51 *(2.20-2.82)*	<0.001
Anion gap, mmol/l	11.0 *(8.5-13.5)*	15.4 *(13.6-17.1)*	0.003
pO_2, mm Hg	104 *(89-119)*	103 *(93-113)*	0.938
Lactate, mmol/l	7.74 *(5.14-10.32)*	15.0 *(12.72-17.36)*	<0.001
Bilirubin, mmol/dL	4.65 *(2.00-7.29)*	8.28 *(4.62-11.92)*	0,104
AST, U/L	690 *(195-1184)*	3042 *(1280-4196)*	0.001
ALT, U/L	193 *(89-296)*	1205 *(691-1718)*	<0.001
GGT, U/L	142 *(66-218)*	83 *(56-110)*	0.079
Thrombocytes,	134 *(99-169)*	97 *(73-121)*	0.075
INR	2.21 *(1.96-2.56)*	2.94 *(2.63-3.33)*	0,002

Data are present as mean and 95% confidential interval (CI)

As shown in the Table 5, at the time when the citrate accumulation was diagnosed, patients revealed severe metabolic acidosis with significantly reduced pH and S-bicarbonate concentration, and base excess; significantly elevated anion gap and lactate concentration; significantly reduced ionized calcium concentration, significantly elevated concentration of a total calcium and, consequently, significantly increased tCa/iCa ratio.

Accordingly, signs of citrate accumulation in actual study consisted of:

1) systemic ionized hypocalcemia (presented in all cases, in 82.9% of cases iCa was < 1.05 mmol/L);

2) elevated total to ionized systemic calcium ratio (tCa/iCa > 2.1 presented in all patients, > 2.25 presented in 78% of cases) and

3) severe metabolic acidosis (presented in 94.3% of cases) with

4) increased anion gap (presented in 78% of cases).

All four metabolic signs of citrate accumulation were present in 62,5 % of cases.

Besides the pathognomonic metabolic signs for citrate accumulation, a significant elevation of AST and ALT, as well as INR, was observed. At the time when citrate accumulation was diagnosed, there was an aggravation of thrombocytopenia and increased bilirubinemia, however the differences were not statistically significant.

All described metabolic disarrangements progressively increased parallel to each other, with the most severe changes occurring over the last 24 hours of RCA-CRRT (Fig. 5 – A, B, C, D). Significant decreases of arterial pH, base excess and bicarbonate (Fig. 5 – A, B, C, respectively), as well as significant increase of anion gap and lactate concentration (Fig. 5 – D and Fig. 6, respectively) occurred twelve hours before the clinical diagnosis of citrate accumulation have been made. Remarkably, all patients had severe lactic acidosis (pH 7.2 ± 0.11 and lactate 15.0 ± 6.8 mmol/l) at the time when citrate accumulation was diagnosed. It is of interest that both gradually decreasing ionized calcium and calcium-substitution were 12-24 hours prior to acid-base disarrangements. However, significant difference was reached only 6 hours prior to diagnosis of citrate accumulation (Fig. 7).

Figure 5 (A, B). Time course of metabolic characteristics of 32 patients with signs of citrate accumulation over a 96-hour observation period (last 48 hours of CVVHD with RCA and 48 hours after switch to conventional CRRT). At time point 0, the citrate accumulation was diagnosed and CVVHD with RCA was switched to conventional CRRT: **- A**) arterial pH; **- B**) base excess. Data are present as mean ± SD. * p <0.05, ** p<0.01, ***p<0.001 versus time point -48 hours. Number of patients at the time point -48, -36, -24, -12, 0, 12, 24, 36, 48 was 12, 16, 21, 34, 35, 24, 15, 11, 9 patients, respectively.

Fig. 5 - C

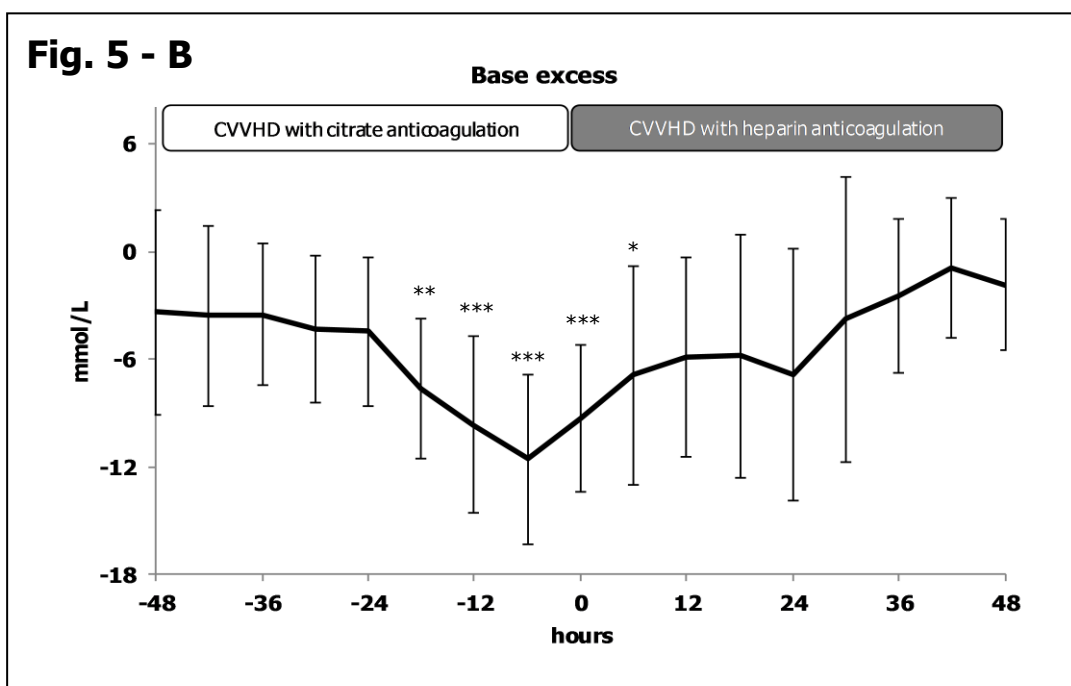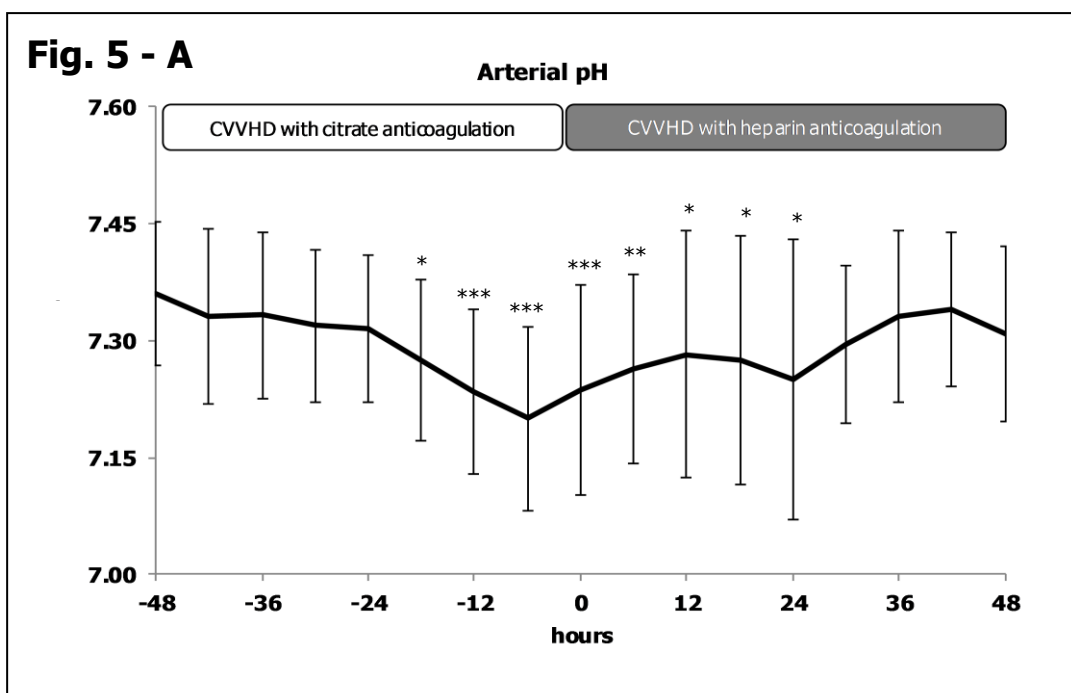**Bicarbonate**

