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ORIGINAL ARTICLE

Artificial Kidney / Dialysis

A new technique for low-volume continuous sampling of spent dialysate: a validation study

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Abstract

The measure of hemodialysis (HD) adequacy recommended nowadays by most guidelines, Kt/V-urea, presents significant drawbacks. Direct dialysis quantification (DDQ) through total dialysate collection (TDC), considered the gold standard measure of HD adequacy, is cumbersome, which precludes its widespread use in clinical practice. The present study aims to validate a low-volume continuous sampling of spent dialysate (CSSD). Cross-sectional study carried out at a university hospital. Throughout 4-h hemodialysis sessions, urea removal was measured by three DDQ methods: TDC, CSSD, and fractional sampling of dialysate (FSD). The primary outcome was the comparison between the total mass of urea removed measured by TDC and the dialysate sampling techniques. The comparison between urea distribution volume (UDV) estimated by anthropometric method and through DDQ was a secondary outcome. The analysis was done through linear regression and Bland–Altman concordance method. Twenty HD sessions were studied. The mean amount of urea collected in TDC and calculated from the 40-mL sample of CSSD were 33.70 ± 11.70 g and 33.90 ± 11.70 g, respectively [r 0.96, $p < 0.0001$; bias -0.2 (95% CI -1.8 to 1.4); limits of agreement -6.8 to 6.4]. The anthropometric measure, when compared with DDQ method, underestimated UDV in patients with smaller body size. This new simple, inexpensive, and small volume CSSD technique can provide accurate information about the total amount of solutes removed by hemodialysis.

Keywords Hemodialysis · Adequacy · Solute removal · Direct dialysis quantification

Introduction

Since its introduction into clinical practice, hemodialysis (HD) has extended and improved the lives of thousands of end-stage renal disease (ESRD) patients. In the earliest times of the procedure, around 70 years ago, technical problems were the major challenge to overcome [1].

Nowadays, once technical issues have been surpassed, the primary goals are to increase the longevity and quality of life of ESRD patients on HD. The publication of the National Collaborative Dialysis Study (NCDS) in 1981, gave rise to the concept of HD adequacy, through the urea kinetic modeling (UKM) [2]. This trial was the first to show the association between minimal dose of dialysis and mortality. Almost 40 years later, the same Kt/V-urea equation derived from NCDS trial is still recommended by the guidelines for HD adequacy [3]. However, HD treatment has expanded from standard thrice a week 4-h therapy to a multitude of patterns and schedules. Convective techniques [4] and the slow continuous therapies [5]

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have been introduced into clinical practice. The hemodializer membranes have become more biocompatible, allowing greater flux and efficiency [6]. After these myriad advances in hemodialysis, the old and good Kt/V-urea equation remains a guide to the minimal dose of therapy, but perhaps not such an all-embracing guide. The accuracy of Kt/V-urea has been questioned in the current scenario of hemodialysis. Kt/V-urea is based on assumptions that are not always true [7].

Urea is even today regarded as the prototype of small molecular weight solutes, but it is now well known that not all small molecules to be removed by dialysis share the same kinetic. To measure the adequacy of elimination of such molecules, such as phosphorus, which have more than one distribution volume, the equation of Kt/V has no value [8].

The estimation of mass removal of solutes through the measurement of its concentration in the total effluent dialysate, called direct dialysis quantification (DDQ), is considered the gold standard measure of hemodialysis adequacy [9]. However, handling more than 100 L of dialysate is cumbersome, which precludes its use in clinical practice. Continuous dialysate sampling to obtain the CSSD was first described in 1989 to try to overcome this limitation [10]. After that, several techniques and equipment for the CSSD, more or less complex and expensive, were validated. However, while requiring the handling of a smaller dialysate volume, all previously described CSSD techniques still use volumes of approximately 1 L [10–14].

The aim of the current study is to describe and validate a new simple and inexpensive technique of continuous sampling of spent dialysate, with collection of a smaller dialysate volume, which could allow its routine clinical application.

Methods

This is a cross sectional study carried out at the dialysis and kidney transplantation unit of a university hospital (a tertiary-care center). Dialysis was performed using On-Line Clearance Monitor (OCM)-equipped Fresenius 4008-S machines (Fresenius, Bad Homburg, Germany), with low-flux polysulfone dialyzers. The target Kt/V-urea [15] was 1.2 or higher.

