


















ORIGINAL ARTICLE

Time-averaged concentration estimation of uraemic toxins with different removal kinetics: a novel approach based on intradialytic spent dialysate measurements

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ABSTRACT

Background. K_t/V_{urea} is the most used marker to estimate dialysis adequacy; however, it does not reflect the removal of many other uraemic toxins, and a new approach is needed. We have assessed the feasibility of estimating intradialytic serum time-averaged concentration (TAC) of various uraemic toxins from their spent dialysate concentrations that can be estimated non-invasively online with optical methods.

Methods. Serum and spent dialysate levels and total removed solute (TRS) of urea, uric acid (UA), indoxyl sulphate (IS) and β_2 -microglobulin (β_2M) were evaluated with laboratory methods during 312 haemodialysis sessions in 78 patients with four different dialysis treatment settings. TAC was calculated from serum concentrations and evaluated from TRS and logarithmic mean concentrations of spent dialysate (M_{inD}).

Results. Mean (\pm standard deviation) intradialytic serum TAC values of urea, UA, β_2M and IS were 10.4 ± 3.8 mmol/L, 191.6 ± 48.1 $\mu\text{mol/L}$, 13.3 ± 4.3 mg/L and 82.9 ± 43.3 $\mu\text{mol/L}$, respectively. These serum TAC values were similar and highly correlated with those estimated from TRS [10.5 ± 3.6 mmol/L ($R^2 = 0.92$), 191.5 ± 42.8 $\mu\text{mol/L}$ ($R^2 = 0.79$), 13.0 ± 3.2 mg/L ($R^2 = 0.59$) and 82.7 ± 40.0 $\mu\text{mol/L}$ ($R^2 = 0.85$)] and from M_{inD} [10.7 ± 3.7 mmol/L ($R^2 = 0.92$), 191.6 ± 43.8 $\mu\text{mol/L}$ ($R^2 = 0.80$), 12.9 ± 3.2 mg/L ($R^2 = 0.63$) and 82.2 ± 38.6 $\mu\text{mol/L}$ ($R^2 = 0.84$)], respectively.

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Conclusions. Intradialytic serum TAC of different uraemic toxins can be estimated non-invasively from their concentration in spent dialysate. This sets the stage for TAC estimation from online optical monitoring of spent dialysate concentrations of diverse solutes and for further optimization of estimation models for each uraemic toxin.

Keywords: chronic haemodialysis, dialysis adequacy, time-averaged concentration, urea, uraemic toxin

INTRODUCTION

Adequate dialysis treatment improves survival and reduces morbidity in haemodialysis (HD) patients. Conventionally, dialysis adequacy is assessed by the clearance of the small molecular weight molecule urea and quantified as Kt/V_{urea} or URR, which is calculated from urea concentrations in pre-dialysis and post-dialysis blood samples [1]. Kt/V_{urea} has helped to standardize HD treatment and define the minimum dose of dialysis needed to avoid morbidity and mortality related to inadequate dialysis, although the Kt/V_{urea} concept has several shortcomings [2–4].

Kt/V_{urea} may be inaccurate for dialysis patients with divergent body compositions [3, 5, 6]. In addition, Kt/V_{urea} does not easily allow comparison of adequacy for patients who receive different dialysis prescriptions with varying duration and frequency [4, 7, 8] and for patients who have acceptable residual renal function [3], as this has a major impact on solute removal [9, 10]. Furthermore, Kt/V_{urea} poorly reflects the removal of solutes other than urea, which are associated with clinical outcomes, such as middle molecules and protein-bound molecules [3, 11–13].

Despite these limitations, Kt/V_{urea} remains the most frequent measure of dialysis adequacy [1], even as high cut-off membranes are available and convective strategies have become common that aim to increase the clearance of middle molecules and protein-bound uraemic toxins [3, 13]. New approaches are therefore needed to quantify the HD dose that also represents removal of uraemic toxins other than urea and can be applied to dialysis sessions with varying settings (modality, frequency, duration) to optimize patient survival and quality of life [3, 14].

Alternative measures of dialysis adequacy have been proposed, e.g. the equivalent renal urea clearance [15], the time-averaged concentration (TAC)/time-averaged deviation concept [16], ionic dialysance [17] and fractional solute removal [18], among other indices. However, a key issue is the existence of many types of uraemic toxins that should be removed by HD but are not assessed by Kt/V_{urea} . The TAC of individual solutes, which strongly depends on the total dialysis time per week and on the weekly dialytic frequency, may provide insights into the clearance of diverse solutes [7, 16, 19]. TAC evaluates changes in uraemic toxin levels over time, even if they have different size and removal characteristics, and allows comparing the effect of different dialysis strategies on individual uraemic toxins [7, 16, 19]. Moreover, TAC integrates the impact of patient parameters, such as residual renal clearance and the rate of generation of uraemic toxins [16]. Indeed, TAC was historically used to assess dialysis adequacy, but it was replaced by the simpler Kt/V_{urea} due to cumbersome calculations [16].

