The Gordian knot of the long-term safety of dialysate citrate: is there really a concern about patient hard outcomes?

Francesco Pizzarelli 1 and Carlo Basile 2,3

1Nephrology Unit, SM Annunziata Hospital, Florence, Italy, 2Clinical Research Branch, Division of Nephrology, Miulli General Hospital, Acquaviva delle Fonti, Italy and 3Associazione Nefrologica Gabriella Sebastio, Martina Franca, Italy

Correspondence to: Francesco Pizzarelli; E-mail: fpizzarelli@yahoo.com

The bicarbonate-based dialysate contains a few millimoles of acetic acid to stabilize the pH. Due to the favourable dialysate–plasma gradient, the plasma acetate levels at the end of the haemodialysis (HD) session are several times higher than pre-treatment, such as to induce the well-known acetate adverse effects [1]. Citric acid is an alternative dialysate buffer that is converted to its anion citrate into the body. Compared with controls with normal renal function, citrate clearance by the Krebs cycle is not impaired in long-term HD patients [2]. As for acetate, the metabolism of citrate is not completed during the dialysis session since the hepatic and muscular metabolism occurs partly after the end of the treatment. In addition, there are fast and slow metabolizing patients, depending on liver function and muscle mass. For instance, in patients with liver insufficiency, citrate clearance is reduced by 50% [3]. Indeed, citric acid not only may substitute acetic acid as a pH stabilizer, but also is an effective anticoagulant on dialysis membranes [4]. Ahmad et al. [5] were the first to demonstrate almost two decades ago that citric acid-based dialysate increases the delivered dialysis dose. These results were confirmed in a large prospective controlled study in 2009 [6]. Moreover, Sands et al. [7] could reduce the heparin dose by up to one-third without any detrimental effect on Kt/V, shifting a large cohort of patients from standard bicarbonate-based dialysate to citric acid-based dialysate. Small, acute studies have challenged the feasibility of heparin-free citric acid-based dialysate in HD [8] and postdilution on-line haemodiafiltration (HDF) [9], respectively. However, the follow-up periods were short.

Apart from anticoagulation and dialysis efficiency, some small crossover studies reported positive effects of citrate on systemic haemodynamics, anaemia, control of acidosis and malnutrition [10–12]. However, the topic of greatest interest is the potential role of citrate on inflammation. Actually, it is difficult to discriminate whether the anti-inflammatory effect of citric acid-based dialysate is due to the absence of acetate [13, 14] or to the presence of citrate as such. Indeed, several in vitro studies prove the anti-inflammatory properties of citrate. Bryland et al. [15] have shown in an elegant study that citrate may have therapeutic potential in diabetic patients by reducing hyperglycaemia-induced endothelial inflammation and by abolishing endothelial dysfunction. In a titration study, Huang et al. [16] found that citrate reduced granulocyte activation and complement activation at the human whole blood concentrations attained during HD. As to clinical reports, several studies found positive effects of citrate on markers of oxidative stress and inflammation such as reduction of oxidized low-density lipoproteins after only 1 week [17], decreased glycoxidation and lipid peroxidation products [18], and reduced intra-dialytic induction of pentraxin-3, a marker of dialysis bioincompatibility associated with cardiovascular disease [19]. Analysing C-reactive protein and interleukin-6 in convective treatments, several studies confirmed the positive effect of citrate on inflammation [12, 20, 21]. However, the aforementioned studies do not fully satisfy evidence-based medicine due to several drawbacks, e.g. a short single-arm study design with a small dataset and/or treatment sequence not randomized, etc.

Assessing the role of citrate in systemic calcification propensity opens up new and exciting scenarios possibly linked to inflammation. Following the preliminary positive results by Lorenz et al. [22], Dellepiane et al. recently studied 45 patients in a 9-month A-B-A study. In comparison with acetate, acid citric-based dialysate improved chemerin-induced endothelial cell dysfunction and vascular smooth muscle cells calcification. Interestingly enough, these in vitro results parallel in vivo results, pointing to a reduction of the inflammatory parameters, namely C-reactive protein and interleukin-6 [23].

As to safety, it is known from regional citrate anticoagulation that citrate is a powerful chelator of divalent cations with complexed calcium removed from the blood during dialysis [24]. Indeed, inducing extracorporeal ionized calcium (Ca++) depletion by regional citrate is a tool for studying the role of Ca++ in neutrophil degranulation during HD [25, 26].

