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Urea- and Creatinine-Based Parameters in the Optical Monitoring of Dialysis: The Case of Lean Body Mass and Urea Rebound Assessment

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.
Ruth Tomson

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Uureal ja kreatiniinil põhinevad parameetrid neeruasendusravi optilises monitooringus: patsiendi lihasmassi ja uurea tagasilöögi efekti hindamine

RUTH TOMSON

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KIRJASTUS

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LIST OF PUBLICATIONS

The current thesis summarises the author's work at the Department of Health Technologies of Tallinn University of Technology, and is based on the following publications referred to in the text by Roman numerals, I-III.

I. **Tomson R**, Fridolin I, Uhlin F, Holmar J, Lauri K, Luman M (2013) "Optical measurement of creatinine in spent dialysate", *Clinical Nephrology*, 79(2): 107 – 117 (DOI: 10.5414/CN107338).

II. **Tomson R**, Fridolin I, Luman M (2015) "Lean body mass assessment based on UV absorbance in spent dialysate and dual-energy x-ray absorptiometry", *The International Journal of Artificial Organs*, 38(6): 311 – 315 (DOI: 10.5301/ijao.5000415).

III. **Tomson R**, Uhlin F, Fridolin I (2014) "Urea rebound assessment based on UV absorbance in spent dialysate", *ASAIO Journal*, 60 (4): 459 – 465 (DOI: 10.1097/MAT.0000000000000091)

Author's contribution to the publications

In all of the publications the author performed the data analysis and wrote up the manuscripts. In Publication II the author planned and participated in carrying out the *in vitro* experiment.

INTRODUCTION

Since the first experimental attempts of haemodialysis (HD) at the beginning of the 20th century [1–3], the advancement of the technology has been remarkable, making it an effective renal replacement therapy (RRT). Over 2.5 million patients worldwide undergo maintenance dialysis as of 2013 [4], and further growth in the number of patients is predicted [5–7]. Yet despite this, trends in the morbidity and mortality of HD patients have not improved accordingly [8,9]. Therefore, in order to enhance treatment quality, patient quality of life, and survival further development is needed.

One of the factors that are associated with morbidity and mortality in dialysis patients is protein-energy malnutrition (PEM). Amongst the causes of PEM are reduced protein and energy intake, metabolic acidosis, hypercatabolism and the dialysis treatment itself [10]. Therefore, regular assessment of the nutritional status of dialysis patients is recommended [11]. As creatinine (MW=113 Da) is produced at a nearly constant rate in the process of creatine metabolism in the muscles [12,13], this serves to reflect total body skeletal muscle mass. Therefore lean body mass (LBM), which is a creatinine-based parameter, is suggested as being one of those measures which is suitable for nutritional status evaluation [14,15]. The established method for LBM assessment is creatinine kinetic modelling (CKM). However, this approach is cumbersome because urine and dialysate collection is needed in addition to blood samples. Alternative methods for LBM assessment include dual-energy x-ray absorptiometry (DXA) and bioelectrical impedance measurement. However, the drawback in both of these approaches is the fact that the altered tissue hydration in dialysis patients has an effect on the measurement results [16–18]. Therefore a method is needed that enables the non-invasive assessment of LBM and that is not influenced by excess body water.

The most used index of dialysis adequacy is Kt/V [19], which is based on the most abundant uremic retention solute urea (MW=60 Da) [20]. The reference method for the calculation of Kt/V, formal single-pool variable volume urea kinetic modelling (spUKM), does not take into account urea compartmentalisation during dialysis and the resulting post-dialysis urea rebound (PDUR) [21] when immediate post-dialysis blood samples are used. Consequently, the dialysis dose is overestimated in this case. A more accurate result is achieved when equilibrated urea concentration is used in the calculations. However, it is inconvenient both for the patients and dialysis facilities to wait for up to an hour in order to take the post-dialysis sample. Therefore, algorithms have been developed that enable the estimation of the equilibrated urea concentration by using the conventional pre- and post-dialysis blood samples [22,23]. However, it would be beneficial if equilibrated urea concentration and PDUR could be assessed consistently and without the need for repeated blood samples to provide more adequate information for each dialysis session.

The optical dialysis monitoring method [24,25] has the potential to offer a solution to the problems which have been outlined above. The general aim of the thesis was to examine the possibility of using the optical dialysis monitoring method to assess urea-based and creatinine-based parameters via the example of lean body mass and urea rebound calculation. The purpose of the study in **Publication I** was to develop a model for the estimation of creatinine concentration by using UV absorbance in spent dialysate. In **Publication II**, the lean body mass estimation in dialysis patients was presented as a possible application of the optical measurement of creatinine concentration. In the future this would provide a systematic and efficient way of reviewing the nutritional status of dialysis patients. The objective of **Publication III** was to explore the possibility of assessing the post-dialysis urea rebound employing UV absorbance in spent dialysate. In the future, this estimate could be used to correct the single-pool Kt/V (spKt/V) values in order to obtain equilibrated Kt/V (eKt/V) values.

Approbation

- **Tomson R**, Fridolin I, Uhlin F, Jerotskaja J, Lauri K, Luman M. "Development of the model for the optical multiwavelength monitoring of creatinine in the spent dialysate". *12th Biennial Baltic Electronics Conference, Tallinn, Estonia, October 4-6, 2010.*

- **Tomson R**, Uhlin F, Holmar J, Larui K, Luman M, Fridolin I (2011) "Development of a method for optical monitoring of creatinine in the spent dialysate", *Estonian Journal of Engineering*, 17(2): 140-150.

- **Tomson R**, Fridolin I, Uhlin F, Holmar J, Lauri K, Luman M. "Development of the model for the optical monitoring of urea in spent dialysate". *13th Biennial Baltic Electronics Conference*, *Tallinn, Estonia, October 3-5, 2012*.

