Estimating total urea removal and protein catabolic rate by monitoring UV absorbance in spent dialysate

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Abstract

Background. Dialysate-based, on-line measurements of Kt/V and protein catabolic rate (PCR) in dialysis patients have been considered more accurate compared with measurements on the blood side during dialysis. The primary aim of this study was to compare total removed urea (TRU) and PCR, normalized to body weight (nPCRw), obtained by three dialysate-based methods: (i) on-line ultraviolet (UV) absorbance of the spent dialysate; (ii) total dialysate collection (TDC), as reference method; and (iii) Urea Monitor 1000 (UM) from Baxter Healthcare Corp.

Methods. We studied 10 uraemic patients on chronic, thrice-weekly haemodialysis. We made absorption measurements (UV radiation) on-line with a spectrophotometer connected to the fluid outlet of the dialysis machine, with all spent dialysate passing through an optical cuvette for single-wavelength measurements. UV absorbance measurements were compared with TDC and the UM.

Results. nPCRw obtained with UV absorbance was 0.82 ± 0.17, that from TDC 0.81 ± 0.18, and that measured by UM 0.87 ± 0.18, which was significantly higher than the results of the other methods. The difference between nPCRw calculated by TDC and by UM was −0.05 ± 0.06, showing a slightly lower SD than the difference between nPCRw by TDC and UV absorbance, −0.01 ± 0.07.

Conclusion. The study demonstrates that TRU, and consequently PCR, can be estimated by on-line measurement of the UV absorption in the spent dialysate.

Keywords: dialysis adequacy; haemodialysis monitoring; protein catabolic rate; total removed urea; urea; UV absorption

Introduction

In general, the efficacy of haemodialysis and protein nutritional status is determined by calculating Kt/V and protein catabolic rate (PCR) from pre- and post-dialysis blood concentrations of urea. Calculations based on data from blood samples are indirect measurements of the primary effect of haemodialysis, and have led to the development of alternative methods of measurement on the dialysate side. At present, there are some commercially available urea monitors for the automatic measurement of urea concentration in the spent dialysate. Parameters such as Kt/V, total removed urea (TRU), PCR, etc. are quantified by direct measurement of urea loss [1], or Kt/V is determined by the ionic dialysance method [2]. On-line monitoring of the spent dialysate has been proposed as a more accurate method to measure dialysis dose [3]. The possibility of utilizing many samples on-line during dialysis has led to fewer measurement errors, e.g. urea rebound and laboratory errors [4], compared with procedures based on single samples.

Direct dialysis quantification (DDQ) from total dialysate collection (TDC) is often mentioned as the ‘gold standard’ for measuring the total amount of urea removed [5]. TDC is cumbersome as a clinical routine; and sources of error affecting TDC, such as urease-producing bacteria [6] and dilution from fresh dialysate during the by-pass mode, have been mentioned [7].

PCR is widely accepted as a marker of protein nutritional status and is equivalent to dietary protein intake in stable dialysis patients [8]. The urea generation rate is not constant over the interdialytic period [9], and day-to-day variations in daily protein intake may result in significant fluctuations in PCR; therefore, it has been proposed to average the values of 7 days to obtain a PCR that more accurately reflects the nutritional status of the patient [10]. The same authors have calculated PCR from TRU and fractional factors for different treatment days in the week, enabling the estimation of PCR for a 7 day cycle with one dialysis...
treatment. A common way to normalize PCR is to use dry body weight (nPCRw), but this could result in an error in patients with abnormal body compositions [8].

Recently, a new technique for on-line monitoring of solutes in the spent dialysate was described, which utilizes UV absorbance and enables a single haemodialysis session to be followed continuously, monitoring deviations in dialysis efficiency [11]. A good correlation between UV absorbance and several removed small waste solutes, such as urea, creatinine and uric acid, has been found [11]. As a consequence of the good correlation between the UV absorbance and urea, urea Kt/V can be estimated from on-line UV absorbance measurements, even if the UV technique does not measure urea itself [12].

The aim of this study was to compare TRU and PCR calculated from the on-line UV absorbance of the spent dialysate with both the total dialysate collection (TDC, reference method) and with the measurements made by the Urea Monitor 1000 (UM).

