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Original Article

ORIGINAL ARTICLE

A prospective study on serum citrate levels and clinical correlations in patients receiving regional citrate anticoagulation

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ABSTRACT

Background. Current ways to diagnose citrate accumulation (CA) in patients receiving regional citrate anticoagulation (RCA) continuous renal replacement therapy (CRRT) are confounded by various clinical factors. Serum citrate measurement emerges as a more direct way to diagnose CA, but its clinical utility and optimal cut-off values remain undefined. This study examined serum citrate kinetics and its diagnostic performance for CA in patients receiving RCA CRRT.

Methods. A multicentre prospective study was carried out in two tertiary referral centre intensive care units in Hong Kong with serum citrate levels measured at baseline and 2, 6, 12, 24, 36, 48 and 72 h after initiation of RCA CRRT and their relationships with the development of CA.

Results. Among the 133 patients analysed, 18 patients (13.5%) developed CA. The serum citrate levels at baseline and 2, 6 and 12 h after initiation of RCA CRRT in patients who had CA were significantly higher than the non-CA group ($P < .001$ for all). The CA group also had higher serum citrate levels than the non-CA group {median 0.93 mmol/L [interquartile range (IQR) 0.81–1.16) versus 0.37 mmol/L (IQR 0.26–0.57), $P < .001$}. Using a cut-off of 0.85 mmol/L, the serum citrate level had a sensitivity of 0.77 and a specificity 0.96 for the diagnosis of CA [area under the receiver operating characteristics curve (AUROC) 0.90, $P < .001$]. The 2-h and 6-h serum citrate levels had good discriminatory abilities for predicting subsequent development of CA (AUROC 0.86 and 0.83 for 2-h and 6-h citrate levels using cut-off values of 0.34 and 0.63 mmol/L, respectively; $P < .001$).

Conclusion. Serum citrate levels were significantly higher in patients with CA compared with patients without CA. Serum citrate levels showed good performance in diagnosing and predicting the development of CA.

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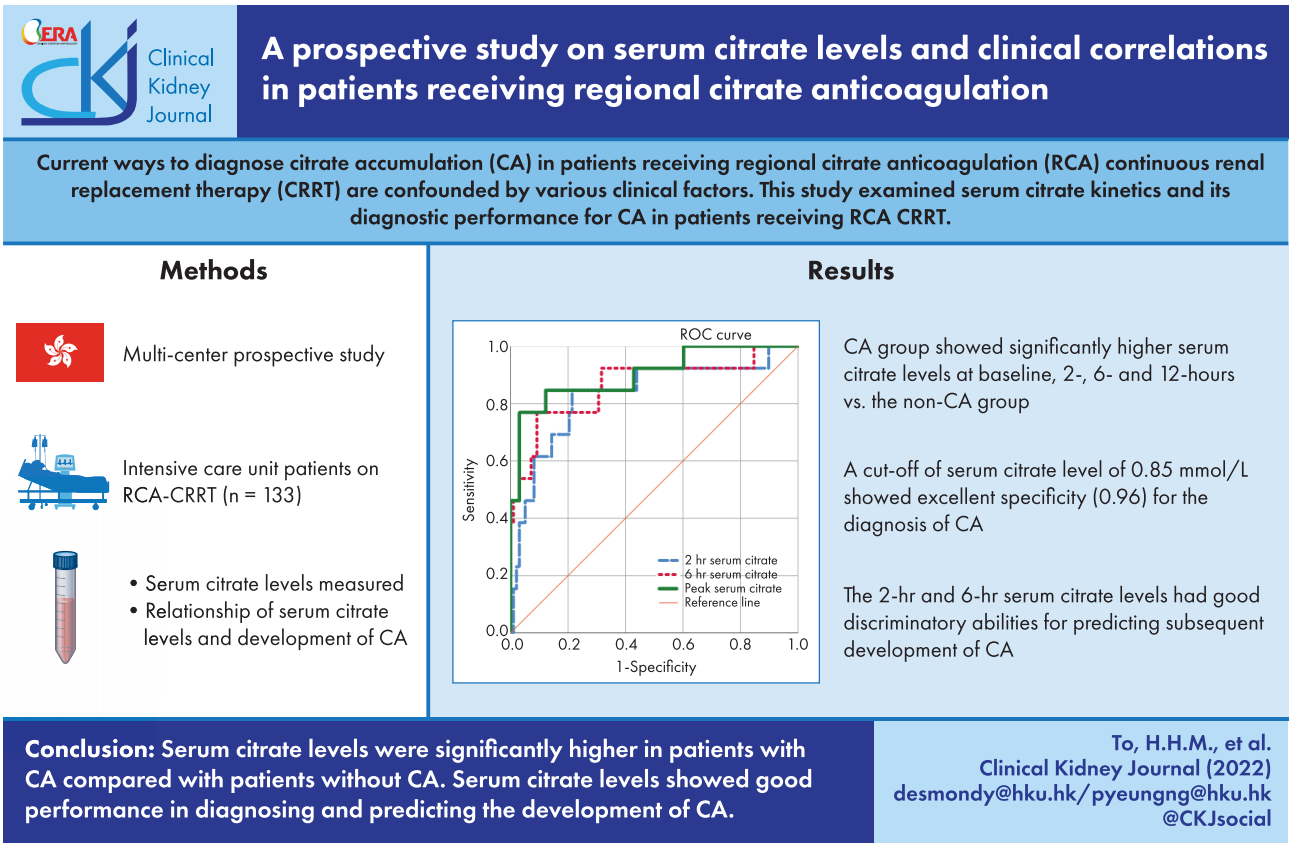
LAY SUMMARY