- **Tomson R**, Fridolin I, Luman M. "Lean body mass estimation utilising UV-absorbance in spent dialysate". *12th Conference of Baltic societies of Nephrology, Molėtai district, Lithuania, June 12-14, 2014*.

- **Tomson R**, Uhlin F, Fridolin I. "Optical urea rebound estimation during dialysis". *16th Nordic-Baltic Conference on Biomedical Engineering*, *Göteborg, Sweden, October 14-16, 2014*.

- **Tomson R**, Fridolin I, Luman M. "Lean body mass assessment based on UV-absorbance in spent dialysate". *14th Biennial Baltic Electronics Conference, Tallinn, Estonia, October 6-8, 2014*.

- **Tomson R**, Fridolin I, Luman M (2015) "Development of an optical monitoring technology for urea rebound assessment", *International Journal of Bioelectromagnetism*, 17(2), 52-56.

- **Tomson R**, Fridolin I, Luman M, Holmar J. "The effect of Kt/V on post dialysis urea rebound in hemodialyis and hemodiafiltration". *53rd ERA-EDTA Congress, Vienna, Austria, May 21-24, 2016*.

- **Tomson R,** Fridolin I, Luman M. "Estimation of the influence of K/V on post dialysis urea rebound in HD and HDF with traditional and optical methods". *13th Baltic Nephrology Conference, Jurmala, Latvia, October 13-15, 2016.*

ABBREVIATIONS

APD – automated peritoneal dialysis
AR - access recirculation
ARF – acute renal failure
BMI – body mass index
C_0 – pre-dialysis concentration of a solute
CAPD – continuous ambulatory peritoneal dialysis
C_{eq} – equilibrated concentration of a solute
CI - creatinine index
CKD – chronic kidney disease
CPR – cardiopulmonary recirculation
CrCl – creatinine clearance
C_T – post-dialysis concentration of a solute
CVD – cardiovascular disease
DXA - dual-energy x-ray absorptiometry
eKt/V – equilibrated dialysis dose
ESRD – end stage renal disease
EUTox – European Uremic Toxin work group
GFR - glomerular filtration rate
HD – haemodialysis
HDF – haemodiafiltration
HF – haemofiltration
hf-HD – high-flux haemodialysis
HPLC – high performance liquid chromatography
Kt/V – dialysis dose efficacy parameter
LBM - lean body mass
nPNA - normalised protein nitrogen appearance
PD – peritoneal dialysis
PDR – post-dialysis rebound
PDUR – post-dialysis urea rebound
PEM – protein-energy malnutrition
PNA – protein nitrogen appearance
RRT – renal replacement therapy
spKt/V – single-pool Kt/V
spUKM – single-pool variable volume urea kinetic modelling
stdKt/V – standard Kt/V
UKM – urea kinetic modelling

1 THE KIDNEYS, RENAL FAILURE AND RENAL REPLACEMENT THERAPY

1.1 The kidneys

The kidneys are part of the urinary system and their functions include [26]:

- the excretion of wastes and excessive liquid;
- the regulation of blood volume, pressure, and electrolyte and acid-base balances;
- contributing to the metabolism by hormone secretion and participation in vitamin D synthesis.

The functional units of the kidneys are the nephrons that produce urine and maintain the homeostasis of blood through three processes: 1) glomerular filtration; 2) tubular reabsorption; 3) tubular secretion. The average volume of glomerular filtrate can reach 180 litres per day [26]. However, following tubular reabsorption the end product is between one and two litres of urine excreted daily.

1.2 Renal failure

Renal failure is a loss of kidney function resulting in the accumulation of water and toxic solutes and can be distinguished as acute [27–29] or chronic [30–33]. Acute renal failure (ARF) is an abrupt and sustained decrease in kidney function that can be caused by intoxication or serious trauma, for example, and may be reversible [27,28]. Chronic kidney disease (CKD) is defined as abnormalities in kidney function or structure that are present for over three months and that cause health implications [33]. CKD is usually irreversible and develops over different time periods. The development of CKD has been classified into five stages based on the progressive decrease of the glomerular filtration rate (GFR) [33]. End stage renal disease (ESRD) is stage 5 of CKD, when GFR is below 15 ml/min/1.73 m^2 and renal replacement therapy is needed [33–35]. The main causes of ESRD are polycystic kidney disease, glomerulonephritis, hypertensive renal disease and diabetic nephropathy [30,31].

1.3 Renal replacement therapy

Three renal replacement therapy options are available for patients with ESRD: peritoneal dialysis (PD), haemodialysis (HD), and transplantation. The best solution is renal transplantation as it reduces dialysis-related comorbidities and offers improved quality of life [36,37]. However, this approach may not be applicable, either due to the patient's condition or because a suitable donor organ is not immediately available.

PD is a home-based RRT option in which the patient's peritoneal membrane is used [38,39]. The peritoneal cavity is filled with dialysate via a catheter for a

certain period of time and then is drained. The process is repeated several times a day. Two PD techniques are available [38]: continuous ambulatory PD (CAPD) and automated PD (APD). The advantages of PD include patient autonomy and independence, as well as the fact that no vascular access is required. However, the technique may fail due to peritonitis, mechanical problems with the catheter, ultrafiltration failure, or the patient becoming tired of having to carry out the changes [38,40].