Patients

The sample of the study consisted of patients with chronic kidney disease (CKD) on HD for over 3 months, with a thrice-weekly 4 h HD schedule. The inclusion criteria were residual diuresis of less than 500 mL/day; hemodynamic stability during treatment (hypotension episodes in < 15% of the sessions); good vascular access (arteriovenous fistula allowing double-needle puncture) allowing a blood flow rate greater than 300 mL/min; a hemoglobin level greater than 9.0 g/dL.

Design

Each recruited patient was monitored during one mid-week hemodialysis session. Throughout this session, removal of urea was measured by DDQ using total dialysate collection (TDC), CSSD, and fractional sampling of dialysate (FSD) (Fig. 1). The blood flow was maintained at approximately 300 mL/min and the dialysate flow at 500 mL/min.

Urea measurements

Plasma urea concentration was measured before and after each HD session. The urea concentration was measured from

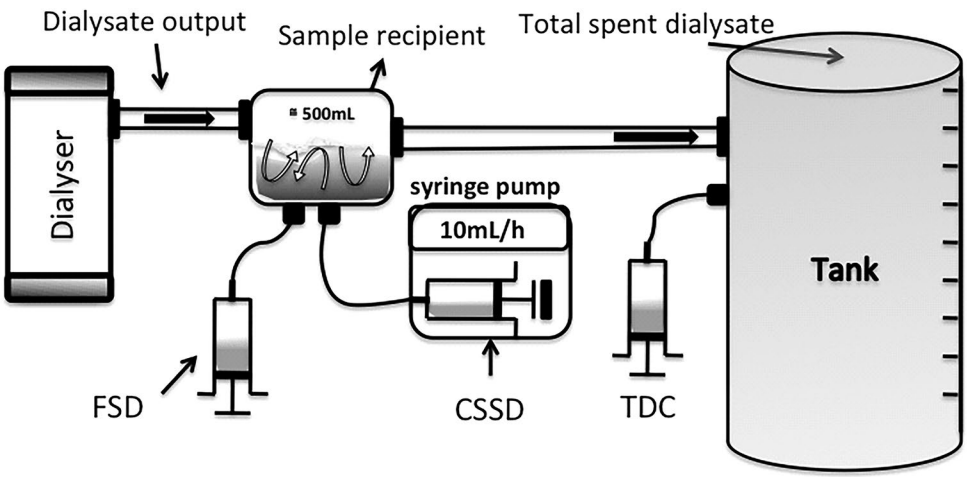

Fig. 1 Schematic diagram of the collection system. *CSSD* continuous spent sampling of dialysate, *FSD* fractional sampling of dialysate, *TDC* total dialysate collection

dialysate samples obtained every 15 min from the drainage tube, which constituted the FSD. It was also measured from the final sample of CSSD, and from the total effluent dialysate (TDC) (Fig. 1). The total mass of urea removed during each HD session was calculated by multiplying the urea concentration by the total volume of effluent dialysate. The urea concentration was measured in Chem Well T Analyzer—Labtest, enzymatic UV method.

Main outcomes

The primary outcome of the study was the correlation between the urea concentration in dialysate samples obtained by TDC or CSSD. The purpose of this correlation was to validate a new CSSD technique, adapted from previously described techniques [13, 16].

The dialysate outflow drain was connected to a sample recipient, which maintain a constant dialysate volume of approximately 500 mL (Fig. 1). Inside this sample recipient, the dialysate is constantly renewing through turbulent flow. The CSSD was obtained at a rate of 10 mL/h by an automatic reverse syringe infusion pump (Lifemed®, Pelotas, Brazil), specially adapted for the study, connected to a sample recipient. After the 4-h HD, a 40 mL dialysate sample had been collected into the syringe (CSSD).

Fractional dialysate sampling (FSD) (5 ml) was collected manually from the 500 mL-recipient every 15 min. The first sample was collected 5 min after HD initiation, and the last one was obtained 10 min before the conclusion of the HD, with a total of 16 fractional samples for each hemodialysis session.

After the HD session, the tank solution (~ 120L) (TDC) was homogenized and an aliquot (40 mL) of total spent dialysate was collected. The tank contains a previously calibrated metric level tube for volume assessment. All dialysate samples were frozen at − 80 °C until biochemical analysis. The blood samples were centrifuged at 2000×*g* for 10 min and plasma was also stored at − 80 °C until biochemical analysis.