Regional citrate anticoagulation (RCA) is gaining popularity across the world as the first-line anticoagulation strategy for continuous renal replacement therapy (CRRT). Citrate accumulation (CA) remains a feared complication limiting the widespread application of RCA. Diagnosis of CA currently requires consideration of multiple biochemical parameters including the total calcium:ionized calcium ratio, which can be complex to interpret and subject to inaccuracies. This prospective study is the first of its kind to examine the serum citrate profile at predefined time points in 133 patients who received RCA CRRT. Serum citrate levels were shown to have good diagnostic value for CA. The 2-h citrate level taken early in the course of RCA CRRT had good predictive value for CA development, suggesting a potential role of direct citrate measurement in patients at high risk of developing CA.

GRAPHICAL ABSTRACT



Keywords: citrate accumulation, citrate toxicity, regional citrate anticoagulation, serum citrate level

INTRODUCTION

Anticoagulation is important for successful continuous renal replacement therapy (CRRT). Regional citrate anticoagulation (RCA) is associated with lower bleeding risks and superior filter life [1], with a consequent reduction in nursing costs and interruption of treatment compared with systemic heparin [2]. Consequently, RCA is advocated as first-line anticoagulation for CRRT [3]. Despite such proven benefits, RCA remains underutilized, especially in developing countries [4, 5]. Barriers to the widespread use of RCA include the complexity of RCA protocols and unavailability of commercially prepared citrate-containing replacement fluid [6]. More importantly, RCA CRRT is associated with the potentially life-threatening complication of citrate accumulation (CA), but accurate diagnosis of CA and identification of at-risk patients remain challenging [7].

Various diagnostic criteria for CA have been used, including surrogate markers for serum citrate such as pH, anion gap, total calcium concentration and total calcium:ionized calcium (tCa:iCa) ratio [8–10]. Among these parameters, only the tCa:iCa ratio has been shown to correlate with serum citrate levels in critically ill patients [11]. Such variability in the definition of CA accounted for the disparity in the CA incidence observed in different cohorts [12]. CA is most commonly inferred by an elevated tCa:iCa ratio (≥2.5) in combination with other parameters such as serum ionized Ca <1.1 mmol/L or an increasing Ca replacement requirement and high anion gap metabolic acidosis [10, 11]. However, many confounding factors such as

hypoalbuminaemia may lead to an elevated tCa:iCa ratio independent of CA [13, 14], resulting in a suboptimal performance of the tCa:iCa ratio >2.5 for the diagnosis of CA [15]. A large retrospective study of 1070 patients on RCA CRRT showed that systemic hypocalcaemia was seen in 82.9% of CA patients, an elevated tCa:iCa ratio in 78% and all of the above parameters at the same time in only 62.5% of the cases with CA [11]. A recent study resorted to machine learning models taking into consideration the complex interaction of multiple biochemical and clinical parameters to provide early warnings of CA [16]. Recognizing these limitations, direct measurement of the serum citrate level has emerged as a promising way to diagnose CA [8, 12, 17].

Previous studies of serum citrate measurements were limited by small patient sample sizes and the clinical utility and optimal cut-off values of serum citrate levels in RCA CRRT remain unknown [12, 17]. In this multicentre prospective study, we examined serial serum citrate kinetics in patients receiving RCA CRRT. The objective was to establish the optimal sampling time and cut-off values for diagnosing and predicting the development of CA.

MATERIALS AND METHODS

Study design and subjects

We conducted a multicentre prospective study to investigate serum citrate kinetics and its relationship with CA in patients receiving RCA CRRT. Critically ill adult patients who were admitted to the intensive care units (ICUs) of Queen Mary Hospital and Tuen Mun Hospital in Hong Kong and received RCA CRRT during the period July 2018–November 2020 were recruited. Both ICUs are tertiary referral centres that take care of patients with both medical and surgical diagnoses. RCA is the first-line anticoagulation in both ICUs for patients without contraindications to RCA and expected to be on CRRT for >24 h. The inclusion criteria were adult (>18 years of age) patients who require CRRT fulfilling at least one of the following clinical criteria: patients satisfying the 'injury' criteria (increase in creatinine by 2-fold or urine output <0.5 ml/kg/h for 12 h) according to the Risk, Injury, Failure, Loss and End-stage kidney disease criteria [18]; hyperkalaemia (K^+ >6.5 mmol/L); severe acidaemia (pH <7.2); urea >25 mmol/L accompanied by uraemic symptoms or clinically significant organ oedema not amenable to diuresis. The exclusion criteria were patients with fulminant hepatic failure (alanine aminotransferase/aspartate transaminase >3000 or international normalised ratio >3); pregnancy or lack of patient or surrogate consent.