HD is a RRT option in which accumulated metabolic waste and excess water are removed from the patient's blood using an artificial kidney [41]. During treatment, the retained solutes move from the blood across a semi-permeable membrane to the dialysis fluid by diffusion and/or convection. Purified blood is returned to the patient's vein and spent dialysate is disposed of by sending it into the drain. Four haemodialysis techniques are available [41]: conventional HD, haemofiltration (HF), high-flux haemodialysis (hf-HD) and haemodiafiltration (HDF). The techniques are differentiated by the characteristics of the membrane that is used, and by whether it is mainly diffusion, convection, or both being applied. The standard HD schedule is three times a week and the duration of one treatment is four hours [19,42]. Usually patients go to a dialysis centre or hospital to have the treatment. Common complications of HD include intradialytic hypotension and cardiac arrhythmias, cardiovascular disease (CVD) and amyloidosis [41].

2 UREMIC SOLUTES, UREA AND CREATININE

Over 270 uremic retention solutes, which accumulate in the case of kidney failure, have so far been identified [43]. According to the European Uremic Toxin (EUTox) work group these solutes can be divided into three major categories [44–46]:

- free, low molecular weight water-soluble molecules (molecular weight (MW) <500 Da, e.g. urea (MW=60 Da), creatinine (MW=113 Da);
- protein-bound solutes (e.g. indoxyl sulphate (MW=251 Da), indole-3-acetic acid (MW=175 Da));
- middle molecules (MW>500 Da, e.g. β_2-microglobulin (MW=11818 Da), cystatin C (MW=13300 Da)).

The present thesis focuses on the kinetics and monitoring possibilities of urea and creatinine, both of which belong to the category of low molecular weight water-soluble molecules.

2.1 Urea

Urea (MW=60 Da) is the principal end product of the catabolism of proteins. It is the most abundant uremic retention solute to appear in the blood of patients who are suffering kidney failure [20]. The acceptance of urea as a uremic toxin is still under debate. It appears that urea at levels of concentrations which are observed in clinical uremia are not directly toxic to the organism, as reports of its adverse effects are scarce [47–49]. However, it has been suggested that carbamylation, an irreversible modification of proteins and amino acids that alters their structure and function, may be a mediator for at least part of the toxic effects of urea [50–53]. Carbamylation results from exposure to isocyanic acid, the active form of cyanate which is a decomposition product of urea. Equilibrium exists between urea and cyanate [54]. Therefore, in the case of CKD and ESRD, as urea accumulates and its concentration in the blood of patients increases, the rate of carbamylation also increases [50,53]. Carbamylation has been linked to increased mortality risk in HD patients [55] and cardiovascular disease (CVD) [52]. Therefore it is thought that carbamylation could possibly be the connection between CVD, inflammation, and protein energy wasting in CKD and ESRD patients [51].

Urea has several favourable properties which are important for a marker solute [19,20]:

- it is water soluble, which means it is distributed in body water;
- it diffuses easily between body compartments and across the dialysis membrane due to its small molecular size and lack of electrical charge; therefore patient urea clearance is closely approximated by dialyser urea clearances;
- it is easily measured due to its abundance in body fluids of uremic patients;

- it provides an index of nutrition, as urea generation is exclusively from protein catabolism;
- it provides a sensitive index of dialyser function due to high clearance by the dialyser.

However, the absolute values of urea concentration in blood are not considered to be a good diagnostic parameter because of their ambiguity. In CKD the use of plasma urea concentration as a diagnostic marker is disputable as it is influenced by several factors (such as changes in urinary excretion, and the nutritional and metabolic state of the patient) [49]. Also, in ESRD patients interpreting urea concentration in blood requires information of the patient's nutritional status, as the relative contributions of urea removal and generation both play a role in determining the outcome [20]. Moreover, it has been shown that urea kinetics during dialysis do not characterise the removal of other low molecular weight water-soluble uremic solutes [56,57]. This calls into question the role of urea as a marker molecule which also represents the behaviour of other uremic solutes.

Nevertheless, due to the previously discussed advantages of urea, the fractional urea clearance, Kt/V, is the most frequently used index of dialysis adequacy [19].

Urea and dialysis adequacy

Urea Kt/V is considered to be the best predictor of dialysis outcome currently available, based on the accumulated evidence of its positive correlation with morbidity and mortality in chronic HD patients [19,58]. It is recommended that Kt/V should be assessed at least on a monthly basis [19].

Kt/V is a dimensionless parameter, where K is the dialyser urea clearance, t is the duration of the HD session, and V represents distribution volume for urea. Calculating Kt/V is traditionally based on urea concentration in pre-dialysis and post-dialysis blood samples. Formal single-pool variable volume urea kinetic modelling, which is recommended by the guidelines as the reference method [19,58], utilises iterative, computer-based mathematical modelling to estimate V and urea generation (G) [59]. In addition to UKM, there also exist simplified equations for the calculation of spKt/V that are applicable to the standard three-times-a-week HD schedule, e.g. the Daugirdas second generation equation [60]:

$$spKt/V = -\ln\left(\frac{C_T}{C_0} - 0.008T\right) + \left(4 - 3.5\frac{C_T}{C_0}\right)\frac{\Delta BW}{BW_{post}} \quad (1)$$

where C_0 is urea concentration before dialysis (in mmol/l), C_T is urea concentration at the end of dialysis (in mmol/l), T is the duration of dialysis (in hours), ΔBW is intradialytic weight loss (in kg) and BW_{post} is body weight at the

end of the session (in kg). For HD therapies with a different frequency and duration the standard Kt/V (stdKt/V) has been proposed [61].

The advantage of the spUKM method is that, in addition to spKt/V, it also provides information about the nutritional status of the patient and an individual treatment time prescription [58]. However, the weak point in this method is the fact that it does not take into account urea compartmentalisation, which causes the development of a disequilibrium in concentrations between the blood and peripheral compartment during dialysis [21]. The single-pool variable volume model that is implemented assumes that urea is equilibrated across a single compartment. Consequently, as the urea concentration in blood during HD is lower than the overall concentration in the total body water, spKt/V overestimates the delivered dialysis dose.