A question arises of whether the much lower dose of acid citric in the dialysate, e.g. one-fifth of that adopted in regional coagulation, is still able to induce Ca++ derangements in maintenance dialysis treatments. Despite low concentrations (0.8–1.0 mmol/L), citrate reduces dialysate calcium (Ca) and magnesium (Mg) concentrations, then diffuses through the dialysis membrane and further decreases plasma Ca++ and ionized Mg concentrations, a mechanism that tends to reduce

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divalent ions mass balance. Specific effects of dialysate citrate on Ca mass balance and serum immunoreactive parathyroid hormone (iPTH) levels have been well described in both acute and long-term settings. In comparison with control dialysate, citric acid-based dialysate was associated with post-treatment lower serum Ca and higher serum iPTH levels, while pre-treatment levels were generally unaffected [19–21, 27, 28]. This holds true in both HD and HDF. The chelation effect of dialysate citrate on divalent ions has prompted the manufacturers to compensate for this negative mass balance effect by increasing dialysate Ca up to 0.15–0.25 mmol/L, not considering adjustment for dialysate Mg.

The first side effect of hypocalcaemia is a prolongation of the QT interval [29]. Clinical signs of hypocalcaemia and hypotension in humans appear at plasma Ca levels of <0.9 mmol/L. The side effects tend to disappear with a dialysate total Ca concentration of 1.65 mmol/L, in this way accounting for 10% of the Ca chelation estimate [21]. In the multicentre randomized crossover study by Schmitz et al. [30], more patients developed fatigue, cramps and pain in the first 2 weeks of exposure to citrate. However, these studies are so short-lived that they are not able to cut the Gordian knot of dialysate citrate safety in the long run.

While lacking robust randomized controlled trials (RCTs), the industry and the nephrological community were gradually convinced of the efficacy and safety of citric acid-based dialysate. Dialysate citrate has been used in the USA for more than a decade [5] and then worldwide, without particular concerns. Indeed, during the 2000s in Europe up to 20% of HD and HDF sessions have been made with acetate-free citric acid-based dialysate. Besides its extensive utilization in the general HD population, there are clinical pictures in which the utilization of dialysate citrate may be preferential over dialysate bicarbonate (Table 1) [31].

Table 1. Clinical pictures in which the utilization of dialysate citrate may be preferential over dialysate bicarbonate in maintenance HD patients*

<table>
<thead>
<tr>
<th>Haemodynamic instability</th>
<th>Low serum iPTH levels or adynamic bone disease</th>
<th>Hypercalcemia</th>
<th>Vascular calcification</th>
<th>Indication to dialysis with a reduced heparin dosage or no heparin at all</th>
<th>Post-HD metabolic alkalosis</th>
<th>Symptomatology associated with increased intra- or post-HD serum bicarbonate levels or aggravated by post-HD metabolic alkalosis: chronic respiratory failure with excess carbon dioxide generation; advanced chronic liver disease; headache</th>
<th>Hypermagnesaemia</th>
<th>Difficulty in achieving the prescribed dose of dialysis</th>
<th>Malnutrition</th>
</tr>
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*Adapted from reference [31].

up to 40% in patients exposed to citrate-based dialysate. These preliminary, not reassuring data prompted the Agence Nationale de Sécurité du Médicament (the French National Agency for the Safety of Medicinal Products) on 6 December 2018 to send a national warning message focused on the citrate-based dialysate [32]. Séret et al. [33] and Potier et al. [34] were the first to grab the relay baton. The study by Séret et al. [33] did not show an increased risk of mortality in incident HD patients (median follow-up 23 ± 18 months) with citrate-based dialysate, despite higher comorbidities in the citrate-exposed group. While Séret et al. [33] published their data as a letter to the Editor, Potier et al. [34] are publishing their results as an original article in this issue of Nephrology Dialysis Transplantation (NDT). The primary goal of their study was to ascertain whether long-term citrate exposure is associated with extra all-cause mortality in maintenance extracorporeal treatments. In this long-term, retrospective, observational study, the authors analysed 1132 incident patients starting dialysis over a time span of 10 years, from 2008 to 2018, in five sanitary territories in the western region of France. Interestingly enough, the prevalence of dialysate citrate in these dialysis centres reached 57% in 2018, e.g. a much higher figure than in Europe. Dichotomizing subjects according to the two different criteria of exposure to citrate, the authors compared patients who spent >80% or <80% of their dialysis time on citric acid-based dialysate (exploratory study), and those who have always been treated with citrate compared with those who have never been (exploratory study). Both populations were analysed before and after propensity score matching [34].

The good news is that (verbatim) ‘CIT-HD exposure up to a 6-year period has no significant effect on all-cause mortality in HD patients’. This result derives from robust statistics such as Kaplan–Mayer and multivariate Cox regression analysis. However, this is a retrospective study, with all the related potential biases [34].