Fig. 5 - D

Anion gap

Figure 5 (C, D). Time course of metabolic characteristics of 32 patients with signs of citrate accumulation over a 96-hour observation period (last 48 hours of CVVHD with RCA and 48 hours after switch to conventional CRRT). At time point 0, the citrate accumulation was diagnosed and CVVHD with RCA was switched to conventional CRRT: **- C**) bicarbonate; **- D**) anion gap. Data are present as mean ± SD. * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$ versus time point -48 hours. Number of patients at the time point -48, -36, -24, -12, 0, 12, 24, 36, 48 was 12, 16, 21, 34, 35, 24, 15, 11, 9 patients, respectively.
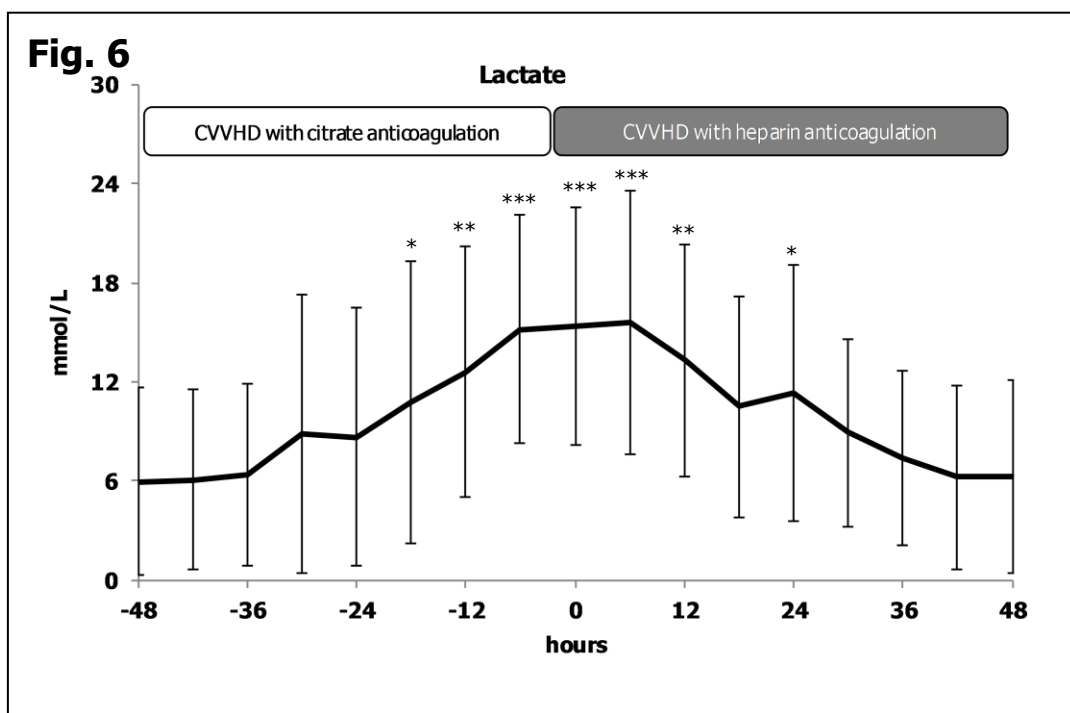
Figure 6. Time course of blood lactate concentration of 32 patients with signs of citrate accumulation over a 96-hour observation period (last 48 hours of CVVHD with RCA and 48 hours after switch to conventional CRRT). At time point 0, the citrate accumulation was diagnosed and CVVHD with RCA was switched to conventional CRRT. Data are present as mean ± SD. * p <0.05, ** p<0.01, ***p<0.001 versus time point -48 hours. Number of patients at the time point -48, -36, -24, -12, 0, 12, 24, 36, 48 was 12, 16, 21, 34, 35, 24, 15, 11, 9 patients, respectively.