Materials and methods

Subjects

We studied 10 patients with chronic renal failure (four females and six males, mean age 62.8 ± 20.9 years) on chronic thrice-weekly haemodialysis at the Department of Nephrology, University Hospital of Linköping, Sweden. Additional information about the studied patients is shown in Table 1. The fact that five patients were anuric and five had a glomerular filtration rate of 1–9 ml/min was not taken into consideration in this study. The patients were monitored during four dialysis sessions each lasting between 240 and 300 min. The sessions studied (n = 40) were not consecutive, but were performed within 3 weeks for each patient. An althane dialyser was used with an effective membrane area of 1.8 m² (AF180, Althin Medical, Ronneby, Sweden). The dialysate flow was 500 ml/min and blood flow 300 ml/min—except in two sessions (230 and 250 ml/min) due to temporary access (needle) problems. Two types of machines were used, AK 200 (Gambro Lundia AB, Sweden) and Fresenius 4008H (Fresenius Medical Care, Germany). The clinical set-up of the experiments is shown in Figure 1.

The Ethics Committee approved the study protocol, and informed consent was obtained from all patients.

Urea monitor

An on-line dialysate monitor, the UM from Baxter Healthcare Corp., USA, with an accuracy of ±5% was used to determine TRU (mmol) and nPCRw (g/kg/day) [1].

Sampling and laboratory analysis

TDC started when the blood filled the dialyser and ended when the blood was returned to the patient at the end of the dialysis. All spent dialysate was collected in a tank equipped with a scale (Figure 1). After its weight was recorded and the collected spent dialysate was carefully stirred, a TDC sample (Dtotal) was immediately sent to the laboratory, and urea concentration (mmol/l) was measured within the following 1–4 h.

Urea concentrations in the dialysate (Durea), later used for transformation, were also determined at scheduled times during dialysis: at 5, 15, 30, 60, 90, 120, 180 and 240 min (also at 270 and 300 min, if the treatment was longer than 240 min) from samples taken from the dialysate drain tube.

Table 1. Patient data (n = 10)

<table>
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<th>Patient number</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Body weight, dry weight (kg)</th>
<th>Weight loss (kg), mean of four sessions</th>
<th>P-albumin (g/l)</th>
<th>Residual renal function, creatinine clearance (ml/min/1.73m²)</th>
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<tr>
<td>Mean ± SD</td>
<td>62.8 ± 20.9</td>
<td>67.2 ± 15.1</td>
<td>1.9 ± 0.7</td>
<td>35.4 ± 5.0</td>
<td>3 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. The experimental set-up.
The concentrations of urea were determined at the Clinical Chemistry Laboratory at Linköping University Hospital using standardized methods. The accuracy of the method for determination of urea in the dialysate was ±5%.

**UV absorbance monitoring**

A double-beam spectrophotometer (UVIKON 943, Kontron, Italy) with an accuracy of ±1% was used for the determination of UV absorbance. During the on-line experiments, the spectrophotometer was connected to the fluid outlet of the dialysis machine, and all spent dialysate passed through the specially designed optical cuvette. The sampling frequency was set at two samples per minute and the wavelength was 285 nm.

**Transformation of UV absorbance into dialysate urea concentration**

In order to transform UV absorbance (dimensionless) to $D_{\text{urea}}$ (mmol/l), a good correlation must exist between the two variables [11]. The transformation is based on the regression line between UV absorbance and $D_{\text{urea}}$ (Figure 2A).

Regression lines from the different sessions in the week were assessed for transformation from one session of each patient: first in study, first in week or mid-week. The equation (Figure 2A) from the first studied dialysis for each patient was used for the subsequent three (non-sequential) treatments when calculating TRU and nPCRw, and was used for the comparisons with TDC and UM.

**Estimation of TRU**

One way to estimate TRU, assuming that the dialysate flow, $Q_d(t)$, is constant and the total ultrafiltration (UF) is known, is to use the following equation [13]:

$$\text{TRU}(\text{mmol}) = \text{urea [mean]} \times (Q_d \times T + \text{UF})$$  \hspace{1cm} (1)

where urea [mean] in mmol/l is the mean urea concentration in the spent dialysate of the particular haemodialysis session. For the TRU calculations, urea [mean] = $D_{\text{total}}$ was utilized.

In a similar way, TRU may be calculated from the on-line UV absorbance (TRUa) curve (Figure 2B) as:

$$\text{TRUa}(\text{mmol}) = (\text{slope} \times \text{meanA} + \text{intercept}) \times (Q_d \times T + \text{UF})$$  \hspace{1cm} (2)

where meanA is the mean of all UV absorbance values from the start to the end of the dialysis, $Q_d$ is the rate of the dialysate flow in l/min, $T$ is the duration of the dialysis session in minutes and UF is the total volume of the ultrafiltrate, in litres, during the session. The regression line between UV absorbance and $D_{\text{urea}}$ from one on-line measurement gives

![Graph A](image1)

**Fig. 2.** (A) The scatterplot of $D_{\text{urea}}$ against UV absorbance (wavelength 285 nm) for a single haemodialysis treatment ($n = 10$). The best-fit regression line presented as $D_{\text{urea}} = \text{slope} \times A + \text{intercept}$. (B) Typical on-line absorbance curve during a single haemodialysis treatment of 5h duration, where UV absorbance at the 285 nm wavelength is plotted against time. The parameters are explained in the text.
Protein catabolic rate based on UV absorbance were finally compared. The means of the TRUs from the three methods estimation of 1.1%. TRU was obtained from the UM part of Equation 2). This approach had a good agreement comparison.