TAC is usually estimated from repeated blood sampling and calculated as the area under the concentration curve over the period of interest. In other words, TAC is the mean concentration of the solute of interest over a period of time, which can be one treatment cycle, e.g. a week, or one intradialytic period, i.e. an individual dialysis session time [7, 15, 18, 20]. However, evaluating intradialytic TAC from repeated blood samples obtained

during the dialysis session is more complex than assessing Kt/V_{urea} , which usually requires only pre- and post-dialysis sampling [7, 20, 21]. This problem becomes more prominent for solutes with higher intercompartmental resistance [20].

Online optical monitoring methods that do not require blood sampling allow the simultaneous monitoring of multiple uraemic toxins in the outflow of effluent dialysate from dialysis machines (spent dialysate) [22–24]. As the mass of toxins removed from blood to the dialysate is proportional to the dialysate flow/dialyzer clearance ratio [21, 25] in the case of using membranes with negligible adsorption capacity, such as polysulfone-based membranes [26], we hypothesized that the concentration of uraemic toxins in spent dialysate could be used to precisely estimate blood TAC values from each dialysis session. So far, dialysate-based methods have enabled evaluation of Kt/V_{urea} , the removal ratio and the total mass of removed solutes, providing additional information about treatment quality [22, 23, 27, 28].

The aim of this work was to estimate intradialytic serum TAC of urea, uric acid (UA), indoxyl sulphate (IS) and β 2-microglobulin (β 2M) from their concentrations in spent dialysate. Urea, UA, IS and β 2M were considered as markers for the three general uraemic toxin groups based on different physicochemical characteristics and removal kinetics, namely small water-soluble compounds, protein-bound compounds and middle molecules [27].

MATERIALS AND METHODS

Clinical data were acquired from four separate dialysis centres from countries with diverse life expectancies, renal replacement therapy incidences and kidney transplant rates: North Estonia Medical Centre, Tallinn, Estonia (22 patients); Linköping University Hospital, Linköping, Sweden (21 patients); Ghent University Hospital, Ghent, Belgium (15 patients) and Fundación Jiménez Díaz University Hospital Health Research Institute, Madrid, Spain (20 patients). The clinical characteristics of the 78 participants monitored for a total of 312 dialysis procedures have been described [28] and are summarized in Supplementary Table 1. All studies were performed in accordance with the Declaration of Helsinki after approval of the study protocol by local ethics committees. Informed consent was obtained from all subjects involved in the study [28].

Inclusion criteria were age >18 years; chronic HD; thrice weekly HD procedures for 3.5–4.5 hours, preferably via arteriovenous fistula or graft; achievable blood flow of at least 300 ml/min; absence of clinical signs of infection or other active acute clinical complications and an estimated life expectancy >6 months.

Each patient underwent four HD sessions, each time using a different HD setting, as summarized in Supplementary Table 2 and described in detail previously [28]. Blood and spent dialysate samples were collected during each dialysis session [28]. Serum and spent dialysate concentrations of uraemic toxins were determined in clinical or analytical laboratories as described earlier [28, 29]. In short, urea, UA and β 2M were assessed in