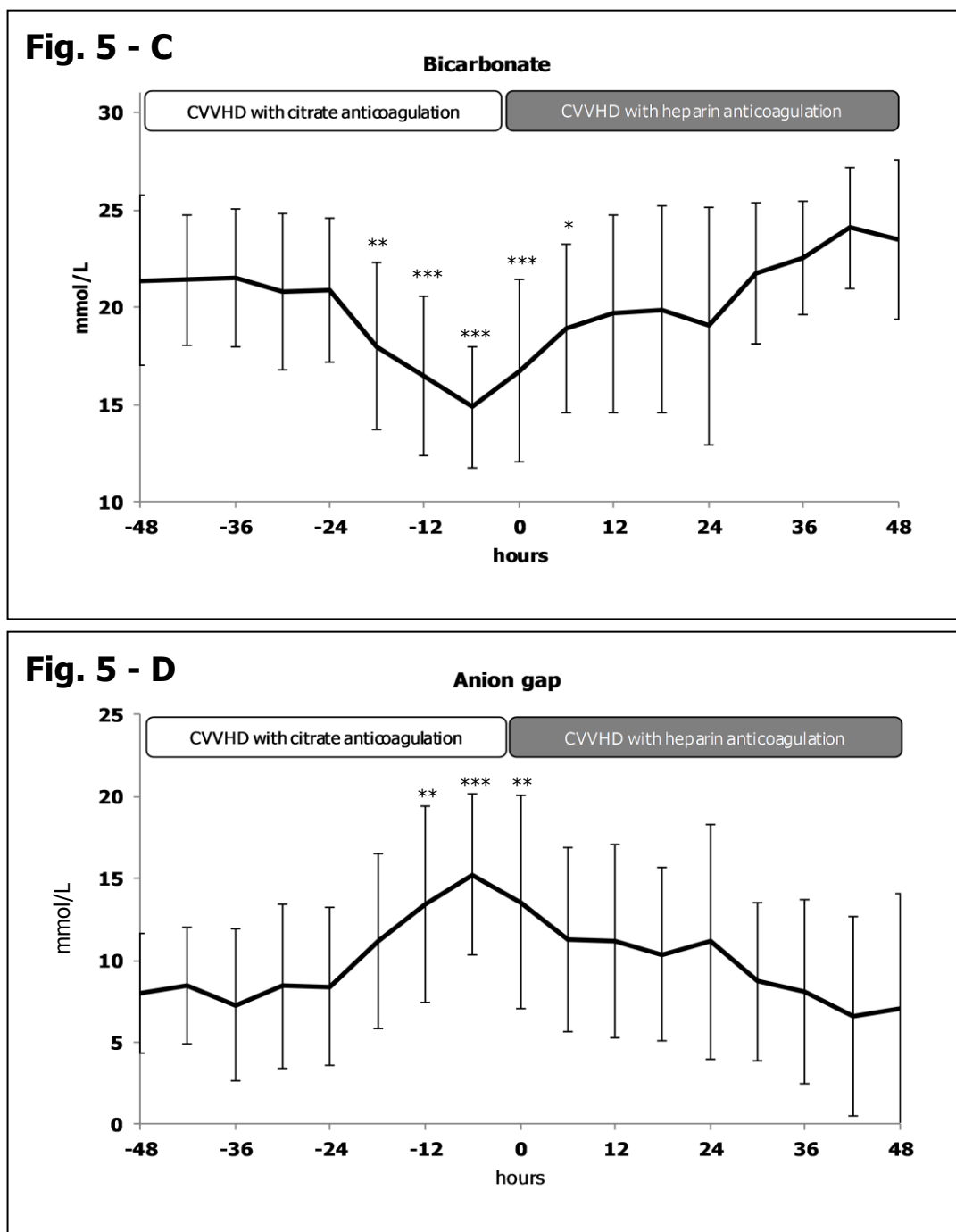
Figure 7. Time course of systemic ionized calcium concentration and calcium substitution rate of 32 patients with signs of citrate accumulation over a 96-hour observation period (last 48 hours of CVVHD with RCA and 48 hours after switch to conventional CRRT). At time point 0, the citrate accumulation was diagnosed and CVVHD with RCA was switched to conventional CRRT. Data are present as mean ± SD. * p <0.05, ** p<0.01, ***p<0.001 versus time point -48 hours. Number of patients at the time point -48, -36, -24, -12, 0, 12, 24, 36, 48 was 12, 16, 21, 34, 35, 24, 15, 11, 9 patients, respectively.
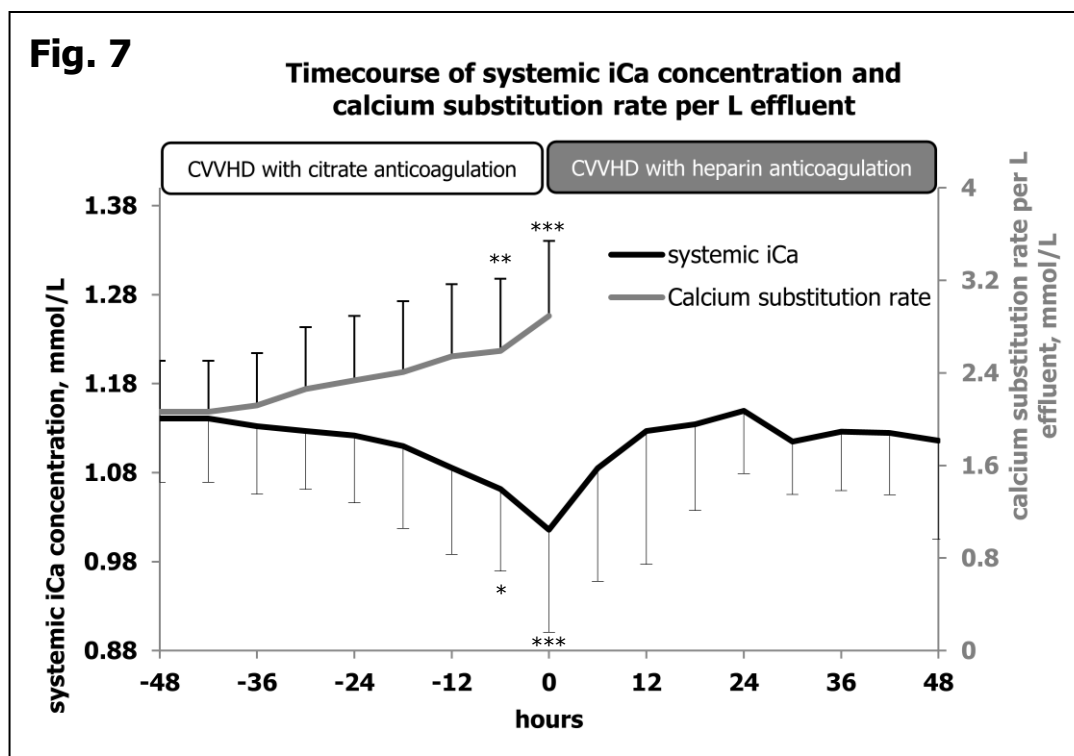
Additionally, in 28 out of 35 cases (80%), patients received a bolus calcium substitution with a mean amount of 8.3 ± 6.8 mmol calcium in the 24-hour period before citrate accumulation was diagnosed. To prevent increasing metabolic acidosis in 30 from 35 cases (85.7%), patients were additionally buffered with sodium bicarbonate (median 100 mmol) or trometamol (median 300 mmol) 24 hours before citrate accumulation was diagnosed (Table 6).