Ethics approval and consent

The study was approved by the local ethics review committees (HKU/HA HKW IRB reference number: UW 18-356; NTWC IRB reference number: NTWC/REC/18104). Informed consents were obtained from patients (or their surrogate if they were not mentally sound to consent at the time of recruitment).

Material, equipment and procedure

Venous access for CRRT was obtained via a 12.5 Fr 16 or 20 cm triple-lumen catheter (MAHURKAR, Covidien, Dublin, Ireland) for internal jugular routes or a 25 cm triple-lumen catheter (MAHURKAR, Covidien) for femoral routes. All RCA CRRT was administered according to the departmental RCA protocol detailed in Appendix 1. Briefly, citrate was delivered either as Prismocitrate 18/0 (Baxter, Deerfield, IL, USA) or as 4% tri-sodium citrate pre-filter. The citrate dose was titrated in both protocols to target a post-filter ionized Ca level of 0.25–0.35 mmol/L. The default starting CRRT intensity was 25 ml/kg/h but could be titrated according to patient needs. All patients received Ca replacement in the form of 10% calcium chloride solution. The calcium infusion rate was titrated to achieve a systemic iCa level of 1.0–1.2 mmol/L. In patients with an elevated tCa:iCa ratio, physicians were given the discretion to alter the circuit setting by reducing the citrate dose, increasing dialysate flow or using alternative anticoagulation.

Data collection and specimen processing

Blood tests were performed for all recruited patients at baseline and every 6 h after commencement of RCA CRRT. Blood tests included arterial blood gas (ABG), renal function tests, non-pH-adjusted iCa level and lactate level. ABG and iCa were also taken at 2 h. Serial serum citrate levels at baseline and 2, 6, 12, 24, 36, 48 and 72 h (or at the end of CRRT if the circuit was stopped prior to 72 h) after initiation were taken. Specimens for serum citrate level measurement were kept at 4°C. Deproteinization was performed with a Pierce Protein Concentrator (10 K, molecular weight cut-off 0.5 ml; catalogue no. 88513, Thermo Scientific, Waltham, MA, USA). Serum citrate levels were measured by adapting a commercially available Citrate Assay Kit (Colorimetric/Fluorometric; catalogue no. ab83396; Abcam, Cambridge, UK). Optical density at 570 nm was measured using the Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA). A calibration curve was constructed by assaying in duplicate six citrate standards ranging from 0 to 10 nmol/well. The concentration of serum citrate was obtained from a standard curve of absorbance against nmol/well. Intra-assay variability was 5.2%. Lactate clearance at *x* hours was defined as (baseline lactate level − lactate level at *x* hours)/baseline lactate level. Ionized calcium levels were measured by a point-of-care test machine (RAPIDPoint 400 analyzer, Siemens Healthineers, Erlangen, Germany).

Outcomes

The primary outcome was CA. CA was established by the presence of at least three of four generally accepted systemic metabolic criteria of a decrease of systemic iCa below 1.1 mmol/L, a concomitant increase of total Ca and thus and an elevated tCa:iCa ratio >2.5, relevant metabolic acidosis (pH <7.2 of base excess below −5 mmol/L) without or with an increased anion gap (normal: 11 mmol/L) [10].

Statistical analysis

The primary analysis was to describe and compare the serum citrate profiles of CA and non-CA patients on RCA CRRT. The secondary analyses were to establish cut-offs for diagnosis and prediction of CA. Based on pilot data showing a difference of 0.4 mmol/L in citrate levels between patients with and without CA, a sample size of 128 had 90% power to detect a difference at a significance level of 0.05. Baseline demographic, laboratory data and plasma citrate levels were compared between patients with and without CA. Categorical variables were expressed as frequencies (percentages) and compared with the chi-squared test or Fisher's exact test where appropriate. Continuous variables were presented as mean ± standard deviation (SD) or median with interquartile range (IQR) and analysed with the Student's

Table 1: Baseline clinical characteristics in patients who have and have not developed CA.