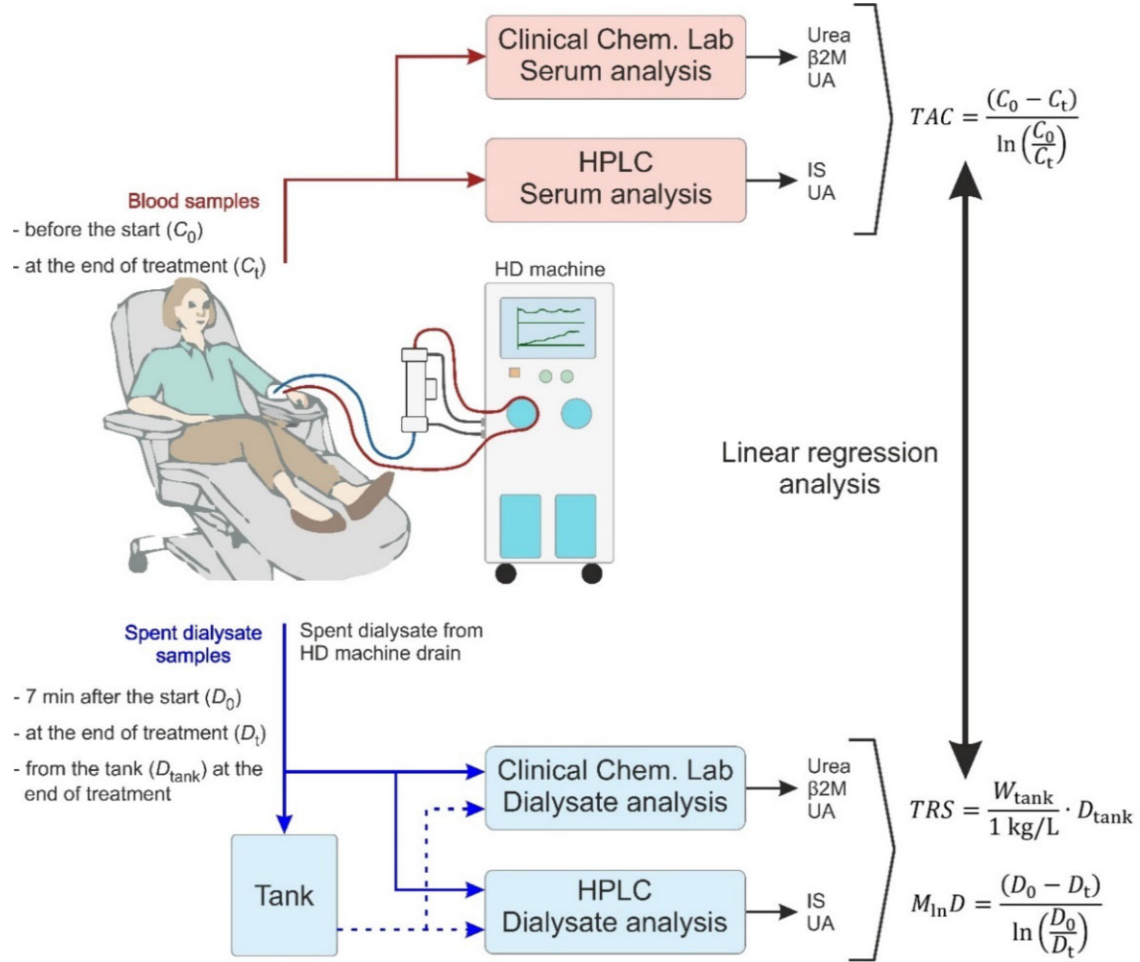


Figure 1: Clinical set-up, sample collection and analysis. The same set-up was repeated four times for each patient, each time using a different HD setting.

clinical biochemistry labs and IS and UA were assessed by high-performance liquid chromatography (HPLC), as shown in Fig. 1. For UA, HPLC results were used in subsequent calculations.

For each dialysis session, the TAC over dialysis sessions was estimated for urea, UA, IS and $\beta 2M$ from serum concentrations, total removed solute (TRS) from total dialysate collection and the mean concentration of uraemic toxins in spent dialysate during dialysis, which was calculated from spent dialysate concentrations.

Values of average effective blood flow (Q_b) during dialysis sessions, dialysate flow, ultrafiltration rate, total ultrafiltration volume and total substitution volume were read from the dialysis machine's screen at the end of the treatment session. The mass of the total waste dialysate collected during the session was measured. It was assumed that the average density of spent dialysate is equal to the density of water at room temperature (1 kg/L). The effective spent dialysate flow rate (Q_d) was calculated from the weight of spent dialysate (W_{tank}) collected during the session, divided by the dialysis session time (240 min), multiplied by the average density of spent dialysate:

$$Q_d = \frac{W_{\text{tank}}}{1 \text{ kg/L} \cdot 240 \text{ min}} \quad (1)$$

The TAC of uraemic retention solutes in serum over dialysis sessions was calculated by the following equation [29], where the

denominator is a simplified single-pool Kt/V :

$$TAC = \frac{(C_0 - C_t)}{\ln\left(\frac{C_0}{C_t}\right)} \quad (2)$$

where C_0 is the serum concentration of uraemic solutes before the dialysis session and C_t is the serum concentration of uraemic solutes at the end of the dialysis session.

Serum TAC values were normalized to a 300 ml/min effective blood flow rate to robustly compensate for dialyzer clearance.

$$TAC_n = TAC \cdot \frac{Q_b}{300 \text{ mL/min}} \quad (3)$$

where Q_b is the effective blood flow rate for the particular dialysis session.

The mean concentration of uraemic toxins in spent dialysate during the dialysis session was calculated as the logarithmic mean concentration ($M_{\ln D}$) using equation (3):

$$M_{\ln D} = \frac{(D_0 - D_t)}{\ln\left(\frac{D_0}{D_t}\right)} \quad (4)$$

where D_0 is the spent dialysate concentration of uraemic solutes in samples taken 7 min after the start of the dialysis session and

D_t is the spent dialysate concentration of uraemic solutes at the end of the dialysis session.

For comparability of dialysis sessions with different treatment settings, spent dialysate $M_{in}D$ values were subsequently normalized to a 300 ml/min effluent dialysate flow rate to compensate for flow rate-dependent dilution of dialysate samples using equation (5), where Q_d is the effluent dialysate flow rate for the particular dialysis session:

$$M_{in}D_n = M_{in}D \cdot \frac{Q_d}{300 \text{ mL/min}} \quad (5)$$

The TRS of each solute was calculated from the total dialysate collection (TDC) as follows:

$$TRS = \frac{W_{\text{tank}}}{1 \text{ kg/L}} \cdot D_{\text{tank}} \quad (6)$$

where D_{tank} is the concentration of uraemic solute in the total dialysate collection and W_{tank} is the weight of total waste dialysate in the dialysate collection tank (kg).

All the results were assessed for possible errors and data conformity. The stability of blood and dialysate flow rates were monitored online (shown in Supplementary Figure 1) throughout each dialysis session, similarly as described before [30]. Dialysis sessions were excluded from the analysis when the sampling of spent dialysate had occurred during notably different flow rates relative to the other sampling points or during self-tests of the HD machine. In addition, data points in which analyte concentrations were below the quantification limit of clinical laboratory methods were omitted.