**PCR calculations from TRU**

The calculation of PCR, from TDC and UV absorbance, was, herein, based on a theory by Garred et al. [10], where a calculation of urea removal is expressed as a fraction of the week’s urea generation. The fraction varies with the day of the week, and was found to be essentially constant for each patient on a given day [10]. The amount of urea could therefore be determined approximately from the measurement of urea concentration in only one of the three treatments of the week, and PCR could be calculated as:

$$\text{NPCRw} = \text{factor 1, 2 or 3} \times \text{TRU 1, 2 or 3} \times \text{BW} + 0.17$$  \hspace{1cm} (3)$$

where TRU 1, 2 or 3 (expressed in g of urea nitrogen) is the TRU from the first (1), mid-week (2) or the last dialysis in the week (3) and factor 1, 2 or 3 is the fractional factor for the first (1), mid-week (2) and last treatment (3) of the week, respectively; factor 1 = 2.45; 2 = 2.89; 3 = 3.10 [10]. Obligatory loss of dietary protein in stools and in shed skin represents the constant term 0.17 (g protein/kg body weight/day). The dry body weight (BW) was used for normalization of PCR (nPCRw).

The nPCRw value presented by UM was used for comparison.

**Statistical analysis**

Results are expressed as mean ± SD. The Student paired t-test (two-tailed) and the Levene test of homogeneity of variances were used to compare means for different methods and SD values, respectively. A P-value < 0.05 was considered significant. The three different methods were compared using Bland and Altman analysis [14]. For the analysis, SPSS (Statistical Package for the Social Sciences, version 11.0) was used. A total of six sessions were excluded due to technical failure of the UM (three of 40 sessions) or the failure of the spectrophotometer (three of 40). Uncertain values marked as ‘fit error’ by the UM (two of 40 sessions) were also excluded.

**Results**

Figure 2A shows the example of one dialysis session, demonstrating the high correlation between UV absorbance (A) at the 285 nm wavelength, and urea measurements on manually taken dialysate samples, D_{urea}, which were used for the transformation. The corresponding regression equation based on 10 manually taken D_{urea} samples was D_{urea} = 4.0368 × A + 0.2641, \( r^2 = 0.995 \). Figure 2B shows the corresponding on-line absorbance curve during a single haemodialysis treatment of 300 min; in it, UV absorbance at the 285 nm wavelength is plotted against time.

Table 2 shows TRUa using Equation 2 applied to different treatment days used for transformation and presented as mean, SD and number of sessions. TRU values from TDC and readings by UM are also shown in Table 2. The results show slight differences in the mean and SD of TRUa of the different dialysis treatments used for transformation. TRUa using the first study session of the patients, which could have been on any day in the week, is also presented with the transformation session excluded. TRUa using the first or the mid-week session for transformation exhibits the same mean value, but mid-week TRUa shows a higher SD compared with the others. However, TRUa using every session for transformation, not suitable in clinical practice, demonstrates values similar to the other methods.

**Discussion**

The results show that it is possible to estimate TRU, and consequently PCR, based on a measurement of urea concentration by UV absorbance. The values
of TRU and nPCRw obtained using UV absorbance measurements were of the same order as TRU and nPCRw calculated from TDC (reference method), and they were significantly lower than corresponding values obtained by UM (Table 2 and Figure 3). However, the SD of the mean value for TRU and nPCRw was of the same magnitude for all dialysate-based methods.

The values of nPCRw in this study are somewhat lower compared with those of other studies, where nPCRw calculation is based on the dialysate side [1,15]. A source of error in the TDC method could be urease-producing bacteria [6]. The reported accuracy of the laboratory method, ~5% for determining urea, cannot explain the differences in the mean values of nPCRw between this and other studies. The lower nPCRw obtained by all three methods in this study may depend on patient selection: high age, nutritional status and the fact that some of our patients have an important residual renal function (Table 1). Two of the patients had low P-albumin (without having any indication of ongoing infection or inflammation) and therefore can be suspected to have malnutrition. Our low nPCRw values clearly reflect the patient’s nutritional condition and residual renal function. In the other studies mentioned above, the residual renal function was considered [1,15]. The somewhat higher SD of the difference in the nPCRw obtained by the TDC and UV method (Figure 4A) compared with the TDC and UM method (Figure 4B) may be explained by the fact that the UM rejects unstable points not fitting the expected exponential decay [1]. In the case of the UV absorbance technique, the only reason for excluding a session was technical failure.