Table 6. Concomitant medication in patients with citrate accumulation

Supplements for metabolic correction	Amount	Frequency of application, % of cases
Calcium gluconate Mean ± SD	8.3 ± 6.8 mmol	80.0%
Sodium bicarbonate Median (range)	100 mmol (0-600)	54.3%
Trometamol Median (range)	300 mmol (0-2160)	62.9%

In twenty-eight out of 32 patients (87.5%) with citrate accumulation, patients received red cell transfusions or other blood products as an additional source of citrate (in median 3 units of red cell packs and 5 units of fresh frozen plasma per patient).

4.4 Clinical outcome

All patients with citrate accumulation died during their ICU stay, with the 28-day mortality rate at 78.1%. Most patients died shortly after citrate accumulation diagnosis (in median, after 22 hours), with only 34.4% of patients surviving 48 hours thereafter. We compared this finding with the clinical outcome of all patients treated with RCA-CRRT. According to our data, an unfavorable outcome on day 28 was found only in 37.5 % of all patients treated with RCA-CRRT (298 out of 1070 patients died on day 28, where 103 patients were not followed up but counted as dead on day 28) (P < 0.001, Figure 8).
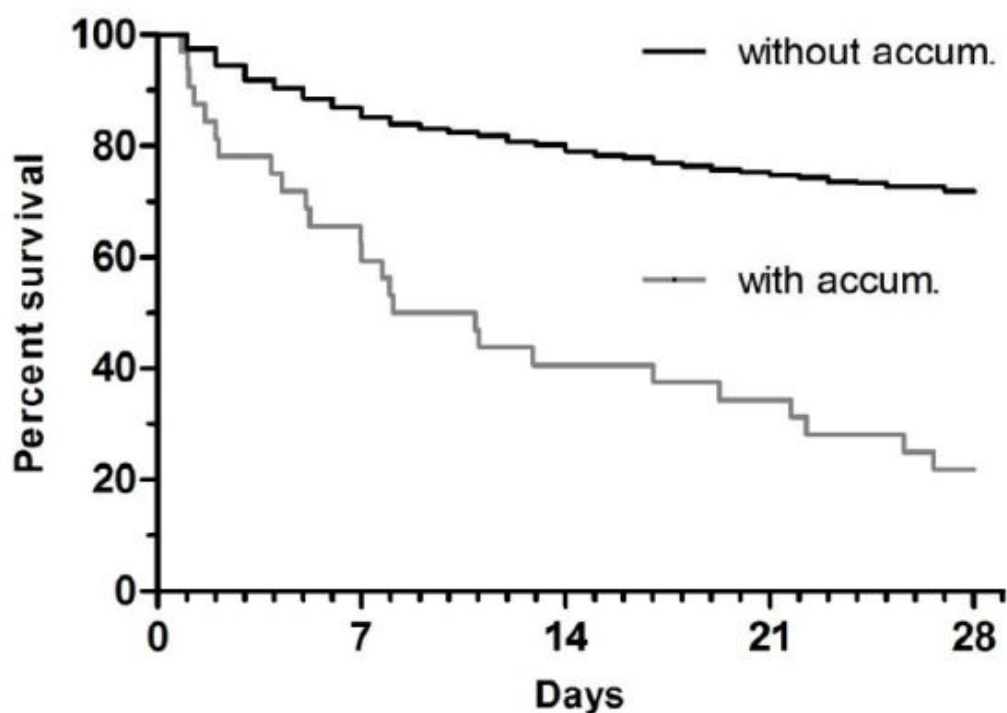
No. of patients at risk	day 0	day 7	day 14	day 28
without citrate accumulation	1038	913	818	669
with citrate accumulation	32	20	14	7

Figure 8. Survival of the patients with citrate accumulation (*grey line*) and total cohort of patients treated with CVVHD with regional citrate anticoagulation (*black line*) after ICU admission. Data were gathered over the years 2008-2010. Total 1070 patients; 298 patients died over the period of 28 days after ICU admission, 103 patients were lost to follow-up during 28 days and were counted as dead. *Log rank, p < 0.001.*

5. Discussion

Taking into consideration the advantages of CRRT, current KDIGO Clinical Practice Guideline for Acute Kidney Injury (2012) suggests using CRRT, rather than standard intermittent RRT, for hemodynamically unstable patients, patients with increased intracranial pressure, or generalized brain edema [3]. The most important disadvantage of CRRT is the need for continuous anticoagulation. For a long time the anticoagulation during the CRRT represented a great challenge. Based on recent clinical trials and meta-analyses, KDIGO Clinical Practice Guideline for Acute Kidney Injury (2012) suggests using RCA rather than heparin for anticoagulation during CRRT in patients who do not have contraindications for citrate, independently of their bleeding risk [3], and who are not already systemically anticoagulated for other reasons. Despite this recommendation and otherwise obvious advantage of RCA over standard heparin anticoagulation, the application of RCA in clinical practice is still limited to 0–20% of the patients/treatments in recently published trials [7]. The main reason for avoiding RCA is the fear of metabolic disarrangements due to RCA in critically ill patients with AKI requiring RRT. However, metabolic complications due to RCA are infrequent in patients without contraindication to RCA. According to the actual KDIGO Clinical Practice Guideline for AKI, severely impaired liver function or shock with muscle hypoperfusion represent the major contraindications for the use of RCA [3]. Both shock and decreased liver function are supposed risk factors for citrate accumulation. In other words, risk for citrate accumulation represents a major contraindication for RCA-CRRT. Thus, it is crucial to reveal those patients and their common metabolic characteristics in order to meet the clinical decision of which anticoagulation to choose during CRRT.