Linear regression analysis was used to investigate the relationship between TAC values in serum and TRS and $M_{in}D_n$ values in spent dialysate. Afterwards, obtained linear regression equations were used to estimate TAC values in serum. Confidence intervals were estimated for regression lines using the predict function in MATLAB R2020b (MathWorks, Natick, MA, USA).

Systematic error (BIAS) was calculated for the results as follows:

$$BIAS = \frac{\sum_{i=1}^N e_i}{N} \quad (7)$$

where e_i is the i th residual (difference between the results) and N is the number of observations [31].

The standard error (SE) of performance corrected for BIAS was calculated as follows [31]:

$$SE = \sqrt{\frac{\sum_{i=1}^N (e_i - BIAS)^2}{N - 1}} \quad (8)$$

Individual differences between the TAC of uraemic toxins in serum and corresponding values estimated from TRS or $M_{in}D$ values in spent dialysate were examined using Bland-Altman analysis [32]. MATLAB R2020b was used for data analysis and data visualization.

RESULTS

Overall, clinical data were available for 78 participants from four HD units from four different countries monitored for a total of 312 dialysis procedures. Clinical characteristics have been described earlier [28] and are summarized in Supplementary

Table 1. TAC values were calculated from intradialytic serum and $M_{in}D$ values were calculated from spent dialysate concentrations for different uraemic retention solutes and normalized by effective blood or spent dialysate flow rates, respectively. TRS was evaluated based on TDC.

There was a generally strong correlation between TRS and intradialytic TAC ($R^2 > 0.59$) values (shown in Fig. 2) and $M_{in}D$ ($R^2 > 0.89$) values (shown in Supplementary Fig. 2) for different uraemic retention solutes, normalized by effective blood or spent dialysate flow rates, respectively. The lowest R^2 values were found for $\beta 2M$, a solute with the highest intercompartmental resistance and molecular weight.

There was also good correlation between intradialytic TAC and $M_{in}D$ values in all cases, regardless of treatment modality (shown in Fig. 3). The correlation was higher for urea (molecular mass 60 g/mol, $R^2 = 0.92$), intermediate for UA and IS (molecular mass 168 g/mol, $R^2 = 0.80$ and molecular mass 213 g/mol, $R^2 = 0.84$, respectively) and lower for $\beta 2M$ (molecular weight 11.8 kDa, $R^2 = 0.63$). For haemodiafiltration and HD modality separately, the strongest correlation was seen for urea [$R^2 = 0.91$ ($n = 152$), $R^2 = 0.96$ ($n = 63$)] and the weakest correlation for $\beta 2M$ [$R^2 = 0.62$ ($n = 168$), $R^2 = 0.83$ ($n = 37$)], respectively.

Table 1 shows the intradialytic TAC values for urea, UA, $\beta 2M$ and IS and the corresponding TAC values calculated from TRS or $M_{in}D_n$ in spent dialysate. TAC values estimated from TRS were calculated by the linear regression equations shown in Fig. 2 and TAC values estimated from spent dialysate $M_{in}D_n$ values were calculated by the linear regression equations shown in Fig. 3.

Bland-Altman plots comparing intradialytic TAC values and TAC values estimated from spent dialysate $M_{in}D_n$ normalized by flow rates or estimated from TRS are shown in Fig. 4. It can be seen from the plot that random error between TAC values and estimated TAC values remains similar over the whole concentration scale, while systematic error is negligible.

DISCUSSION

To our knowledge, serum TAC of uraemic toxins have not been previously estimated from spent dialysate. The main finding of the present report is that the concentration of diverse uraemic solutes in spent dialysate can be used to estimate serum TAC values for multiple uraemic solutes, minimizing blood sampling needs and blood loss. This finding sets the stage for online optical monitoring of serum TAC from spent dialysate concentrations of multiple uraemic toxins that would allow real-time, point-of-care decision making regarding HD adequacy [23, 24, 30].

The high correlation coefficients found between intradialytic TAC and spent dialysate $M_{in}D$ or TRS values support the potential to estimate the intradialytic TAC of uraemic solutes with different removal kinetics from their concentrations in spent dialysate.

It is noteworthy that the midweek mean intradialytic serum TAC value of urea (10.4 ± 3.8 mmol/L) was well aligned with the average equivalent measures of the HEMO study standard and high-dose arm, corresponding to a HEMO high weekly TAC value ≤ 11.6 mmol/L [33], very similar to the median TAC values presented by Kloppenburg et al. [34], and the plasma TAC (11.7 mmol/L) for a study exploring increasing HD frequency versus HD duration [7], respectively. Furthermore, the achieved serum urea TAC was very close to the lowest TAC value (≈ 10 mmol/L) for the most efficient treatment modes in the 'Lopot plot' [35] modelled from data with varying duration, frequency and spacing of treatments based on a study using a