PCR fluctuates significantly from day to day, and, therefore, a mean PCR value from several measurements over time has been suggested as being a better indicator of PCR [10]. From that point of view, the UV method should have advantages, because integration with the dialysis machine makes it possible to make measurements and save information, from every treatment, and present mean values with chosen periodicity.

Blood urea PCR was not considered in this study, since (i) different sampling time periods were used for the dialysate and the blood sides; and (ii) fraction factors [10] were used on the dialysate side but not on the blood side.

Studies have shown that Kt/V and PCR correlate with clinical outcome in dialysis patients [16], but the correlation between these two parameters seems to disappear with higher Kt/V values [4,17]. Kt/V only partially reflects a patient’s status; it must be used in addition to PCR evaluation [10]—and perhaps nutritional parameters are stronger predictors of outcome than Kt/V. This indicates that both PCR and Kt/V, and not just one of them, are important

Fig. 3. Mean values of nPCRw in g/kg/day derived from TDC (n = 40), from UV absorbance (n = 37) and from UM (n = 35).

Fig. 4. Bland–Altman plot of the differences between (A) nPCRw derived from TDC and by using UV absorbance (n = 37) and (B) nPCRw obtained by using TDC and by using UM (n = 35), plotted against nPCRw derived from TDC.
to follow in the treatment of dialysis patients. The corresponding equilibrated $K_t/V$ (mean $\pm$ SD) in this study was 1.23 $\pm$ 0.17, using pre- and post-dialysis blood urea concentration, 1.16 $\pm$ 0.18, using the slope of UV absorbance, and 1.24 $\pm$ 0.18, using readings from UM [12]. The somewhat lower values obtained by UV absorbance, compared with the other methods, may be explained by different elimination rates between solutes measured by UV absorbance and urea measured by the chemistry laboratory or urea determined by UM [12]. With commercially available on-line monitors based on the ionic dialysance method, $K_t/V$ can be estimated [2]. Using methods based on direct measurement of urea loss [1] and the new UV absorbance technique, it is possible to estimate both $K_t/V$ and PCR.

Figure 2B illustrates the exponential decrease of UV-absorbing solutes during a dialysis treatment. This indicates that it is possible to follow a single haemodialysis session continuously and monitor deviations in the clearance of UV-absorbing solutes. The calculations of TRUa were based on the correlation between UV absorbance and $D_{\text{urea}}$ (Figure 2A). This probably describes a relationship between UV-absorbing solutes and urea that could be specific for each patient. A number of transformation methods were evaluated (Table 2), and there were only small differences between them regarding mean values and SD. Therefore, in this study, we used the first study session as the most applicable in clinical practice.

The need to have individual dialysate samples makes estimating TRU with the UV method a tedious and laborious procedure. A general regression model based on the correlation between UV absorbance and $D_{\text{urea}}$ for all subjects should be preferred, and such efforts are underway. Investigations of factors that may interfere with the UV absorbance, e.g. drugs or temperature variations, are also of great importance and should be considered.

The primary aim of this study was to compare three dialysate-based methods. The UM utilizes dry BW for normalization of PCR; therefore, BW was used for all methods. Normalization by lean body mass has been recommended as a means of eliminating errors caused by abnormal body composition [8]. In the present study, the dialysis treatments were not consecutive, instead the fractional factors for urea were used for TRU when calculating PCR [10]. Due to the good correlation between UV absorbance and urea [11] (Figure 2A), the same fractional factors were used when calculating PCR by the UV method.

Earlier studies using high-performance liquid chromatography (HPLC) have shown that UV-absorbing solutes, that were removed by haemodialysis, were of a higher molecular weight than urea [18]. Retention of solutes in uraemic patients is a complex problem that concerns many solutes and wide variations in their concentrations [19]. The role of urea in the uraemic patient has been discussed; its lack of toxicity has made urea relatively unimportant in the development of uraemia [19]. However, urea is still a surrogate marker in dialysis patients.

In conclusion, this study indicates that TRU and PCR can be assessed by the UV technique, even when the technique does not measure urea itself. Despite the good correlation between UV absorbance and the concentrations of molecules other than urea [11], whether the total removal of, for example, creatinine, uric acid and also other solutes mentioned by the EUTox group [19] can be used as a measure of dialysis adequacy in the same manner as urea should be assessed.

The UV method may be more reflective of a total elimination process compared with the measurement of single solute elimination. Perhaps this new methodology will be applicable in the future in the management of dialysis patients.

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Conflict of interest statement. None declared.

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