During the last years some clinical evidence has been gathered showing that shock and liver impairment do not necessary lead to citrate accumulation during RCA-CRRT [3, 20, 46, 64, 67]. Thus, those factors should be considered as only a relative, and not absolute, contraindication for RCA. Furthermore, consequently excluding hemodynamically instable patients in shock for treatment with RCA-CRRT, one actually excludes a significant part of patients who, according to KDIGO Clinical Practice Guideline for AKI, would especially profit from CRRT and not from intermittent RRT.

Under these circumstances, it is of particular interest to know what is the actual incidence of citrate accumulation in critically ill patients undergoing RCA-CVVHD. In our opinion, that question cannot be addressed at this time, as there is a lack of scientific

evidence. All available studies describing citrate accumulation during CRRT might have, at least to some extent, a selection bias (by including only the patients at risk for bleeding or excluding certain patients because of the liver dysfunction, risk for citrate accumulation, etc.) and, thus, might cause an underestimation of the incidence of this complication. Moreover, some trials have a limited observational period, which could lead to an underestimation of the incidence rate of citrate accumulation [20]. In Charité university hospital, RCA-CVVHD is the modality of choice for hemodynamically instable patients with AKI since 2006. Until the actual recommendations of KDIGO Clinical Practice Guideline for AKI published in 2012, each patient requiring CRRT at our center was initially treated with RCA-CRRT independently of bleeding risk, liver function, muscle hypoperfusion, or shock. We performed a retrospective study analyzing the incidence of citrate accumulation in a three year period (2008-2010).

The aim of the present study was to determine the incidence of metabolic disarrangements consistent with citrate accumulation and to assess the common metabolic findings of this complication. The study was performed in a large, representative group of critically ill patients requiring RRT and treated with standardized RCA-CRRT.

5.1 Incidence of metabolic signs of citrate accumulation

The most important finding of the present study is that the incidence of citrate accumulation in patients treated with CVVHD and RCA was, with only 2.99%, rather low. This finding is comparable with previously reported frequencies of citrate accumulation, however represented in cohorts with a significantly smaller number of patients (Table 7).

Table 7. Comparison of incidence clinically diagnosed citrate accumulation to other published data[*]

Reference	Number of patients with RCA	Mode of CRRT	Applied citrate dose per liter of blood	Incidence of citrate accumulation, n (%)
Kutsogianis et al. [16]	16	CVVHDF	3.9 mmol	None
Morgera et al. [10]	161	CVVHD	4 mmol	2 (1.24%)
Oudemans-van Straaten et al. [11]	97	CVVH	3 mmol	1 (1.03%)
Hetzel et al. [8]	87	CVVH	4 mmol	1 (1.15%)
Slowinski et al. [20]	133	CVVHD	4 mmol	3 (2.26%)
Mariano et al. [68]	31	CVVHDF	~ 2 mmol	None
Kalb et al. [9]	75	CVVHD	4 mmol	1 (1.33%)
Morabito et al. [69]	33	CVVH	3 mmol	1 (3.03%)
Link et al. [61]	208	CVVHD	4 mmol	None
Present study	1070	CVVHD	4 mmol	32 (2.99%)

* due to the differences in the study designs, applied RCA protocols and the delivered citrate dose, the comparison yields only for the general overview.

It is noteworthy that the previously reported incidence of citrate accumulation is approximately similar in all reports, independently of neither CRRT modality, nor applied citrate dose.

Moreover, the demonstrated incidence of suspected citrate accumulation as an adverse event is in the similar magnitude as heparin-induced thrombocytopenia in patients treated with CRRT and heparin anticoagulation [70, 71].

5.2 Metabolic signs of citrate accumulation

Only 34% of patients with citrate accumulation had a pre-existing liver impairment. This finding conflicts to some extent with the common concern that patients with liver failure are the most relevant patient group at risk to develop citrate accumulation. On the other hand, our finding is in line with a recent prospective observational multicenter study conducted at our department, where the safety and efficacy of regional citrate anticoagulation in ICU patients having normal and impaired liver function was evaluated [20]. Together with other European centers, we have demonstrated that CVVHD with RCA can be safely used in patients with different grades of liver dysfunction, defined, as well as in our study, according to the total serum bilirubin. Interestingly, the 2.26% incidence of clinical diagnosis of citrate accumulation was even slightly lower than that reported in the current study. Although the attempt to include patients with elevated S-bilirubin in that study could have been expected to result in a higher incidence rate, the observation period was limited to first 72 hours of CVVHD treatment with a possible underestimation of the citrate accumulation frequency in that patient population.

In the present study, citrate accumulation was diagnosed based on ionized hypocalcemia in combination with elevated total/ionized calcium ratio, metabolic acidosis with elevated anion gap, and severe lactic acidosis. Despite the calcium substitution via the dialysis device that increased by a mean of 28.7% compared to baseline, the majority of patients (80%) required additional bolus calcium substitution. 85.7% of the patients required additional buffering with sodium bicarbonate, trometamol, or both. Hypocalcemia with sustained increase of calcium substitution by the dialysis device as well as a parallel continuous increase of lactate concentration, were the first clinically recognizable signs of imminent citrate accumulation. The increased calcium substitution requirement as a sign of possible citrate accumulation was also described in 1999 by Meier-Kriesche et al. [12].

Although most experts and guidelines on citrate anticoagulation report on an association between citrate accumulation and lactic acidosis, to the best of our knowledge the incidence, clinical outcome and laboratory findings of citrate accumulation have been poorly described in the literature so far. There is a very plausible biochemical rationale for a causal relationship. Both anions require oxygen during metabolism, which is obvious in the case of complete oxidation but also evident for other metabolic pathways. For instance, gluconeogenesis from lactate requires more

ATP-equivalents than those generated in anaerobic glycolysis. This clearly suggests that ATP from oxidative metabolism is required to convert larger quantities of lactate into glucose. After entering the Krebs cycle, citrate is metabolized via the isocitrate-dehydrogenase with hydrogen being transferred to NADH. As the coenzyme NADH needs to be regenerated to NAD^+ in the respiratory chain, the metabolism of citrate is therefore oxygen-dependent. Thus, the parallel accumulation of citrate and lactate likely indicates an insufficient oxidative metabolism, e.g. a blockade in the regeneration of NAD^+ from NADH via the respiratory chain. Remarkably, after a switch to conventional CRRT, the resolution of metabolic disorders was observed. That could be explained by 1) application of bicarbonate- and calcium-rich dialysate, 2) decreasing number of patients due to death, with only 11 out of 32 patients surviving > 48 hours, and 3) partial recovery of citrate metabolism in short-term survivors. One of the further assumptive explanations for lactate levels reduction could be an improvement of cardiac contractility after normalization of acid-base status and/or resolution of hypocalcaemia.