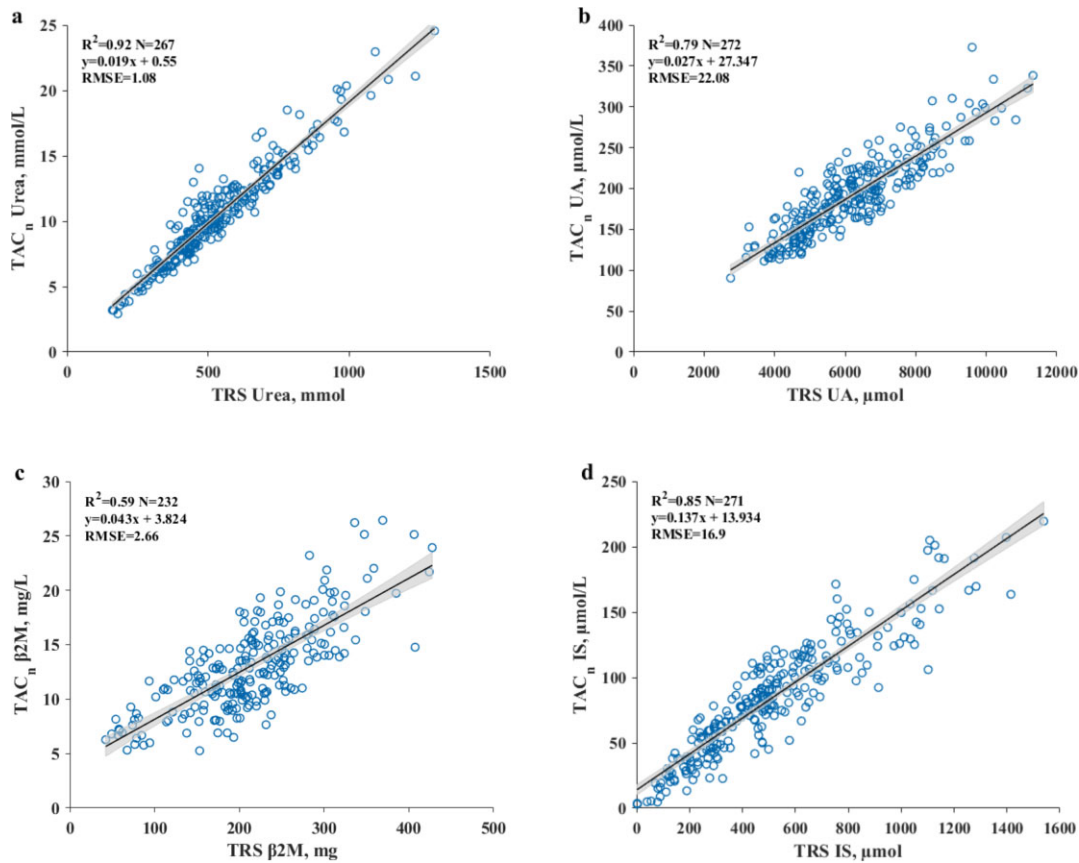


Figure 2: Correlation between total dialysate collection based on TRS and intradialytic TACs of (a) urea, (b) UA, (c) β2M and (d) IS in serum (TAC_n) normalized by effective blood flow rate. Black line indicates the regression line and grey area indicates the 95% CI of the slope.

variable-volume two-pool urea kinetic model [8]. Even so, serum urea TAC values remained higher than those obtained with high-efficiency daily HD, which are close to those of healthy kidneys, in which serum urea TAC is <4 mmol/L [36].

The strongest correlation between intradialytic TAC and spent dialysate M_{inD} values was observed for urea ($R^2 = 0.92$) and the weakest for β2M ($R^2 = 0.63$). The main reason for the different correlation coefficients of different uraemic solutes is probably related to the solute-dependent kinetic behaviour. Urea has negligible resistance to intercompartmental shifts compared with other solutes, which are therefore more difficult to remove by dialysis [4, 12, 13]. This causes a rapid decline of the serum concentration of other solutes at the beginning of the HD session, especially for solutes with slow intercompartment clearance such as β2M, and therefore double-pool kinetics should be used to describe the removal of such solutes [12, 37].

As serum TAC values over the dialysis session were calculated in the current work using equation (2), where the denominator is a simplified single-pool Kt/V [38], serum TAC of uraemic toxins with slower intercompartment clearance was probably overestimated due to the pronounced decrease of serum levels at the start of dialysis [12, 37]. The divergence between serum TAC and corresponding values estimated from M_{inD} was likely further amplified by the difference in the timing of the sampling time of blood and spent dialysate. While the first serum samples were taken prior to the start of dialysis when serum and extracellular compartments were equilibrated, the first spent

dialysate samples were taken 7 minutes after starting the dialysis session, when an intercompartmental concentration gradient had already been developed to some extent. This effect even overestimates intradialytic serum urea TAC when using only pre-dialysis and post-dialysis serum samples to calculate TAC [7, 20, 21], but a larger effect can be expected for solutes with higher intercompartmental resistance.