Characteristics	CA group ($n = 18$)	Non-CA group ($n = 115$)	All ($N = 133$)
Patients			
Age (years), mean ± SD	70.4 ± 10.1	64.7 ± 14.3	65.5 ± 13.9
Male, *n* (%)	11 (61.1)	76 (66.1)	87 (65.4)
MAP (mmHg)	67 (64–83)	78 (65–86)	74 (65–85)
Heart rate (beats/min)	98 (81–106)	98 (79–109)	98 (79–109)
pH	7.29 (7.23–7.43)	7.35 (7.26–7.43)	7.34 (7.25–7.43)
Body temperature (°C)	36.2 (35.5–37.2)	36.7 (36.1–37.2)	36.6 (36.0–37.2)
Noradrenaline (μg/kg/h)*	18.0 (7.5–26.4)	3.27 (0.00–11.1)	3.69 (0.00–14.8)
LVEF (%)	52.5 (32.5–58.8)	50.0 (35.0–55.0)	50.0 (35.0–55.0)
Packed cells transfused (*n*)	0.0 (0.0–0.5)	0.0 (0.0–1.0)	0.0 (0.0–1.0)
Baseline biochemistry			
Sodium (mmol/L)	139.5 (135.0–145.0)	136.0 (132.0–141.0)	136.0 (132.0–141.0)
Chloride, mmol/L)	102.0 (94.3–111.0)	97.0 (92.0–101.0)	96.5 (91.3–99.0)
Potassium (mmol/L)	4.6 (4.0–5.6)	4.6 (4.0–5.4)	4.6 (4.0–5.4)
Bicarbonate (mmol/L)	17.0 (13.0–20.0)	19.0 (16.0–24.0)	19.0 (16.0–23.5)
Base excess (mmol/L)	−8.0 (−11 to −4.75)	−6.0 (−10.0 to −0.8)	−6.0 (−10 to −1.9)
Creatinine (μmol/L)	376.5 (201.8–489.0)	479.0 (290.0–668.0)	437.0 (282.5–661.0)
Urea (mmol/L)	31.3 (19.4–42.3)	30.2 (21.3–39.3)	30.3 (21.3–39.5)
Baseline lactate* (mmol/L)	4.3 (2.6–11.4)	1.8 (1.1–2.9)	2.00 (1.2–3.7)
Peak lactate** (mmol/L)	14.5 (9.7–18.7)	2.5 (1.7–4.3)	2.9 (1.8–7.3)
APACHE IV score**	138.0 (112.5–164.8)	99.0 (82.0–117.0)	104.0 (85.0–124.0)
APACHE II score**	39 (28–46)	29 (24–34)	30 (25–36)
Lactate clearance at 6 h**	−0.39 (−1.05–0.04)	0.00 (−0.20–0.20)	−0.05 (−0.29–0.17)
Lactate clearance at 12 h**	−0.95 (−2.78–0.08)	0.00 (−0.33–0.22)	−0.07 (−0.44–0.21)
Bilirubin** (μmol/L)	23.0 (13.8.0–92.3)	12.0 (7.00–39.5)	13.0 (7.3–43.8)
ALT (U/L)	79.0 (49.0–471.0)	65.0 (26.8–244.0)	33.0 (16.0–120.0)
AST (U/L)	74.0 (41.5–351.0)	66.5 (29.3–252.3)	66.0 (30.0–249.0)
INR**	1.70 (1.60–1.80)	1.20 (1.10–1.50)	1.3 (1.1–1.6)
PT** (s)	18.6 (16.3–21.7)	14.1 (13.2–17.1)	14.4 (13.3–17.8)
APTT** (s)	42.9 (36.4–51.0)	35.0 (29.3–41.6)	35.7 (29.5–42.6)
Mortality, *n* (%)			
ICU mortality**	16 (88.9)	30 (26.1)	46 (34.6)
1-month mortality**	18 (100)	47 (40.9)	65 (48.9)
3-month mortality**	18 (100)	52 (45.2)	70 (52.6)

Values are presented as median (IQR) unless stated otherwise.
*$P < .05$, **$P < .01$.
ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate transaminase; INR, international normalised ratio; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; PT, prothrombin time.

t-test or Mann-Whitney *U* test where appropriate. Variates with *P*-values <.05 in univariate analyses were included in multivariate logistic regression performed to identify independent predictors for CA. Non-parametric receiver operating characteristics (ROC) curve analysis was used to assess the sensitivity and specificity of different serum citrate thresholds for the diagnosis and prediction of CA. Correlation between tCa:iCa ratios and serum citrate levels was assessed with Spearman correlation analysis. Analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). Optimal cut-offs for ROC curves were identified by criterion values with the largest Youden index. This, along with comparisons of the areas under the receiver operating characteristics curve (AUROCs) were performed with MedCalc version 20 (MedCalc Software, Ostend, Belgium).