One possibility to overcome the shortcomings of spUKM would be the use of the equilibrated urea concentration (C_{eq}) instead of the customary immediate post-dialysis urea concentration. In this way the eKt/V value is calculated, which represents the effective urea clearance and is a more accurate measure of the dialysis dose [21]. As it takes between 30 to 60 minutes for urea concentration to equilibrate between the compartments, taking the post-dialysis sample requires waiting up to an hour after the completion of HD [62]. Therefore this approach is not applicable in every day clinical practice due to its inconvenience for patients and the added work load for staff. An alternative method has been proposed by Daugirdas and Schneditz [63] that allows the calculation of eKt/V based on conventional blood samples:

$$eKt/V = spKt/V - \left(0.6\frac{spKt/V}{T}\right) + 0.03 \quad (2)$$

The current NKF KDOQI Clinical Practice Guidelines for Haemodialysis Adequacy recommend that for standard thrice weekly dialysis the target spKt/V should be 1.4 per session, with a minimum of 1.2 [19]. The European Best Practice Guidelines by ERA-EDTA recommend that eKt/V should be used to express the dialysis dose and a minimum target eKt/V of 1.2 per session for a thrice weekly dialysis schedule [58].

Urea and nutrition

ESRD patients are at risk of protein-energy malnutrition and uremic wasting due to spontaneous dietary protein intake restriction, chronic inflammation and insulin resistance, which are observed in case of uremia [64–66]. Moreover, beside uremic toxins the HD procedure also removes important nutrients via the filter such as vitamins, amino acids, and glucose [67]. The guidelines recommend several assessment tools for diagnosing malnutrition [11]. One of the measures included in the panel is the normalised protein nitrogen appearance (nPNA).

Protein nitrogen appearance (PNA) is a indirect measure of dietary protein intake, which is based on the premise that in HD patients who are in a steady state the nitrogen balance is zero or is slightly positive [11,68]. PNA is generally normalised (nPNA) for the patient's body weight as dietary protein requirements and net protein breakdown in fasting conditions are greatly influenced by body mass [11]. The calculation of PNA is based on spKt/V and pre-dialysis urea concentration in blood (C_0) [11]. It has been shown that a low PNA level is associated with mortality in HD patients [68].

The European Best Practice Guidelines recommend that in a clinically stable chronic HD patient the nPNA should be above 1.0 g/kg ideal body weight/day, and that nPNA should be measured every three months [11].

Standard biochemical methods for urea measurement

The most commonly used assays for the determination of urea are based on enzymatic methods that employ urease and measure the released ammonia [69]:

$$\text{Urea} + 2H_2O \xrightarrow{\text{urease}} NH_3 + CO_2$$

The advantages of these methods are high specificity and sensitivity, simplicity and adaptability. However, enzymatic methods are prone to interference from endogenous ammonia and compensation for this needs to be factored in [69].

Colorimetric methods for the determination of urea include the reaction with diacetyl monoxime [70] and o-phthalaldehyde [71]. There is no interference from ammonia with the diacetyl monxime methods. However, heating is required and noxious chemicals are used [69]. The o-phthalaldehyde methods do not require heating, but can have interference problems [69].

2.2 Creatinine

Creatinine (MW=113 Da) is the product of creatine and creatine phosphate catabolism in muscles [12,13]. As the conversion process has an approximately steady rate, creatinine constantly diffuses into blood from the tissues and is excreted into urine by the kidneys [12]. Creatinine shows limited biological toxicity [57]. However, it has been suggested that creatinine may have a role in the development of uremic encephalopathy [72]. Also, it is the precursor of the uremic toxin methylguanidine [12].

Creatinine, muscle mass assessment methods and nutrition

Approximately 95% of the precursor of creatinine, creatine, is located in the skeletal muscles [13]. Therefore, urinary creatinine excretion [73,74], serum [13], and plasma [75] creatinine concentration have been proposed as measures of total body skeletal muscle mass. The gold standard for the assessment of urinary creatinine excretion is considered to be the 24-hour urine collection [74].

However, in practice this method proves inconvenient as multiple days of urine collection are required [73] and it is prone to error due to the risk that collection may be carried out incorrectly. Furthermore, creatinine excretion is influenced by dietary protein intake, physical activity, and the health condition of the individual [76], so these factors need to be taken into account in all proposed methods for the assessment of muscle mass. Yet, in the case of age-related muscle loss, it has been suggested that creatinine excretion provides a better estimate than conventional dual-energy x-ray absorptiometry (DXA) [73].

DXA is used primarily to measure bone mineral density with the purpose of diagnosing osteoporosis [77–79]. Additionally, it can be employed in body composition assessment [78–81]. DXA technology is based on the principle that photon attenuation is dependent upon tissue composition [82]. The DXA scanners measure the ratio of photon attenuation in the supine body at two energy levels, e.g. 40 keV and 70 keV, which allows for the determination of two components within each pixel [82]. It is assumed that the body consists of bone mineral, lean soft tissue, and fat. Therefore lean and fat fractions can be determined from areas that do not contain bone [79–82]. Subsequently, the composition of the soft tissue which overlies bone can be extrapolated from these results. This enables the quantification of total lean soft tissue, fat, and bone mineral. The effective doses in DXA measurements are small, e.g. 5-7 μSv in the case of whole-body bone mineral density measurements [81]. This, together with the fact that the procedure is non-invasive and easily applied, makes DXA a favourable method for body composition assessment [78].