These inaccuracies can be avoided by measuring intradialytic serum and spent dialysate concentrations with higher frequency to accurately describe the concentration profile and TAC or M_{inD} of solutes. While additional blood sampling is inconvenient and burdensome for patients, continuous monitoring of different uraemic toxins simultaneously in spent dialysate can be achieved non-invasively with online optical monitoring methods [22–24]. Continuous monitoring of uraemic toxin concentrations in spent dialysate could be used to obtain precise TAC values from TRS [23, 30, 39].

In addition, online monitoring of effluent dialysate concentrations can help to detect interruptions in treatment, sudden changes of dialysate and blood flows and clinical alarms and determine effective dialysis time [30, 40] more accurately, which could reduce errors in TAC estimation. Furthermore, the accuracy of TAC estimation could be increased by using real-time values of dialysis machine treatment settings and considering dialyzer specifications in the modelling of dialyzer clearance. Whereas in this study membranes with negligible adsorption capacity were used, it is important to note that the use of

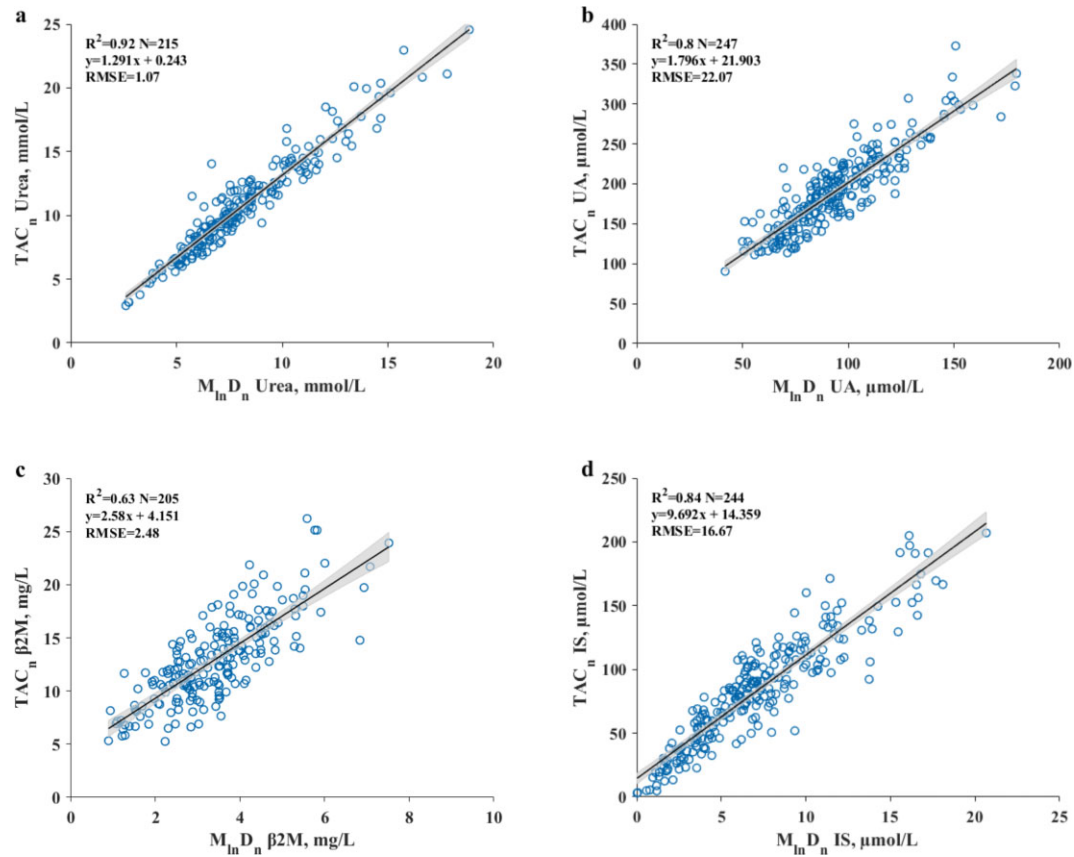


Figure 3: Correlation between intradialytic TACs of (a) urea, (b) UA, (c) β 2M and (d) IS in serum (TAC_n) and logarithmic mean concentration ($M_{ln}D_n$) in spent dialysate normalized by effective blood or spent dialysate flow rates, respectively. Black line indicates the regression line and grey area indicates the 95% CI of the slope.

Table 1: Mean \pm SD values of intradialytic TACs (TAC_n) normalized by blood flow rate and estimated from TRS and $M_{ln}D_n$ in spent dialysate normalized by spent dialysate flow rate for urea, UA, β 2M and total IS).

	TAC_n	TAC_n from TRS	TAC_n from $M_{ln}D_n$
Urea (mmol/L)	10.4 ± 3.8 ($n = 274$)	10.5 ± 3.6 ($n = 267$)	10.7 ± 3.7 ($n = 215$)
UA (μ mol/L)	191.6 ± 48.1 ($n = 273$)	191.5 ± 42.8 ($n = 272$)	191.6 ± 43.8 ($n = 247$)
β 2M (mg/L)	13.3 ± 4.3 ($n = 264$)	13.0 ± 3.2 ($n = 232$)	12.9 ± 3.2 ($n = 205$)
IS (μ mol/L)	82.9 ± 43.3 ($n = 273$)	82.7 ± 40.0 ($n = 271$)	82.2 ± 38.6 ($n = 244$)

negatively charged adsorptive membranes such as polymethyl methacrylate or adsorbent columns can additionally adsorb uraemic toxins before passing across the membrane into spent dialysate. This may cause additional errors in the dialysate-based readings and the commonly used set-up of optical sensors should be modified to take this effect into account.