RESULTS

Patient characteristics

A total of 133 patients were recruited in the study period (Table 1, Appendix 3). The mean age was 65.5 ± 13.9 years and the median Acute Physiology and Chronic Health Evaluation (APACHE) IV score was 104.0 (IQR 85.0–124.0). The leading indications for CRRT were fluid overload (32.3%), metabolic acidosis (29.0%) and hyperkalaemia (20.3%). The median circuit lifespan was 40.0 h (IQR 22.5–54.5) for all sessions and was 48 h (IQR 27.8–72.0) after excluding cases electively terminated for imaging or achievement of goals. A total of 18 patients (13.5%) developed CA, and they had significantly higher peak vasopressor requirements, higher APACHE scores, higher lactate levels and more negative lactate clearance than patients without CA ($P < .05$ for all). Among the 18 patients with CA, the median iCa was 0.98 mmol/L (IQR 0.94–1.08) and the median maximum tCa:iCa ratio was 2.79 (IQR 2.66–3.04). The median iCa was 1.13 mmol/L (IQR 1.07–1.16) and the median maximum tCa:iCa ratio was 2.45 (IQR 2.35–2.54) for patients without CA. The first alteration made to the circuit setting as a result of elevation of the tCa:iCa ratio was undertaken at a median of 8.5 h (IQR 3.2–12.3). CA occurred in 11 of 34 patients (32%) whose circuit was altered. Among the remaining seven patients who had CA, four showed an elevation in the tCa:iCa ratio, but no change to the circuit setting was made, and three did not have an elevation of the tCa:iCa ratio >2.5.

Longitudinal profiles of serum citrate levels

The CA group had higher baseline serum citrate levels than the non-CA group [median 0.17 mmol/L (IQR 0.08–0.44) versus

Table 2: Serum citrate and calcium profiles in patients who have or have not developed CA.

Characteristics	CA group ($n = 18$)	Non-CA group ($n = 115$)	All ($n = 133$)	P-value
Baseline iCa (mmol/L)	1.06 (1.00–1.08)	1.08 (1.01–1.16)	1.07 (1.01–1.16)	.31
Baseline tCa (mmol/L)	1.99 (1.92–2.30)	2.12 (1.93–2.38)	2.12 (1.93–2.37)	.47
Baseline tCa:iCa ratio	1.94 (1.83–2.14)	1.99 (1.82–2.14)	1.98 (1.83–2.14)	.66
Maximum tCa:iCa ratio	2.79 (2.66–3.04)	2.45 (2.35–2.54)	2.47 (2.36–2.61)	<.001
Mean tCa:iCa ratio	2.48 (2.42–2.57)	2.26 (2.19–2.34)	2.28 (2.20–2.36)	<.001
Baseline serum citrate	0.17 (0.08–0.44)	0.08 (0.06–0.13)	0.10 (0.06–0.14)	.01
Peak serum citrate	0.93 (0.81–1.16)	0.37 (0.26–0.57)	0.39 (0.27–0.66)	<.001
2-h serum citrate	0.56 (0.34–0.74)	0.23 (0.18–0.32)	0.25 (0.19–0.36)	<.001
6-h serum citrate	0.72 (0.50–1.04)	0.27 (0.20–0.41)	0.28 (0.21–0.52)	<.001
12-h serum citrate	0.93 (0.40–1.26)	0.29 (0.22–0.43)	0.30 (0.23–0.45)	<.001
Mean serum citrate	0.73 (0.54–0.92)	0.29 (0.21–0.41)	0.31 (0.22–0.49)	<.001
24-h serum citrate	–[a] ($n = 3$)	0.30 (0.24–0.48)	0.31 (0.24–0.49)	
36-h serum citrate	–[a] ($n = 2$)	0.35 (0.24–0.45)	0.36 (0.24–0.45)	
48-h serum citrate	–[a] ($n = 1$)	0.35 (0.20–0.45)	0.35 (0.21–0.47)	
6-h slope intercept	0.08 (0.05–0.12)	0.03 (0.02–0.05)	0.03 (0.02–0.05)	<.001
12-h slope intercept	0.07 (0.03–0.09)	0.02 (0.01–0.02)	0.02 (0.01–0.03)	<.001

[a]Data not available, as RCA CRRT was terminated/switched to alternative anticoagulation.
Serum citrate levels in mmol/L. Values presented as median (IQR).