From our study, it is not possible to determine a threshold for lactate, which predicts impaired citrate metabolism, although the mean lactate concentration of 14.99 mmol/L at the time of diagnosis of citrate accumulation was very high. While a high lactate concentration at the start of CRRT should be an alert of the risk of citrate accumulation, certainly not all patients with lactic acidosis will obligatorily accumulate citrate. On the contrary, considerable proportion of shock patients with a high lactate level do metabolize citrate remarkably well if circulation and oxygen delivery improves during treatment of shock. Thus we suggest that additional attention and close monitoring of calcium homeostasis should be assured in patients suffering from a severe lactic acidosis and treated with any RCA-CRRT set-up.

Finally, it is worth mentioning that the majority of patients with citrate accumulation received a considerable amount of blood products as an additional source of citrate over the last 24 hours before this complication occurred. One unit of packed red blood cells (RBC) contains approximately 16 mmol of citric acid. Thus, patients with massive blood transfusions are at an additional risk of developing citrate accumulation, regardless of RCA required for CRRT. This finding correlates with previously published studies [56-58, 72].

5.3 Clinical outcome in patients with metabolic signs of citrate accumulation

Another important finding of the actual study is that all patients with citrate accumulation had, in comparison to the whole study population, significantly higher APACHE II and SAPS II scores that are well-established as being linked to higher mortality rates. All of these patients had severe therapy resistant shock with multiple organ dysfunctions and died during their ICU stay. These findings indicate that those patients did have a very limited prognosis, *per se*, and citrate accumulation might simply reflect the severity of the underlying disorders and the breakdown of cellular respiration. Our results are in line with the recently published work of Link et al [61]. The authors proved that in patients on CRRT with RCA total-to-ionized calcium ratio correlates with the clinical outcome and is an independent predictor of 28-days mortality.

One would argue the 28-days mortality rate (37.5%) of the patients in actual study to be low in comparison with international prospective multicenter studies on mortality of critically ill patients with need for RRT [1, 73]. The BEST-Kidney Study included 1753 critically ill patients with AKI, a study population that was comparable with the study population in actual survey (mean age 63.2 years, 64% males, mean SAPS II Score 50.3, mean SOFA Score 10.5). Indeed, in the BEST-Kidney Study with mainly conventional anicoagulated RRT, the total 28-days mortality rate was more than 60% [73]. There are several explanations for the considerably different 28-days mortality rate between both studies. First of all, the BEST-Kidney Study included patients from different European centers, thus providing very heterogenic outcomes from center to center, with hospital mortality rate from 22.2 to 100% [1]. Furthermore, in the BEST-Kidney Study the proportion of the surgical reasons for ICU admission was considerably lower than in the actual investigation (41.1 % vs 53.2%). Besides, in the BEST-Kidney Study 80% of patients received the CRRT with very heterogeneous anticoagulation modalities. Regional citrate anticoagulation could have a positive effect on the survival rate due to reduced bleeding risk, especially in patients after surgical intervention [8, 16, 51]. Furthermore, due to the fact that in our study all patients were initially treated exclusively with RCA, the other possible explanation of a better outcome data from our study is pro-inflammatory effects of heparin and/or anti-inflammatory properties of citrate itself [4]. Some data shows that heparin possess potentially pro-inflammatory effects by, for example, releasing the mediators of inflammation from leucocytes and thrombocytes [37, 38, 74-76]. On the other hand it has been assumed that a local

hypocalcemia in the area of dialysis membrane could reduce the release of inflammatory cytokines from the cells adhered to the membrane [77, 78].

Noteworthy, survival rate in patients treated with RCA-CRRT have been reported recently in the prospective randomized trial [11]. Authors have shown a significantly better outcome in the group of patients treated with RCA-CRRT compared to those treated with CRRT with LMWH-anticoagulation. Remarkably, those effects were independent from the differences in bleeding incidence. A better outcome of the patients treated with RCA-CRRT as those, anticoagulated with heparin during CRRT, described by Oudemans-van Straaten et al, was questioned by Hetzel et al. In the multicentre, controlled, randomized, open, prospective clinical trial comparing RCA and systemic anticoagulation with heparin during CRRT. The authors didn't confirm the survival benefit of patients treated with RCA-CRRT.

5.4 Limitations of the study

There are several limitations of the present study to be stressed and addressed. Firstly, it has the usual limitations of single-center retrospective studies. However, the number of patients we retrospectively analyzed is considerably large - inclusion of 6 ICUs with different specializations allowed the involvement of a multitude of morbidities covering the range of what could possibly be present in an ICU, and our data are comparable with citrate accumulation incidences reported in other prospective trials using smaller patients numbers (Table 7). Additionally, using citrate as the standard anticoagulation method for CVVHD in every patient treated with CRRT and without any exclusion criteria except clinically diagnosed citrate accumulation, allowed us to eliminate any selection bias, which may be present even in some prospective trials.

Secondly, we used the surrogate criteria to define citrate accumulation without actually measuring the citrate level. Indeed, the measurement of blood citrate concentration is, at least in Germany, not clinically approved, and, thus, not available in laboratory routine, making such investigation infeasible for a large cohort of patients. On the other hand, the parameters we used are widely accepted. The cut-off levels of total-to-ionized calcium ratio, the most specific criteria for diagnosis of citrate accumulation, vary. To increase sensitivity of our monitoring we accepted a cut-off level ratio of >2.1, as previously described [13, 62].

Thirdly, a possible limitation of the actual study is the use of total serum bilirubin as a main parameter for liver function. However, there is no physiologic parameter applicable for clinical use that allows for early detection of hepatic dysfunction; indeed all current diagnostic criteria are based on laboratory evaluation. Serum bilirubin, as a steady marker of hepatic impairment [79], is a key component of prognostic scores of chronic liver disease and cirrhosis as well as prognostic models in patients with acute liver failure [80]. It could be assumed that international normalized ratio (INR), as a parameter of liver synthesis, may be a better predictor of the liver ability to metabolise citrate. However, INR is elevated in the advanced stages of liver failure and only represents very severe forms of liver dysfunction. Furthermore, the substitution of plasma products as well as disseminated intravascular coagulopathy, often observed during severe sepsis, can influence INR. Moreover, serum bilirubin was used for classification of liver dysfunction in others study as well [20, 60].