It has been suggested that lean body mass (LBM), which reflects muscle mass, and creatinine index (CI), which is the sum of the creatinine extrarenal degradation rate and the appearance rate in dialysate, ultrafiltrate and urine, are reliable and stable measures of long term nutritional status in dialysis patients, as they represent the somatic protein stores [14–16,83,84]. The established approach to the assessment of CI and LBM is the creatinine kinetic modelling [15,83], which requires the measurement of creatinine excretion in urine and dialysate. The advantage of CKM in LBM estimation is that it is not affected by the tissue hydration status of dialysis patients, which can pose a problem in the case of alternatively suggested methods for LBM assessment, e.g. DXA measurements [16–18] and bioelectrical impedance analysis [16]. As CKM may not be available in all dialysis facilities, formulas have been developed for the assessment CI and LBM based on biochemical and anthropometric measurements [14]:

$$LBM = 0.029 \times CI \times BW_{post} + 7.38 \tag{3}$$

$$CI = 162.7 \times \frac{G_{Cr}}{BW_{post}} + 0.00429 \times TAC_{Cr}lm \tag{4}$$

$$G_{Cr}male = 0.8 + \frac{\left(BW_{pre} \times C_{pre} - BW_{post} \times C_{post}\right) \times (1 - R_{Cr})}{-\ln(R_{Cr}) \times BMI \times 152} \tag{5a}$$

$$G_{Cr}female = 0.8 + \frac{\left(BW_{pre} \times C_{pre} - BW_{post} \times C_{post}\right) \times (1 - R_{Cr})}{-ln(R_{Cr}) \times BMI \times 172.7} \quad (5b)$$

$$TAC_{Cr}lm = \frac{C_{pre}(1 - R_{Cr})}{-ln(R_{Cr})} \quad (6)$$

$$R_{Cr} = \frac{C_{pre}}{C_{post}} \quad (7)$$

where BW_{pre} and BW_{post} are body weight (in kg) before and at the end of the dialysis session, respectively; G_{Cr} is creatinine generation rate (in mg/day); $TAC_{Cr}lm$ is logarithmic mean-based, time-averaged creatinine concentration (in µmol/l); C_{pre} and C_{post} are the concentrations of creatinine in blood (in µmol/l) before and at the end of the treatment, respectively. Body mass index (BMI) (in kg/m^2) is calculated based on BW_{post} and the patient's height.

Creatinine and renal function

For the diagnosis and assessment of the progression of renal disease it is necessary to estimate the GFR, which is most precisely done by utilising exogenous filtration markers, e.g. inulin [85]. However, in everyday practice this approach is too complex and expensive, as it requires intravenous infusion under standardised conditions and complex chemical assays for inulin measurement [76].

Because creatinine is non-toxic, and is freely filtered at the glomerulus, is not protein bound, is physiologically inert, and is not metabolized by the kidneys, it complies with the majority of requirements for a ideal filtration marker [76]. Also, as the progression of renal disease has been associated with decreasing renal clearance and an increasing serum concentration of creatinine, serum creatinine concentration and creatinine clearance (CrCl) are frequently used as markers for renal function [12]. However, the European Best Practice guidelines do not recommend the estimation of renal function from blood creatinine measurements alone [86], as the production and elimination of creatinine varies amongst individuals, and over time, even if there is no change in renal function [76]. The major drawback of CrCl estimation is the requirement of 24-hour urinary creatinine excretion measurement [85]. Consequently, methods based on serum creatinine have been proposed to facilitate the estimation of CrCl and GFR [85,86], where body weight, age, gender, and race are used to account for differences in muscle mass. European Best Practice guidelines recommend the estimation of GFR based on the mean of urea and creatinine clearance, which are calculated from a 24-hour urine collection, and by normalising it to body surface area (1.73 m^2) [86].

However, the shortcomings in terms of creatinine as a filtration marker are that CrCl overestimates GFR both in healthy individuals and patients with renal

disease due to the tubular secretion of creatinine into urine [76]. Also, tubular reabsorption of creatinine is possible in the case of low rates of urine flow [76]. Therefore, CrCl and GFR which is estimated based on creatinine concentrations provide only an approximate guide to the renal function [76,85].

Standard biochemical methods for creatinine measurement

The most commonly used assays for the determination of creatinine are based on the Jaffe reaction [76,87,88]. It is a colorimetric method, where picric acid reacts with creatinine under alkaline conditions and as a result the bright red Janovsky complex is formed [89]:

$$\text{Creatinine+picric acid} \xrightarrow{\text{alkaline pH}} \text{Janovsky complex}$$

The absorbance of the produced colour, which is proportional to creatinine concentration, is measured at the wavelengths 490-520 nm [90,91]. The drawback in the use of this method is the fact that the colour-forming reaction is non-specific and interference from several other substances is experienced (e.g. carbonyl compounds, bilirubin, dopamine) [87,92]. Therefore modifications in the method have been developed in order to increase specificity (e.g. deproteinisation, kinetic measurements, and varying the pH) [76,87,88,92,93].

In addition, enzymatic methods have been developed for the determination of creatinine that are based mostly on the following reaction sequences [87,88,92]:

$$\text{Creatinine+}H_2O \xrightarrow{\text{creatininase}} \text{creatine}$$

$$\text{Creatine+}H_2O \xrightarrow{\text{creatinase}} \text{sarcosine+urea}$$

$$\text{Sarcosine+}O_2\text{+}H_2O \xrightarrow{\text{sarcosine oxidase}} \text{formaldehyde+glycine+}H_2O_2$$

Although, theoretically, the enzymatic assays are more specific, interference problems can also be experienced (e.g. from creatine, sarcosine, or bilirubin) [88,92].

2.3 Rebound of solutes

Post-dialysis rebound (PDR) is a rapid increase in plasma concentration of solutes removed by HD immediately after the completion of a dialysis session.