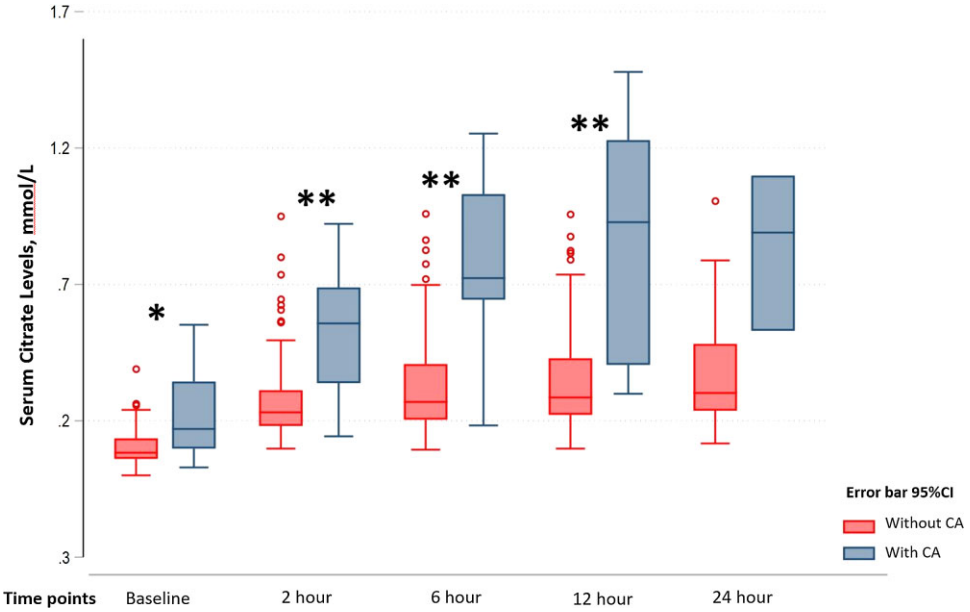


Figure 1: The serum citrate levels at different time points after initiation of RCA CRRT in patients who have and have not developed CA. Serum citrate levels at various time points in patients with CA and patients without CA. Serum citrate levels were significantly higher in the CA group at baseline and 2, 6 and 12 h after initiation of regional citrate anticoagulation (*$P < .05$, **$P < .001$; CA versus non-CA group). CA: patients with CA; no CA: patients without CA.

0.08 (0.06–0.14), respectively; $P = .014$] (Table 2). In the CA group, serum citrate levels increased progressively at 2 and 6 h until the CRRT was terminated. The serum citrate levels at 2, 6 and 12 h after initiation of RCA CRRT in the CA group were all significantly higher than the non-CA group ($P < .001$ for all) (Fig. 1). In patients who tolerated RCA, serum citrate levels remained low over time with a median mean citrate level of 0.29 mmol/L (IQR 0.21–0.41) compared with 0.73 mmol/L (IQR 0.54–0.92) in patients with CA ($P < .001$). Slope intercepts including serum citrate levels at 6 and 12 h were significantly higher in patients with CA (Table 2). All patients with CA had their RCA CRRT runs terminated by 12 h.

Cut-off values of serum citrate for diagnosis of CA

The peak serum citrate level was significantly higher in the CA group than in the non-CA group [median 0.93 mmol/L (IQR 0.81–1.16) versus 0.36 (0.26–0.57), $P < .001$]. With a cut-off of 0.85 mmol/L, the peak serum citrate level has an AUC of 0.90 ($P < .001$) in identifying patients with CA, with a sensitivity of 0.77 and a specificity of 0.96 (Fig. 2, Table 3). The positive predictive value (PPV) was 0.67 and the negative predictive value (NPV) was 0.97. The threshold of the peak citrate level (0.85 mmol/L) was breached at a median of 3 h (IQR 0–6) before the occurrence of CA.

Cut-off values of serum citrate for prediction of CA

The performance of 2- and 6-h serum citrate levels to predict subsequent development of CA were evaluated. Using a cut-off value of 0.34 mmol/L, the sensitivity and specificity of 2-h serum citrate levels to predict subsequent development of CA were 0.77

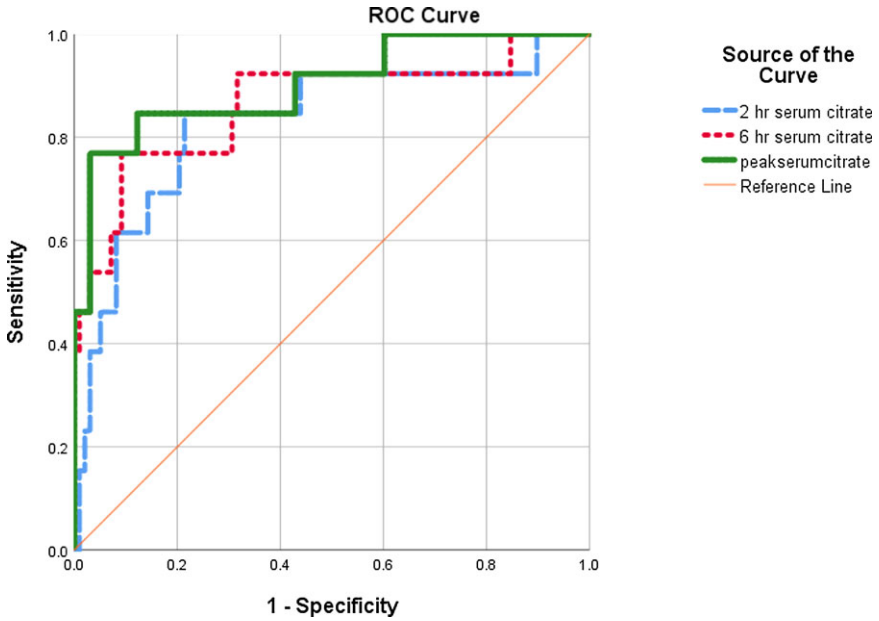


Figure 2: ROC curves of peak, 2-h and 6-h serum citrate levels in the diagnosis and prediction of CA.

Table 3: The performance of serum citrate levels at different time points for the diagnosis of CA.