Secondary outcomes

The secondary outcome was the comparison between the urea distribution volume (UDV) based on DDQ, calculated using Eq. 1, and the UDV based on an anthropometric formula (55% of the body weight). To calculate the urea distribution volume (VDQ) at the start of the treatment Eq. 1 compares the extracted urea mass, corrected for that amount of urea contained in the fluid that has been extracted during the treatment (QF × tdial × Upw,tf), to the concentration change in the body generated by the mass extraction (Upw,t0 − Upw,tf).

The derivation of the Equation is: [16]

$$\begin{aligned} &Vtf \times Upw,tf = Vt0 \times Upw,t0 - Rdialyze + G - Rres \\ &Vtf \times Upw,tf = Vt0 \times Upw,t0 - \Delta MUrea \\ &Vtf = Vt0 - QF \times tdialVt0 = VDQ \\ &\quad = (\Delta MUrea - QF \times tdial \times Upw,tf)/(Upw,t0 - Upw,tf) \end{aligned} \tag{1}$$

The urea concentrations in the plasma water at times t0 (start of dialysis) and tf (end of dialysis) are inserted. MUrea is the total urea mass found in the spent dialysate.

Using the single pool model, the lack of availability of the residual renal urea removal (Rres) and the urea generation (G) are neglected in this analysis, and MUrea is calculated by Eq. 2.

$$MUrea = UDDQ \times VDDQ \tag{2}$$

UDDQ represents the urea concentration in dialysate and VDDQ means the total volume of dialysate from each HD session.

The dialyzer clearance of urea (K) derived from the DDQ (KDQ) was calculated according Eq. 3.

$$KDQ = (VDQ/tdial)\ln(Upw,t0/Upw,tf) \tag{3}$$

Finally, Kt/V derived from DDQ (Kt/V_{DQ}) is calculated according Eq. 4.

$$Kt/VDQ = KDQ\ t/VDQ \tag{4}$$

Statistical analysis

All samples were analyzed in duplicates and the mean and standard deviation of each point was used and presented. The GraphPad Prism 5 and Stata 11.2 statistical software packages were used for analysis. Correlation analysis was performed using linear regression. Bland–Altman graphs with estimation of bias (95% CI), limits of agreement, and Pitman's Test of difference in variance was also used. *P* values less than 5% were considered significant.

Ethical aspects

The local Research Ethics Board approved the protocol (2010/49). All patients provided informed consent.

Results

Sample characteristics

The sample constituted of twenty patients on hemodialysis treatment. There were two losses, one of them because of accidental sample thawing and another due to early interruption of the HD session. Eighteen patients

(eight women) were evaluated during a single HD session. The sample mean age was 49.66 ± 16.09 years, mean HD vintage was 23.50 ± 17.48 months. Mean systolic blood pressure was 137.33 ± 18.31 mmHg, diastolic blood pressure 83.33 ± 12.34 mmHg, and body mass index 24.70 ± 9.60 kg/m^2. The diseases that caused the CKD were glomerulonephritis (5), diabetic nephropathy (4), polycystic kidneys (3), hypertensive nephropathy (1), congenital obstructive uropathy (1), and undetermined (4).

Correlation and concordance between urea concentrations

The mean concentration of urea in the CSSD samples and in the tank (TDC) was 29.77 ± 9.17 mg/dL and 29.55 ± 8.95 mg/dL, respectively (Fig. 2a). The urea concentrations in the CSSD and the TDC samples presented strong correlation ($r = 0.94$, $p < 0.0001$) (Fig. 2b), and concordance [limits of agreement − 6.3 to 5.8, bias − 0.2 (95% CI − 1.7 to 1.3), and Pitman's test of difference in variance p 0.78] (Fig. 2c).

The mean plasma urea concentration before and after the HD session was 97.28 ± 22.68 mg/dL and 14.00 ± 10.18 mg/dL respectively (Fig. 3).

The urea concentration in the FSD collected throughout the HD sessions is shown in Fig. 3. Sixteen dialysate samples were obtained, at 15 min intervals, during each HD session. The urea concentration in these samples ranged from 42 to 14 mg/dL, decreasing continuously over time.