Fourthly, in our study all patients were treated with CVVHD as a basis CRRT

modality, which might have had an impact on citrate clearance. Yet, citrate has a low molecular weight and its sieving coefficient is close to 1, and thus, its clearance remains the same during diffusion or convection [81, 82]. In fact, Morgera et al. [10] had based our RCA-CVVHD protocol on moderately high blood flows as a lower blood flow reduces citrate requirements for anticoagulation and with a blood-to-dialysate flow ratio of 3:1, as this allows the assumption of near complete equilibration of low-molecular weight solutes. Therefore, the citrate exposure during CVVHD should be mainly dose-dependent and almost irrespective from the renal replacement technique used (CVVH, CVVHDF), and thus, similar to such described in present study.

And finally, in our center we used only one protocol according to Morgera et al with a fixed starting dose of citrate of approximately 4 mmol per liter of blood [10]. Indeed, in patients with ionized calcium concentrations in the normal range, 4 mmol citrate per liter blood is sufficient to completely inhibit the coagulation cascade in the extracorporeal circuit. Ionized calcium and coagulation are not linked in a linear proportion. On the contrary, ionized calcium has to be lowered to <0.40 mmol/l to provide any anticoagulation, and coagulation ability is completely unaffected with ionized calcium concentrations above 0.40 mmol/l [49]. To reach a reasonable range (0.25-0.35 mmol/l) in the extracorporeal circuit from normal blood calcium concentrations (1.1-1.3 mmol/l), approximately 4 mmol of citrate per liter blood is needed [48]. Therefore, citrate-sparing protocols are not feasible or at least not ideal for anticoagulation. In patients with clinical signs or a known risk for citrate accumulation, a strategy to reduce a citrate load should be applied: reduction of blood flow with subsequent decrease in citrate dose, or/and increase of extracorporeal citrate clearance by increasing the dialysate flow [61]. Noteworthy, increasing the dialysate flow, at least in our protocol, will subsequently lead to worsening of the metabolic acidosis, due to the reduced bicarbonate concentration in the dialysate. However, this could be, at least partially, compensated by additional bicarbonate infusion. Our retrospective analysis shows that reduction of citrate load was not consequently applied in each patient with suspected citrate accumulation. Due to retrospective character of the study, we can only speculate about the reasons: lack of experience in handling the RCA in early years; rapid onset of metabolic disarrangements with consequent need of prompt change of CRRT modality; very limited prognosis of four patients where the CRRT was discontinued; worsening of the metabolic acidosis when elevating the dialysate flow with reduced bicarbonate

concentration; and reduced dialysis efficacy when decreasing the blood flow in terms of hyperkalemia. According to the actual knowledge and our present results, we would strongly recommend applying strategies to reduce the citrate load in all patients where citrate accumulation would even be suspected.

6. **Summary**

Continuous renal replacement therapy (CRRT) is increasingly applied in critically ill patients with acute kidney injury who requires renal support. CRRT is often preferred to intermittent dialysis because of better hemodynamic stability and metabolic control. Regional citrate anticoagulation (RCA) during CRRT (RCA-CRRT) is a common alternative to systemic heparin anticoagulation, especially by patients with increased bleeding risk. Indeed, in recently published guidelines RCA is now recommended as the anticoagulation strategy of choice in patients undergoing CRRT without contraindication to citrate. According to KDIGO guidelines, the major contraindications for RCA are: severely impaired liver function or shock with muscle hypoperfusion, both representing a risk of citrate accumulation.

Citrate accumulation is a serious complication of CRRT with RCA. It could cause severe metabolic acidosis, decrease cardiac contractility or cause arrhythmias, as symptoms of systemic ionized hypocalcemia. Acute or chronic impaired liver function, shock with arterial hypoxia and reduced tissue perfusion are the major risk factors for citrate accumulation. Unfortunately measurement of citrate concentration in blood is not available on a daily routine basis, and, at least in Germany, available test kits are not approved for clinical use. There are however commonly accepted markers for citrate accumulation, such as: metabolic acidosis with or without increased anion gap, ionized hypocalcemia with simultaneous increased levels of total calcium and, an increased total calcium to ionized calcium ratio (tCa/iCa). Those laboratory parameters do not confirm citrate accumulation in all cases, and often lead to false positive results, what makes the diagnosis of citrate accumulation a complex clinical issue. More over, information about the incidence of citrate accumulation in general cohort of ICU patients undergoing RCA-CRRT is relatively limited, due to the fact that patients with risk factors for citrate accumulation were usually whether excluded from the prospective trials, or the observation of those patients were limited to the study period.

In this monocentric retrospective study we collected and analyzed all patients on RCA-CRRT over a three-year period (from 2008 to 2010) to identify risk factors for citrate accumulation. The primary objective was to reveal the incidence rate of citrate accumulation in cohort of non-selected critically ill patients receiving RCA-CRRT. The secondary objective was to assess the clinical characteristics and outcome related to citrate accumulation based on a representative population of patients.

The results of the present research show that the incidence of metabolic disarrangements consistent with citrate accumulation was rather low since it affected only 2.99% of all RCA-CRRT patients. Our findings show that RCA during CRRT is feasible and complications are uncommon independently of a liver function, shock or presented muscle hypoperfusion. Diagnosis of citrate accumulation was found exclusively in severely ill patients with multiorgan failure and severe lactic acidosis, thus representing a group of patients with a very poor prognosis per se. All patients with suspected citrate accumulation died during their ICU stay. Our findings suggest that citrate accumulation occurs secondary to severe disability of cellular respiration and that citrate accumulation could be interpreted as a strong indicator for patient's poor outcome. We suggest that close attention should be paid to patients treated with RCA-CRRT who have a severe lactic acidosis and an elevated demand for calcium substitution.