Two factors contribute to the genesis of the early phase of PDR (≤ 3 min after dialysis) – access recirculation (AR) and cardiopulmonary recirculation (CPR) [94]. In the process of recirculation the dialysed blood is returned to the dialyser without prior equilibration in the systemic arterial circulation [95]. Therefore due to AR and CPR, the concentration of solutes in blood samples which are drawn immediately after the end of a dialysis session is lower than it is in systemic venous blood [94]. This leads, for example, to an artificially

elevated estimate of spKt/V when the solute under consideration is urea. In order to avoid this and to allow the rebound caused by AR and CPR resolve the slow flow/stop pump sampling technique is recommended by the guidelines [96,97].

The late phase of PDR (>3 min) is caused by the compartmentalisation of solutes [62]. Due to this, solutes are effectively removed from the easily accessible compartment but not from the sequestered compartment. For various solutes, the degree of compartmentalisation, and the size and number of compartments, may be different [62]. For example, as urea equilibrates rapidly between red blood cells and plasma [98] it is cleared from both during dialysis. Creatinine diffusion from red blood cells, on the other hand, is slow [98] with an equilibration half-life of 13.8±2.8 minutes at 37º [99] and it is therefore cleared only from plasma. Consequently, once the dialysis session is ended, the entry of solutes from the relatively undialysed tissues into blood starts and concentrations across body water spaces equilibrate [62,94]. The duration of this period is between 30 to 60 minutes for urea [62] and approximately 60 minutes for creatinine [100]. Therefore, in order to obtain the most accurate solute concentrations (e.g. for Kt/V calculation) the post-dialysis blood samples would have to be drawn 60 minutes after the completion of the dialysis session. However, as this approach is not practical for dialysis facilities, mathematical modelling is applied in order to overcome the problem.

Mathematical models of solute kinetics during dialysis

In order to describe solute kinetics during dialysis, two two-compartment models – the serial two-compartment model and the regional blood flow model – have been proposed.

The serial two-compartment model assumes diffusive transport of solutes between the intracellular and the extracellular fluid compartment. For example, in the case of urea interstitial water, blood plasma and red blood cell water constitute the extracellular compartment and intracellular water forms the intracellular compartment [101]. Dialysis occurs from the extracellular compartment, with intercompartmental clearance governing the transport of solutes between the two compartments [62]. Accordingly, the disequilibrium of solutes during dialysis is explained by the lag in the decrease of solute concentrations in the intracellular fluid compartment [101]. As a result, solutes continue to diffuse into the extracellular compartment after dialysis until the concentrations of the compartments are equal [22].

The regional blood flow model, where organs are divided into a low-blood-flow group and a high-blood-flow group based on perfusion, assumes a parallel arrangement of compartments and mainly convective transport of solutes between them [62]. According to this model the disequilibrium in solute concentrations during dialysis is explained by a delay in transporting solutes from organs with low perfusion (i.e. skin, muscle, bone, and adipose tissue) to the dialyser [100]. The advantage of the regional blood flow model is that it provides an explanation how the rebound of solutes can be reduced by

increasing the perfusion of muscles, e.g. with intradialytic exercise [102,103]. While both models explain urea kinetics equally well [104], in the case of creatinine the regional blood flow model needs to be modified so that it also incorporates diffusion (the diffusion-adjusted regional blood flow model [100]) to account for the limited diffusion of creatinine from red blood cells.

Algorithms for estimating the equilibrated urea concentration

Based on the previously discussed models algorithms for anticipating the equilibrated urea concentration have been developed so that Kt/V could be accurately estimated while avoiding the need to wait for an equilibrated post-dialysis blood sample.

The Smye algorithm [22] is based on the serial two-compartment model, and the post-dialysis equilibrated concentration of urea in blood is estimated based on conventional pre-dialysis and post-dialysis blood samples plus an additional intradialytic blood sample. The base of this algorithm is the premise that after an intercompartmental concentration gradient has been established during the initial period of dialysis, the decrease of the equilibrated urea concentration in body water and the decrease of the extracellular urea concentration have equal log linear slopes [101]. Therefore, the slope (λ) can be estimated based on urea concentration in the intradialytic blood sample (C_{int}) and post-dialysis sample (C_T). Consequently, the equilibrium concentration (C_{eq}) of urea can be calculated as [22]:

$$C_{eq} = C_0 e^{-\lambda T} \tag{8}$$

$$\lambda = \frac{1}{T - t_{int}} ln\left(\frac{C_{int}}{C_T}\right) \tag{9}$$

where t_{int} is the time from the beginning of dialysis to the point at which the intradialytic blood sample is taken (in minutes), and T is the duration of dialysis (in minutes). It is suggested that urea concentration begins to follow a monoexponential decay towards the end of the first hour of dialysis [62]. Therefore, the intradialytic blood sample to determine C_{int} should be obtained between 60 to 80 minutes after the start of the session [105].