Post-hoc correlation and concordance analysis were performed between the mean of 3-point urea concentration in the FSD samples [first point (at 20 min of HD), midpoint (at 125 min) and last point (at 185 min)] and the mean concentration of urea in the TDC sample. The mean of 3-point urea concentration was 28.8 ± 7.7

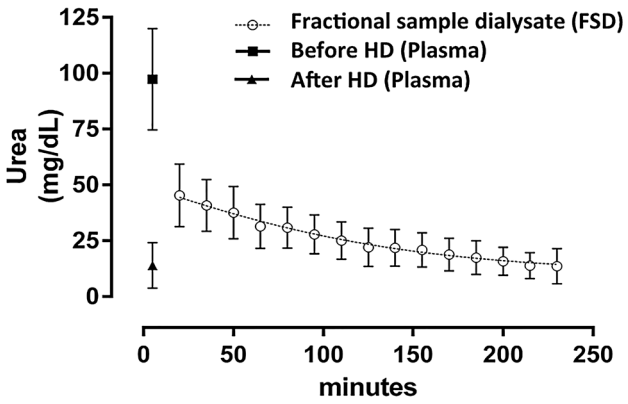

Fig. 3 Plasma urea concentration before and after HD session and urea concentration in dialysate measured over HD session by FSD technique

(mg/dL). There was strong correlation and concordance between the urea concentration in three-point FSD and total dialysate (TDC) [$r = 0.87$, $p < 0.0001$, limits of agreement − 8.1 to 9.6, bias 0.75 (95% CI − 1.4 to 2.9), Pitman's test of difference in variance p 0.24].

Correlation and concordance between urea mass

The mean volume of total dialysate collected in the tank was 112.75 ± 13.32 liters. The total mass of urea removed in a dialysis session, based on the TDC urea concentration, was 33.70 ± 11.70 g. The mean total urea removal calculated from CSSD and calculated from 3-point FSD was 33.90 ± 11.70 g and 32.95 ± 10.63 g, respectively. The correlation (r 0.96, $p < 0.0001$) and concordance [limits of agreement − 6;8 to 6.4, bias − 0.2 (95% CI − 1.8 to 1.4), Pitman's test of difference in variance p 0.95] between total mass of urea removed in a dialysis session calculated with base in urea concentration in TDC and CSSD were strong (Fig. 4a,

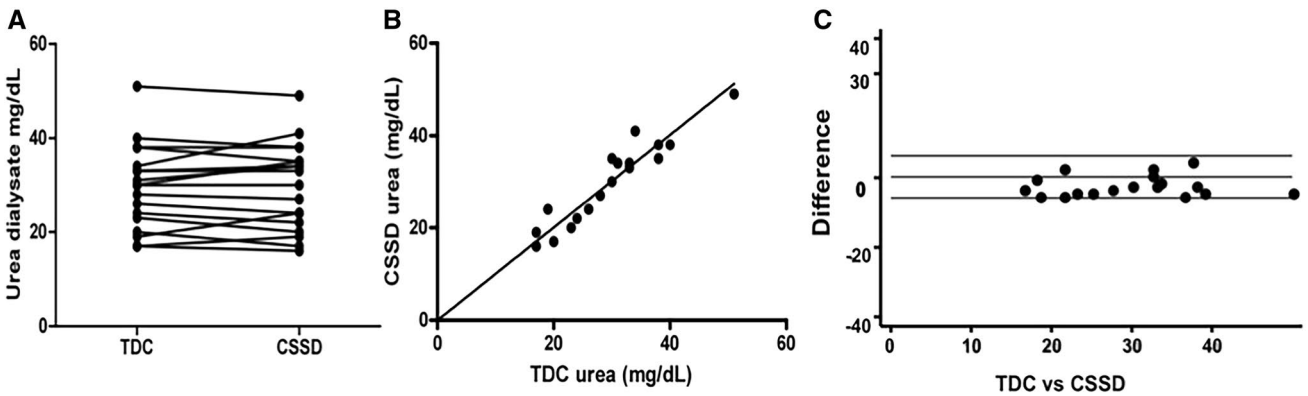

Fig. 2 **a** Urea concentration in total dialysate collection (TDC) and the correspondent point found in continuous spent sampling of dialysate (CSSD). **b** Correlation between urea concentration in CSSD and TDC ($r = 0.94$, $p < 0.0001$). **c** Bland–Altman between urea concentration in CSSD and TDC [limits of agreement − 6.3 to 5.8, bias − 0.2 (95% CI − 1.7 to 1.3)]