Also, patient-specific parameters such as dialyzer recirculation [41] and haematocrit [4] influence the clearance of uraemic toxins and thus proportionality [25, 42] between serum concentration of uraemic toxins and their concentration in spent dialysate [30]. In this regard, urea is removed from both erythrocytes and plasma water as blood passes through the dialyzer, but this is not the case for other uraemic toxins that are only removed from plasma, and thus their clearance depends on haematocrit and cannot exceed plasma flow [4]. Therefore, for uraemic toxins without facilitated transport in and out of erythrocytes, i.e. other than urea [4], plasma concentrations should

be used in the estimation of TAC [4]. This can additionally explain why the strongest correlation between serum and spent dialysate TAC values was observed for urea, as we used serum concentrations, and clearance of urea is mainly limited by extracorporeal blood and dialysate flows [12].

Notwithstanding these limitations, the study demonstrates the feasibility of obtaining reasonable estimates of serum TAC values from spent dialysate. Moreover, preliminary (unpublished) data show that the modality does not affect the accuracy of optical estimation of uraemic toxin concentrations in spent dialysate in the tested range including higher dialysate and substitution flow rates. Before clinical implementation, these general models should be optimized for each uraemic toxin, considering treatment settings, dialyzer membrane specifications, patients' body parameters and using plasma values, which would allow a more precise estimation of serum TAC values from spent dialysate concentration values. Additionally,