The Tattersall method [23] also originates from the serial two-compartment model and Smye's observation that the log linear slopes of urea concentrations decline in body water and blood are equal. This approach employs a patient and solute-specific constant called patient clearance time (t_p), which is independent of the duration and rate of dialysis [23]. t_p is the time separating the two log linear slopes that were demonstrated by Smye (in minutes) [101]. It is suggested that in routine practice the mean value of t_p for urea (35 min) should be used together with an occasional assessment of t_p in all patients [23]. Therefore according to Tattersall [23] C_{eq} can be calculated as:

$$C_{eq} = C_0 \left(\frac{C_T}{C_0}\right)^{\frac{T}{T+t_p}} \tag{10}$$

$$t_p = T \frac{ln\left(\frac{C_{eq}}{C_T}\right)}{ln\left(\frac{C_0}{C_{eq}}\right)} \tag{11}$$

The Daugirdas method [63] applies an empiric regression model that is in compliance with the regional blood flow model. In this approach urea rebound is expressed as the difference ΔKt/V between eKt/V (calculated based on C_{eq}) and spKt/V [63]:

$$\Delta Kt/V = -0.6\left(\frac{spKt/V}{T}\right) + 0.03 \tag{12}$$

It has been shown that the Smye, Tattersall and Daugirdas methods are equivalent on the basis that the decrease of urea concentration during the later stages of dialysis is a single exponential [106]. The advantage of the Smye algorithm is that conceptually it is the most rigorous approximation of C_{eq} based on the serial two-compartment model [101]. Also, this method could be used in the future to estimate the rebound of solutes other than urea without the need for adaptation [106], unlike the alternative Tattersall [23] and Daugirdas equations [63].The shortcoming of the Smye method is that in practice it suffers from inaccuracies which originate from small urea concentration measurement errors in the laboratory [101]. However, this source of error is common for all blood-based methods. In addition, the problems which are associated with the two-sample blood-based Smye method can be overcome by using on-line measurements to determine the slope [105].

3 THE OPTICAL METHOD FOR THE ASSESSMENT OF UREMIC SOLUTES

3.1 Light and matter

Light that interacts with a translucent medium can be reflected at the surface or transmitted through it; inside the medium the light can be scattered, absorbed, or internally reflected [107]. In tenuous media, scattering can be ignored and it can be assumed that the incident light is transmitted, absorbed, or reflected [108]. As spent dialysate is considered to be a weakly scattering medium, absorbance spectroscopy methods can be used for monitoring it [109].

3.2 Absorbance and the Bouguer-Beer-Lambert Law

Absorption is the transfer of photonic energy to electrons, atoms, or molecules, and its conversion into the internal energy of the absorber (e.g. heat) [110]. The absorbance of a medium is a measure of its capacity to absorb incident light, which is expressed by the Bouguer-Beer-Lambert Law (also abridged to Beer-Lambert Law) [111]. According to the law's derivative form, absorbance (A) (in arbitrary units) is given as:

$$A = log_{10} \frac{I_0}{I} \tag{13}$$

where I_0 is the incident intensity and I is the intensity of light transmitted through the medium.

Absorbance can also be expressed through the molar extinction coefficient ε (in $m^{-1}(mol/l)^{-1}$), the concentration of the absorbing compound C (in mol/l), and optical path length d (in m) [109]:

$$A = \varepsilon C d \tag{14}$$

Therefore, if ε and d are known and A is obtained by spectroscopic measurement, the concentration of a solute can be calculated as:

$$C = \frac{A}{\varepsilon d} \tag{15}$$

When the medium contains several different absorbing compounds, the overall extinction coefficient is the sum of each compound's contribution [112]:

$$A = log_{10} \left[\frac{I_0}{I}\right] = (\varepsilon_1 C_1 + \varepsilon_2 C_2 + \cdots + \varepsilon_n C_n) d \tag{16}$$

The Beer-Lambert Law applies only when specific assumptions are fulfilled [111,112]:

- incident light is collimated and monochromatic;

- scattering and reflections are excluded, so that light attenuation occurs only due to absorption;
- absorbing molecules are distributed homogeneously in the solvent;
- the concentration of the absorbing compound is not very high.

3.3 Optical monitoring of uremic solutes in spent dialysate

The first time optical monitoring was proposed for estimating dialysis efficiency was in 1980, by Gal and Grof [113], who suggested the continuous transmittance measurement of spent dialysate at 254 nm. A patent covering the spectrophotometric determination of waste products in spent dialysate was filed in 1999 by a Swedish research group [114]. The first results from the method and related equipment development were presented two years later, describing the possibility of being able to assess urea and creatinine removal during dialysis via UV absorbance monitoring in spent dialysate [115]. The technique was later described in depth [24], and the selection of a suitable wavelength range was discussed more specifically [116]. It was also demonstrated that the on-line measurement of UV absorbance can be used to reliably estimate Kt/V [117]. Since then several aspects of the optical monitoring technique and possibilities related to being able to apply it in everyday clinical practice have been explored and published, e.g.:

- urea [118,119], creatinine [120,121], and uric acid [122,123] quantification;
- the contribution of different uremic solutes to UV absorbance [124];
- the estimation of dialysis quality [109,125] and urea rebound [126,127];
- the monitoring of clinical events [128];
- the assessment of the nutritional status of dialysis patients [129–132].

Other research groups have also explored the possibilities raised by optical dialysis monitoring [25,133–135].

Currently the technology has been integrated into routine clinical practice for the estimation of Kt/V [136,137]. Moreover, the current NKF KDOQI Clinical Practice Guidelines for Haemodialysis Adequacy point out the optical method as being a possible technique to be used when it comes to monitoring the delivered dialysis dose [19].

4 EXPERIMENTAL STUDIES: METHODS, RESULTS AND DISCUSSION

4.1 Methods

Clinical studies

Haemodialysis patients from Linköping, Sweden and Tallinn, Estonia participated in the studies. A summary of the patients, their treatment parameters, and dialysate sampling times are presented in Table 1. The study protocols were approved by the Regional Ethical Review Board, Linköping, Sweden and by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. Informed consent in written form was obtained from all of the participating patients.

The concentrations of creatinine, urea, and uric acid in the collected samples were determined in the Clinical Chemistry Laboratories at the Linköping University Hospital and North Estonia Medical Centre. Standardised methods were used. The accuracy of the methods for creatinine and urea estimation in blood and dialysate was ±5%.