7. Zusammenfassung

Die kontinuierliche Nierenersatztherapie wird beim Großteil der kritisch kranken Patienten mit dialysepflichtigen akutem Nierenversagen auf Intensivstationen verwendet. Die kontinuierliche Nierenersatztherapie wird insbesondere bei hämodynamisch instabilen patienten gegenüber der intermittierenden Dialyse wegen der schonenderen Ultrafiltration und der besseren Stoffwechselkontrolle bevorzugt. Die regionale Zitratantikoagulation während der kontinuierlichen Nierenersatztherapie ist eine zunehmend häufiger eingesetzte Alternative zur systemischen Antikoagulation, insbesondere bei Patienten mit erhöhtem Blutungsrisiko. In den kürzlich veröffentlichten Leitlinien zur Antikoagulation bei kontinuierlicher Nierensesartztherapie (KDIGO 2012) wird die regionale Zitratantikoagulation empfohlen als die Antikoagulation der Wahl bei Patienten ohne Kontraindikation für Zitratantikoagulation und ohne systemische Antikoagulation aus anderen patientenbezogenen Gründen. Die wichtigsten Kontraindikationen für RCA laut den KDIGO Leitlinien sind folgende: stark eingeschränkte Leberfunktion oder Schock mit Muskelminderdurchblutung. Beide Risikofaktoren entsprechen den Risikofaktoren der Zitratakkumulation.

Die systemische Zitratakkumulation stellt eine Komplikation kontinuierlichen Nierenersatzverfahrens mit regionaler Zitratantikoagulation dar. Die möglichen klinischen Folgen der Zitratakkumulation sind schwere metabolische Azidose, verminderte Herzkontraktilität und Herzrhythmusstörungen. Leider ist die Messung der Zitratkonzentration im Blut im klinischen Alltag nicht verfügbar. Allgemein anerkannte Marker der Zitratakkumulation sind rasch fallende Konzentration des ionisierten Kalziums bei gleichzeitig steigendem Kalziumsubstitutionsbedarf, erhöhter Quotient aus Gesamtkalzium zu ionisierten Kalzium, sowie metabolische Azidose mit oder ohne erweiterter Anionenlücke. Diese Laborparameter bestätigen die Zitratakkumulation nicht in allen Fällen und führen häufig zu falschen positiven Ergebnissen. Somit ist die Diagnose der Zitratakkumulation ein komplexes klinisches Problem. Weiterhin ist die Information über die Inzidenz der Zitratakkumulation in allgemeiner Kohorte von dialysepflichtigen kritisch kranken Patienten auf der Intensivstation gering, weil die Patienten mit Risikofaktoren für Zitratakkumulation in der Regel aus den bisherigen prospektiven Studien ausgeschlossen wurden oder der Beobachtungszeitraum vergleichsweise kurz war.

In dieser monozentrischen retrospektiven Studie wurden alle Patienten die mit

kontinuierlicher Nierenersatztherapie mit regionaler Zitratantikoagulation auf der Intensivstationen behandelt worden über einen Zeitraum von drei Jahren (von 2008 bis 2010) identifiziert und analysiert. Das primäre Ziel war es, die Häufigkeit der Zitratakkumulation in einer Kohorte von nicht selektierten kritisch kranken Patienten, die mit RCA-CRRT behandelt wurden, zu ermitteln. Das weitere Ziel war es, die klinischen Merkmale sowie das Outcome der Zitratakkumulation aufgrund einer repräsentativen Patientenpopulation zu bewerten.

Die Ergebnisse der vorliegenden Arbeit zeigen, dass die Inzidenz der Zitratakkumulation mit 2.99 % gering war. Die Ergebnisse zeigen weiterhin, dass die Zitratantikoagulation während der kontinuierlichen Nierenersatztherapie, unabhängig von einer Leberfunktion, Schock oder Muskelminderdurchblutung, möglich sowie komplikationsarm ist. Die Zitratakkumulation ist auschließlich bei Patienten mit schwerster Laktatazidose bei Multiorganversagens und starkeingeschränkter Prognose aufgetreten. Die Ergebnisse deuten auf das Auftreten der Zitratakkumulation als Folge des schwerwiegenden Versagens der Zellatmung hin. Die Zitratakkumulation könnte damit als ein starker Prädiktor für eine schlechte Prognose des Patienten interpretiert werden. Die Patienten, die mit RCA-CRRT behandelt werden und gleichzeitig eine schwere Laktatazidose mit erhöhtem Kalziumsubstitutionsbedarf haben, sind besonderes zu beachten.

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Eidesstattliche Versicherung

„Ich, Dmytro Khadzhynov, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Incidence and outcome of citrate accumulation in critically ill patients undergoing continuous renal replacement therapy with regional citrate anticoagulation" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)" des ICMJE - www.*icmje*.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst."

Datum   Unterschrift

Anteilserklärung an etwaigen erfolgten Publikationen

Dmytro Khadzhynov hatte folgenden Anteil an den folgenden Publikationen:

Publikation: **Khadzhynov D**, Schelter C, Lieker I, Mika A, Staeck O, Neumayer HH, Peters H, Slowinski T: Incidence and outcome of metabolic disarrangements consistent with citrate accumulation in critically ill patients undergoing continuous venovenous hemodialysis with regional citrate anticoagulation. J Crit Care 2014, 29(2):265-271.

Beitrag im Einzelnen (bitte kurz ausführen): Studiendesign, Datenerhebung, Datenanalyse, Publikationsentwurf, Veröffentlichung.

Unterschrift, Datum und Stempel des betreuenden Hochschullehrers

Unterschrift des Doktoranden/der Doktorandin

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

List of publications

1. **Khadzhynov D**, Slowinski T, Lieker I, Neumayer HH, Peters H: Evaluation of acid-base control, electrolyte balance, and filter patency of a prismaflexbased regional citrate anticoagulation protocol for pre-dilution continuous veno-venous hemodiafiltration. Clin Nephrol 2014, *In Press*

2. **Khadzhynov D**, Schelter C, Lieker I, Mika A, Staeck O, Neumayer HH, Peters H, Slowinski T: Incidence and outcome of metabolic disarrangements consistent with citrate accumulation in critically ill patients undergoing continuous venovenous hemodialysis with regional citrate anticoagulation. J Crit Care 2014, 29(2):265-271.

3. Wang-Rosenke Y, **Khadzhynov D**, Loof T, Mika A, Kawachi H, Neumayer HH, Peters H: Tyrosine kinases inhibition by Imatinib slows progression in chronic anti-thy1 glomerulosclerosis of the rat. BMC Nephrol 2013, 14:223.

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