Characteristics	AUC (95% CI)	Cut-off (mmol/L)	Sensitivity	Specificity	P-value	PPV	NPV
Peak serum citrate	0.90 (0.80–1.00)	0.85	0.77	0.96	<.001	0.67	0.97
2-h serum citrate	0.83 (0.69–0.97)	0.34	0.77	0.80	<.001	0.29	0.95
6-h serum citrate	0.86 (0.74–0.99)	0.63	0.78	0.91	<.001	0.50	0.97
tCa:iCa ratio	0.86 (0.72–1.00)	2.5	0.89	0.64	<.001	0.28	0.97
		2.6	0.89	0.85		0.47	0.98

and 0.80, respectively (AUROC 0.83, $P < .001$). Using a cut-off value of 0.63 mmol/L, the sensitivity and specificity of 6-h serum citrate levels to predict subsequent development of CA were 0.78 and 0.91, respectively (AUROC 0.86, $P < .001$) (Fig. 2, Table 3).

tCa:iCa ratio and serum citrate levels

The 2- and 6-h serum citrate levels showed a positive correlation with the tCa:iCa ratio at the corresponding time points ($r = 0.34$ and 0.50, respectively; $P < .001$ for both), while the serum citrate levels at other time points did not show any significant relationship with the tCa:iCa ratio (Appendices 2 and 4).

Using the commonly accepted cut-off of the tCa:iCa ratio (i.e. 2.5), the sensitivity and specificity for diagnosis of CA in this cohort were 0.89 and 0.64, respectively. Further analysis of our data showed that the optimal cut-off value of the peak tCa:iCa ratio to diagnose CA was 2.6 (95% confidence interval CI 2.56–2.64) (sensitivity and specificity were 0.89 and 0.85, respectively; AUROC 0.86, $P < .001$). When the cut-off of the tCa:iCa ratio for diagnosis of CA was increased from 2.5 to 2.6, the PPV increased from 0.28 to 0.47 while the NPV remained unchanged at 0.98. Serum citrate showed a numerically higher AUROC than the tCa:iCa ratio in the diagnosis of CA, but the difference did not reach statistical significance (AUROC 0.90 versus 0.86; $P = .48$). However, the serum citrate level had a higher sensitivity and PPV than the tCa:iCa ratio in the diagnosis of CA (Table 3).

DISCUSSION

RCA CRRT is gaining popularity as the choice of treatment in critically ill patients. The development of CA remains an important concern in RCA CRRT and the current ways of diagnosing CA can be confounded by multiple clinical factors. Measurement of serum citrate level has emerged as a direct way to establish a diagnosis of CA, but such data remain limited to small cohorts with no cases of CA among them [12, 17]. Here we report the results of the largest prospective cohort that examined serum citrate profiles and their clinical associations in patients receiving RCA CRRT. Our data suggest that patients who develop CA have significantly higher serum citrate levels. The 2- and 6-h serum citrate levels exhibited excellent performance in the prediction of CA and should be considered in the management of patients undergoing RCA CRRT.

In CA patients, an abrupt increase in serum citrate levels occurred as early as 2 h after initiation of RCA CRRT, reaching a peak at 12 h. Such a pattern was in stark contrast to the non-CA group, in which serum citrate levels remained stable throughout the treatment period with a mean of 0.29 mmol/L, in keeping with previous study findings [12, 17]. Previous studies on serum citrate levels during RCA CRRT consisted of small cohorts of patients with minimal data on patients who developed CA [8, 12, 17]. Patients who developed CA in this cohort were more critically ill compared with patients without CA, as exemplified by higher vasopressor requirements, APACHE scores and lactate

levels [9, 10]. CA should be regarded as more of an indicator of severe cellular metabolic dysfunction than being causative in death. Furthermore, we also noted that the CA groups showed higher baseline serum citrate levels than non-CA group before initiation of RCA CRRT. The two groups did not differ significantly in blood transfusions after ICU admission. Citrate is metabolized in the Krebs cycle via isocitrate dehydrogenase, with hydrogen being transferred to nicotinamide adenine dinucleotide hydride (NADH). The regeneration of co-enzyme NADH to NAD^+ is an oxygen-dependent process [10]. It remains speculative that these patients had a tendency towards impaired metabolism of naturally occurring citrate in the body from oxygen deficiency at the microcirculatory level and hence were more susceptible to CA during RCA CRRT. Whether baseline serum citrate levels help identify at-risk patients for closer monitoring and earlier interventions to prevent CA remains to be determined by further studies.

Our results demonstrate that using a cut-off of 0.85 mmol/L, the serum citrate level showed excellent performance in diagnosing CA. One should appreciate that the threshold of 0.85 mmol/L was breached early, approximately 6 h after initiation of RCA CRRT, allowing an early, straightforward and definitive diagnosis of CA. This is in contrast with the prevailing means to diagnose CA that mandates consideration of multiple clinical parameters, including elevated tCa:iCa ratio, high anion gap metabolic acidosis and systemic hypocalcaemia [10, 11]. The wide variation in reported incidence of CA from 2.3 to 23% could be partly explained by the variable inclusion and exclusion of the aforementioned laboratory parameters, which reflects the lack of an universally accepted standard of diagnosis [9, 11]. There is an unmet need for an accurate and simple way to diagnose CA.