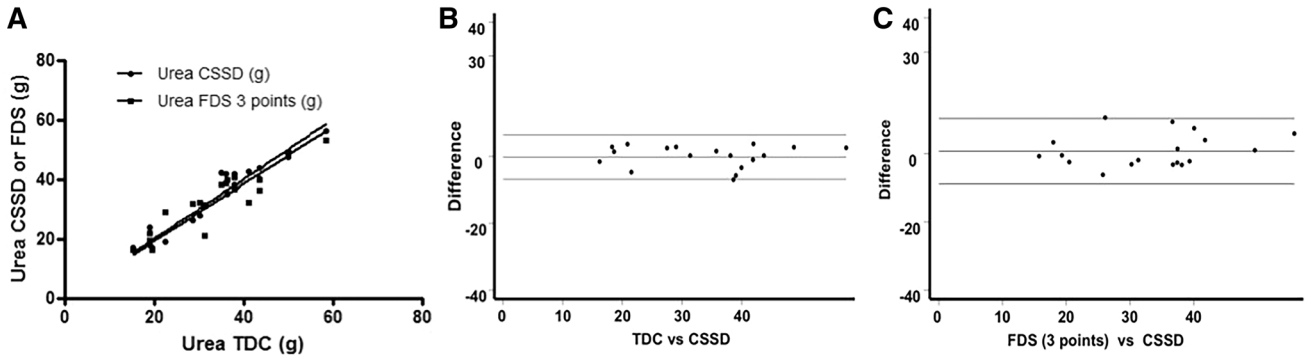

Fig. 4 **a** Correlation between mean of total mass of urea removed in a dialysis session, in grams, in TDC and CSSD ($R^2 = 0.92$) and TDC and three-point FSD ($R^2 = 0.83$). **b** Bland–Altman of TDC vs CSSD [limits of agreement −6;8 to 6.4, bias −0.2 (95% CI −1.8 to 1.4)]. **c** Bland–Altman of TDC vs 3 points of FSD [limits of agreement −8.9 to 10.4, bias 0.7 (95% CI −1.6 to 3.1)]

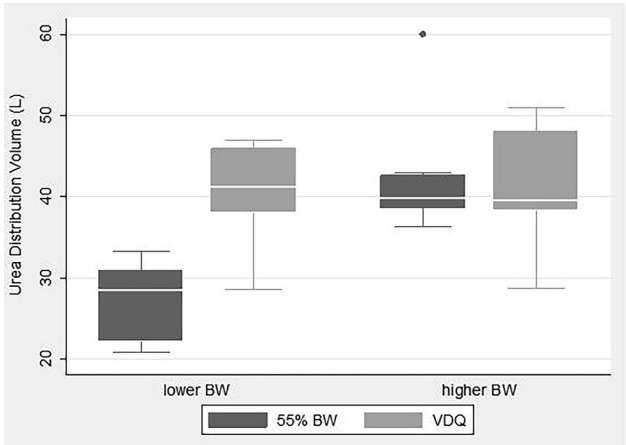

Fig. 5 Difference between UDV calculated by anthropometric measure (55% BW) and V_{DQ} stratified by BW

b). The correlation (r 0.91, $p < 0.0001$) and concordance [limits of agreement −8.9 to 10.4, bias 0.7 (95% CI −1.6 to 3.1), Pitman's test of difference in variance p 0.36] between total mass of urea removed calculated with base on urea concentration in TDC and 3-points mean of FSD was also strong (Fig. 4a, c).

Correlation and concordance between UDV estimations

The mean UDV calculated as 55% of the body weight was 35.16 ± 9.40 L, and the UDV calculated from DDQ was 40.82 ± 7.85 L. Despite statistically significant concordance [limits of agreement −16.8 to 26.4, bias 4.7 (95% CI −1.5 to 11.0), Pitman's test of difference in variance p 0.42], the visual inspection of Bland–Altman graph suggested different bias along the UDV scale. Due to this finding, it was carried out an analysis post-hoc stratified by body weight. This analysis found that the difference between UDV estimated by DDQ and by the anthropometric method was significantly higher among patients with lower body weight [< 66Kg: + 14.31 ± 8.17 Kg; ≥ 66 Kg: − 0.54 ± 8.24 Kg; $p = 0.007$ for DDQ vs anthropometric methods] (Fig. 5).