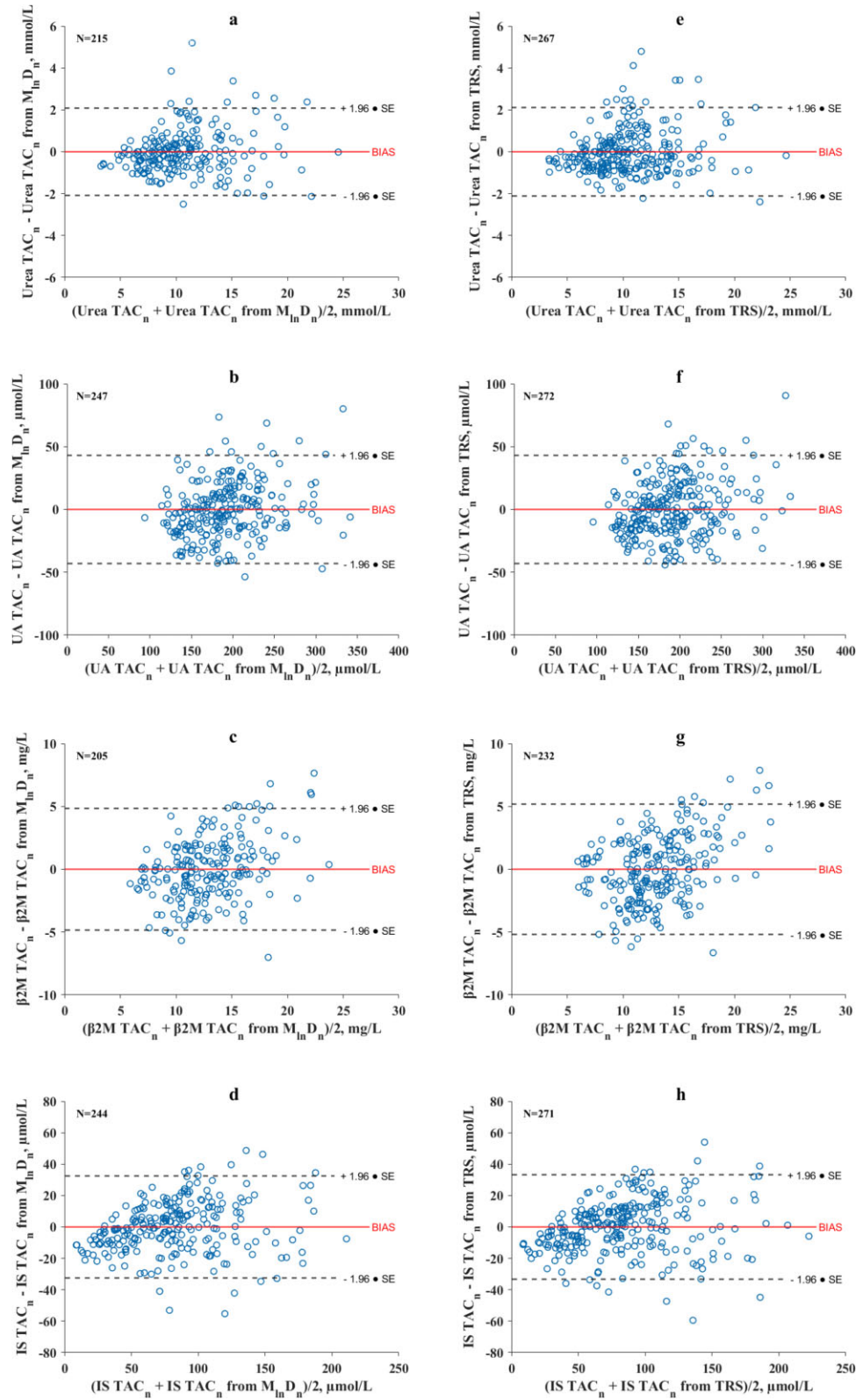


Figure 4: Bland-Altman plots comparing intradialytic TAC values in serum normalized by effective blood flow rate (TAC_n) and corresponding TAC_n values estimated from logarithmic mean concentrations (M_{lnD_n}) in spent dialysate normalized by spent dialysate flow or from TRS for (a, e) urea, (b, f) UA, (c, g) β_2M and (d, h) total IS.

clinical implementation would be facilitated by estimating uraemic toxin concentrations in spent dialysate from continuous optical online monitoring of spent dialysate, providing a more convenient, less labour-intensive method [22–24, 30], which may allow optimization of the HD prescription.

Although the TAC concept offers additional information on HD adequacy, HD adequacy should be multitargeted and cover all patient needs and clinical goals that improve outcomes [14, 43, 44]. Kt/V has been criticized for ignoring the question of how much uraemic toxin is left in the patient [4]. The serum TAC concept can address this question. Moreover, Kt/V does not consider fluid management nor residual kidney function [45]. The latter should also be reflected in TAC values, which has been illustrated for β 2M [46]. TAC is therefore a good parameter for comparison of the status of patients with varying residual kidney functions and diets on different dialysis strategies.

In conclusion, the present study demonstrates the feasibility of evaluating serum TAC of uraemic toxins from uraemic toxin concentrations in spent dialysate. In the future, automatic evaluation of intradialytic serum TAC values from optical online monitoring of spent dialysate could provide a more convenient and precise measure of the impact of treatment on TAC values and allow a real-time, point-of-care adjustment of the dialysis prescription. In this regard, for clinical implementation, the general models described herein should be optimized for each uraemic toxin considering treatment settings and patient parameters.

SUPPLEMENTARY DATA

Supplementary data are available at [ckj](#) online.

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AUTHORS' CONTRIBUTIONS

A.F., I.F., G.G., M.L., M.S., F.U. and A.O. were responsible for conceptualization. A.A., J.A., A.D., A.F., I.F., G.G., L.L., M.L., J.P., E.G.-P., V.M.P.-G., M.S., F.U. and A.O. were responsible for the methodology. J.P., A.A., J.A., J.H., A.D., G.G., L.L., V.M.P.-G., D.S.-O., E.G.-P. and F.U. were responsible for the investigation. J.P., A.A., J.A., A.D., L.L., V.M.P.-G., D.S.-O., K.P. and F.U. were responsible for data curation. J.A., J.P. and I.F. were responsible for validation. J.P., J.A., I.F. and K.P. were responsible for formal analysis. J.P., A.A., J.A., A.D., A.F., E.G.-P., J.H., I.F., G.G., J.H., L.L., M.L., V.M.P.-G., M.S., K.P., F.U. and A.O. were responsible for original draft preparation. J.P., A.A., J.A., A.D., A.F., E.G.-P., J.H., I.F., G.G., J.H., L.L., M.L., V.M.P.-G., M.S., K.P., F.U. and A.O. were responsible for review and editing. J.P. and K.P. were responsible for visualization.

DATA AVAILABILITY STATEMENT

The data are owned by a third party. The data underlying this article were provided by OÜ Optofluid Technologies by permission. Data will be shared upon request to the corresponding author with permission of OÜ Optofluid Technologies.

CONFLICT OF INTEREST STATEMENT

A.F. has received consultancy or speaker fees from Otsuka, AstraZeneca, Vifor Pharma and Alnylam. A.O. is the former CKJ Editor-in-Chief and has received grants from Sanofi and consultancy or speaker fees or travel support from Advicene, Astellas, AstraZeneca, Amicus, Amgen, Fresenius Medical Care, GlaxoSmithKline, Bayer, Sanofi-Genzyme, Menarini, Kyowa Kirin, Alexion, Idorsia, Chiesi, Otsuka, Novo Nordisk and Vifor Fresenius Medical Care Renal Pharma and is director of the Catedra Mundipharma-UAM of diabetic kidney disease and the Catedra AstraZeneca-UAM of chronic kidney disease and electrolytes. V.M.P.-G. has received grants from Catedra Mundipharma-UAM and Catedra AstraZeneca-UAM and consultancy or speaker fees or travel support from Kyowa Kirin, Alexion and Otsuka AstraZeneca and Sanofi-Genzyme. M.S. has received grants from Hansa Biopharma and consulting fees from Hansa Biopharma, Chemocentryx, AstraZeneca and Vifor Pharma. All other authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

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