The exploratory population face a survivorship bias [35]. Dialysis patients have waited some time, even years, before starting the treatment with dialysate citrate. They did not die while waiting, thereby introducing a selection bias with those patients who did. No amount of adjustment for baseline factors can overcome this bias. In contrast, immortal time bias does not belong to the explanatory bias that has always been treated with citrate or with other buffers without ever shifting from one to the other. Therefore, the strength of the study is in the matched explanatory population.

This being a retrospective study, of course, (verbatim) ‘Choice of dialysis modality and facility type relied on a medical decision…’ [34]. Confounding by indication may arise when clinicians use their expertise to decide whether a patient has an indication for a certain treatment [36]. Moreover, unmeasured or unknown confounders may persist despite statistical procedures aimed at eliminating them. When the propensity score model leaves out relevant parameters, matching may not yield the balance in the confounder distribution that is aimed at [37]. In the Potier et al. study, dialysis modality and facility type were not entered as matching variables. As a result, the propensity cohort ‘always on citrate’ was more likely treated with online...
HDF and tended to have a better survival rate than the cohort ‘never on citrate’ [34]. Many RCTs report survival advantages of adequate convection [38–40] and this retrospective study was not intended to add anything in this regard. The problem remains that it is hard to dissociate retrospectively the merits of the buffer from those of convection, as the utilization of both techniques has grown in parallel during the time period of the Potier et al. [34] study.

Regarding the biochemical data, the multivariable analysis would have been more informative if the authors had adjusted for time-averaged instead of baseline values. In comparing time-averaged biochemical values in the matched cohorts treated or not with citrate, the authors attribute the statistically significantly lower Erythropoietin Resistance Index of the former cohort to the possible anti-inflammatory effect of citrate. However, C-reactive protein is not significantly reduced, which is evidence of how it is hard to glance retrospectively the complex inflammatory network by relying on some surrogate parameters. Moreover, albumin reduction does not fit with the possible beneficial effect of citrate on nutrition [12]. Finally, the study was not designed to probe the role of citrate on divalent ions as proved by the lacking data on serum Ca\(^{++}\) and Mg. However, it is reassuring that over the years, the serum iPTH level was not different between citrate and non-citrate cohorts [34].

While the editing process of the present Editorial was going on, another relevant contribution to the topic was submitted and is being published in this issue of NDT [41]. Neri et al. report their experience in dialysis patients treated at the Fresenius Medical Care network in France, the Czech Republic and Turkey. Association between citrate-based dialysate and mortality was assessed in incident patients from January 2014 to October 2018. The authors applied propensity score matching and assessed citrate exposure within different study designs. In the primary analysis, patients were assigned to the exposed group if they were treated with citrate-based dialysate in >70% of sessions during the first 3 months of dialysis, whereas non-exposed patients received no citrate-based dialysate at all. The citrate group had more comorbidities (higher Charlson index, lower serum albumin levels) than the control cohort. Since dialysate composition may change over time, Neri et al. accounted for time-dependent variations in dialysate composition exposure during follow-up. In secondary analyses, the authors assigned patients to the citrate group if even just one session/month was administered with citrate-based dialysate. Mortality was attributed to the last exposure assignment. With the same very wide criteria for citrate assignment, the authors extended the ascertainment time also to 6-month intervals. Analysed both at monthly and 6-month intervals, the data excluded an extra mortality attributable to the use of citrate-based dialysate [41]. Neri et al.’s [41] experience shares with the Séret et al. [33] and Potier et al. [34] studies some weaknesses peculiar to retrospective analyses, in particular, residual confounding. Moreover, the ways in which Neri et al. [41] defined exposure to citrate may be arguable. However, the convergence of the results of the three studies here analysed should make the nephrology community confident about the following issue: no increased risk of mortality among patients receiving citrate-based dialysate was shown compared with patients on standard bicarbonate-based dialysate [33, 34, 41]. These data are even more striking when considering that the authors of these studies were more inclined to prescribe citrate-based dialysate to the patients with the worst clinical pictures.

In conclusion, the use of citric acid as dialysate buffer is constantly growing in European dialysis centres despite the lack of a magic bullet that proves its superiority compared with traditional dialysates. Given the current economic difficulties, we doubt that multicentre, adequately powered RCTs will be carried out on the efficacy and safety of citrate-based dialysate over the long run. Finally, we must be grateful to the authors of the studies here analysed for reporting on the impact of the long-term use of citrate-based dialysate on hard outcomes such as all-cause mortality in HD patients [33, 34, 41]. We hope that others will follow their path, trying to correct some of the biases typical of retrospective studies.

**CONFLICT OF INTEREST STATEMENT**

The authors have no conflicts of interest related to this manuscript.

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