Discussion

The present study describes and validates a new simple and inexpensive technique of continuous sampling of spent dialysate comparing the urea concentration in a 40 mL aliquot of effluent dialysate with the urea concentration in total effluent dialysate, approximately 110 L. The strong correlation between the two measures demonstrates that this small dialysate sample can give a reliable and accurate estimation of the total mass of urea removed by each dialysis session.

Since the description of urea kinetic modeling, almost 40 years ago, [2] several new modalities and technologies have been incorporated into dialysis treatment [4–6]. Although Kt/V-urea may remain useful in conventional HD therapy, its paradigm probably not apply to other dialysis strategies and to other solutes besides urea.

Nowadays it is well known that the single pool Kt/V-urea, the most used method to estimate HD adequacy, does not apply to solutes that have more than one distribution compartment, like phosphorus, despite its small molecule condition [7, 8]. Lately, increasing attention has been directed to phosphorus kinetic as a non-traditional adequacy parameter of dialysis, given its implication on vascular calcification and cardiovascular morbidity and mortality of ESRD patients [17]. Despite the major role of calcium and phosphorus in the ESRD, the kinetic modeling of these solutes have not been used in dialysis adequacy estimation. The direct dialysis quantification, if routinely applied in clinical practice,

could allow quantification and comparison of the adequacy of different dialysis schedules and modalities in terms of phosphorus [18] or any other potentially significant solute.

The Hemodialysis (HEMO) Study is the largest trial to date to investigate the effect of HD dose (measured through Kt/V-urea calculated by Daugirdas second-generation formula: $Kt/V = -\ln(R - 0.008 \times t) + (4 - 3.5 \times R) \times UF/W$) on patient outcomes. Almost two thousand HD patients were randomized to a target Kt/V of 1.3 or 1.7. In the primary analysis, there was no difference in survival between higher and lower dialysis doses, [19] except for women, who had smaller body size [20].

Patients with smaller body size seems to be under-dialyzed in Kt/V-driven dialysis prescriptions [21]. Based on this observation, a new hypothesis was proposed about the possibility of organs and tissues with different rates of production, dilution and even inactivation of uremic toxins, and about the greater proportion of high-metabolic-rate organs in smaller patients [22]. The concept of volume of distribution for urea adopted in the current urea kinetic modeling does not take these differences into account and could lead to insufficient dialysis in patients with small body size.

In the present study, despite the finding of similar urea distribution volume estimated by anthropometric method (55% of the body weight) or calculated from DDQ, there was a tendency to find higher UDV by DDQ. When this difference was stratified by the median of the sample body weight, we found that this difference was significantly greater for smaller patients, for whom the anthropometric method underestimates the UDV. This finding corroborates and helps explain the findings of the HEMO study, showing that, at least for smaller patients, measures of HD efficacy based on DDQ should be advisable.

The increasing awareness about the complexity of generation and removal of toxins in ESRD patients on dialysis led to the search for customized dialysis prescriptions, considering the requirement of each patient. To meet this aim, we first need to know the minimal required dialysis dose for each patient.

One model fits all is surely not the best model, neither to prescribe nor to measure the adequacy of dialysis. It is necessary to search for more flexible ways to measure adequacy, that allow to incorporate other solutes and other dialysis modalities and schedules.

One such way could be through DDQ using the CSSD or FSD. Several techniques for CSSD have been described since late 1980s [10]. However, their use has not ever got popular. The reasons for this lack of popularity may be related to the need for technical apparatus or modules, [12] need to manage with large volumes of dialysate, [11, 12] reduction of the general interest in UKM after HEMO trial results [19].

We found that the concentration of urea in a small aliquot of dialysate or even three-points sampling of dialysate alone achieves a strong concordance with the urea concentration in the total effluent dialysate. Such a simple and inexpensive technique could allow for more accurate estimates of adequacy, based on different solutes, leading to a proper and customized dialysis prescription. Whether this new way of prescribing dialysis would have positive effects on patient outcomes will require testing in future clinical trials.

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Author contributions ORB and BM contributed to conception and design, analysis and interpretation of data, drafting and revising the article; OJP and AMF contributed to design, acquisition of data, analysis and interpretation of data, and revising the article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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