Apart from diagnosis of CA, predicting the development of CA is also of clinical importance. In this context, although the peak citrate level was strongly associated with CA, the uncertain time point of its occurrence may limit its applicability. Our results suggest that serum citrate levels taken at 2 and 6 h after initiation of RCA CRRT are useful in predicting subsequent development of CA. In this study, the first alteration made to the circuit setting as a result of suspicion of CA was undertaken at a median of 8.5 h, and had serum citrate levels been routinely measured at 2 and 6 h, there may be more timely modification of RCA CRRT regimens in at-risk patients. In addition, the cut-off values at 2 and 6 h established by our data are simpler to use than the prevailing means for clinical assessment that involve a combination of surrogates for CA plagued with confounding factors. Between the two, the 2-h citrate level appears to be more practical, taking into consideration the turnaround time for measurement, to facilitate earlier alterations to the regimen/circuit.

In addition, we investigated the relationship between serum citrate levels and the tCa:iCa ratio and compared their performances in diagnosing CA. Although our study was largely exploratory, with an aim to describe the serum citrate profile of patients on RCA CRRT, there was significant correlation between the tCa:iCa ratio and serum citrate levels only at 6 and 12 h after initiation of RCA CRRT but not at other time points. Indeed, the association between serum citrate levels and the tCa:iCa ratio is controversial. Conflicting results in previous studies might be related to the lack of any CA in previous cohorts and confounding factors like variable serum albumin levels in critically ill patients [8, 12, 13, 15]. Serum citrate measurement exhibited distinct properties that may complement the tCa:iCa ratio in diagnosing CA. Serum citrate levels have the merit of higher specificity and PPV in diagnosing CA and thus give clinicians more confidence in reducing unnecessary circuit alterations caused by non-specific elevations in the tCa:iCa ratio. In our cohort, CA occurred in only 32% of cases whose circuit was altered because of clinical suspicion of CA, suggesting that the majority of circuit alterations could be avoided with a more accurate diagnostic test. Rather than replacing existing diagnostic criteria/tests, serum citrate may complement current means such as the tCa:iCa ratio. This is particularly useful in situations where there is major confounding for the tCa:iCa ratio (e.g. albumin level) or lactate levels (e.g. use of high-dose adrenaline) or when patients are at high risk of CA (e.g. hepatic dysfunction). Further studies should focus on identifying particular patient subgroups that may benefit from targeted citrate level monitoring.

Important limitations of our study were its observational nature and the inclusion of only a derivation cohort without a separate validation cohort. Moreover, we did not collect data on blood transfusion prior to ICU admission as a potential source of citrate that could potentially affect baseline serum citrate levels. As our data were derived from patients receiving continuous venovenous haemodiafiltration (or continuous venovenous haemodialysis), these results may not be generalizable to centres that perform only continuous venovenous haemofiltration. Despite a low absolute number of patients who developed CA, the incidence rate of CA appeared to be quite high in our cohort. Previous studies have reported variable rates of CA, which may be related to the different diagnostic criteria used and patient heterogeneity. Our cohort also represents a sicker cohort, as reflected by a higher median APACHE II score than that reported in a large retrospective cohort [APACHE II score of CA group in this cohort was 39 (IQR 28–46) versus 34 (IQR 31–38) in a historical cohort while that in the non-CA group was 29 (IQR 24–34) versus 26 (IQR 25–26) in a published cohort] [11]. Our study is by far the largest prospective cohort that has investigated serum citrate kinetics in critically ill patients receiving RCA CRRT. In contrast to previous studies that focused only on patients with septic shock or burns [12, 17], our inclusion of patients with different medical and surgical diagnoses renders our observations a representation of real-world data and more applicable to various clinical contexts. Other issues include the difficulty in standardization of citrate measurement, as most commercially available assays use enzyme-linked immunosorbent assay or two-step enzymatic techniques. Further studies are worthwhile to evaluate the cost-effectiveness of incorporating serum citrate in a standardized monitoring algorithm during RCA CRRT.

CONCLUSION

Serum citrate levels were significantly higher at different time points in patients who developed CA and showed good performance in the diagnosis and prediction of CA. The measurement of serum citrate levels can facilitate patient monitoring and treatment modification in RCA CRRT.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

H.H.M.T. wrote the manuscript. H.H.M.T., A.M.C.K., N.Y.Y.L. and D.Y.H.Y. designed the study. P.Y.N., C.W.N., W.M.C., A.S.K.W. and T.S.K.M. contributed to data acquisition. I.Y. contributed to data interpretation and analysis. All authors reviewed the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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