UV absorbance of the collected spent dialysate samples was determined using a double-beam spectrophotometer (UVIKON 943, Kontron, Italy, in Linköping, and Shimadzu UV-2401 PC, Shimadzu, Japan, in Tallinn). Spectrophotometric analysis was carried out over the wavelength range of 190-380 nm; an optical cuvette with a path length of 10 mm (Publications I & III) or 5 mm (Publication II) was used.

On-line UV-absorbance monitoring (Publication III) was carried out by connecting a spectrophotometer (UVIKON 934, Kontron, Italy in Linköping, and HR2000, Ocean Optics Inc., USA in Tallinn) to the fluid outlet of the dialysis machine. Spent dialysate passed through an optical flow cuvette with a path length of 10 mm. UV absorbance was registered at the wavelength 297 nm with a sampling frequency of two samples per minute.

DXA measurements (Publication II) were carried out as whole body scans on an interdialytic day using the Lunar iDXA system (GE Healthcare, UK) with the purpose of examining the body composition of the patients.

Table 1. Patients, treatment parameters and sampling times

Publication	**I**	**II**	**III**
Total number of patients	29	9	25
- from Linköping (male/female), mean age ± SD	5 (2/3), 68.8±11.4 6 (2/4), 56.8±23.0 10 (5/5), 62.8±20.9 7 (4/3), 58.1±22.5 10 (6/4), 60.1±19.1	-	10 (6/4), 60.1±19.1
- from Tallinn (male/female), mean age ± SD	10 (7/3), 62.4±11.7	9 (5/4), 58.8±8.6	15 (9/6), 59.7±11.2
Number of sessions (type)	168 (HD)	28 (9 HD/19 HDF)	52 (46 HD/ 6 HDF)
Dialysis machine	Gambro AK200 Fresenius 4008H	Fresenius 5008	Fresenius 4008H Fresenius 5008
Blood flow, ml/min	146...350	250...300	200...350
Dialysate flow, ml/min	500	500	500
Session's length, min	240...300	180...240	210...270
Sampling time, min – dialysate	2, 4, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300 5, 15, 30, 60, 90, 120, 150, 180, 210, 240, 255, 270, 300, TDC 5, 60, 90, 120, 150, 180, 210, 240, 255, 270, TDC 10, 60, 90, 120, 150, 180, 210, 240, TDC	10, end of dialysis	5, 60, 120, 180, 240, 255, 270
Sampling time, min - blood	0, 4, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240 0, 4, 10, 20, 30, 60, 120, 180, 240, 270, 300 0, 15 60, 180, 240, 270, 300 0, 15 60, 180, 240, 255, 270, 300 0, 60, 120, 180, 240, 255, 270 0, 240	Pre-and post-dialysis	0, 60, 120, 180, 240, 255, 270, 30 post-dialysis Pre- and post-dialysis, 30 post-dialysis

TDC – total dialysate collection

In vitro experiments (Publication II)

The purpose of the experiment was to investigate the influence of tissue hydration on DXA measurement results. Four mixtures of minced pork (Rakvere Meat Processing Plant, Estonia) and NaCl 0.9% solution (B.Braun, Germany) were prepared (Table 2) in a 2 l PMP beaker (LP Italiana, Italy). A digital kitchen scale (Soehnle Siena, Leifheit, Germany) was used to weigh the components. The DXA scans for each mixture were run in triplicate on the Lunar iDXA system (GE Healthcare, UK).

Table 2. Composition of the mixtures [131]

Mixture	M I	M II	M III	M IV
Minced pork, g	1200	1200	1200	1200
NaCl 0.9%, ml	-	65	135	305
Ratio tissue:fluid	100:0	95:5	90:10	80:20

Data analysis

Creatinine reduction ratio (RR) (Publication I) was calculated as

$$RR = \frac{C_{pre} - C_{post}}{C_{pre}} 100\% \quad (17)$$

based on creatinine concentration in blood (RR_b), creatinine concentration estimated by the single wavelength (SW) model (RR_SW) and the multi-wavelength model (RR_MW). In the case of RR_SW and RR_MW, the UV absorbance values of the 4, 5, and 10 min dialysate samples were used for the calculation of C_{pre}.

The urea reduction ratio (URR) (Publication I) was calculated as

$$URR = \frac{C_0 - C_T}{C_0} 100\% \quad (18)$$

based on urea concentration in the blood.

In Publication I the spKt/V was calculated according to the Daugirdas second generation equation [60] (Equation 1).

Total removed creatinine (TRCr) (Publication I) was calculated as

$$TRCr = C_{total} W \quad (19)$$

where C_{total} is creatinine concentration in the TDC sample (in µmol/l) and W is the weight of the collected spent dialysate (in kg). It was assumed that for dialysate 1 kg=1 l. For the calculation of TRCr creatinine concentration in the TDC sample (TRCr_lab), creatinine concentration estimated by the single wavelength (SW) model (TRCr_SW), and the multi-wavelength model (TRCr_MW) were used.

In Publication II LBM was calculated using the method that was developed by Desmeules et al [14] (Equations 3-7), based on creatinine concentration in

blood (LBM_{blood}) and the SW model estimate (LBM_a). In the case of LBM_a the UV absorbance values of the 10 min dialysate samples were used for the calculation of C_{pre}.

In Publication III C_{eq} was calculated using the Smye algorithm [22] (Equations 8-9) based on urea concentration in blood and dialysate (Figure 1). One intradialytic sample (60 min) as well as all available samples from 60 min were used in the estimation of λ. UV absorbance in spent dialysate samples and on-line UV absorbance measurements were also used to calculate a substitute value for C_{eq} for the urea rebound calculation (Figure 1).

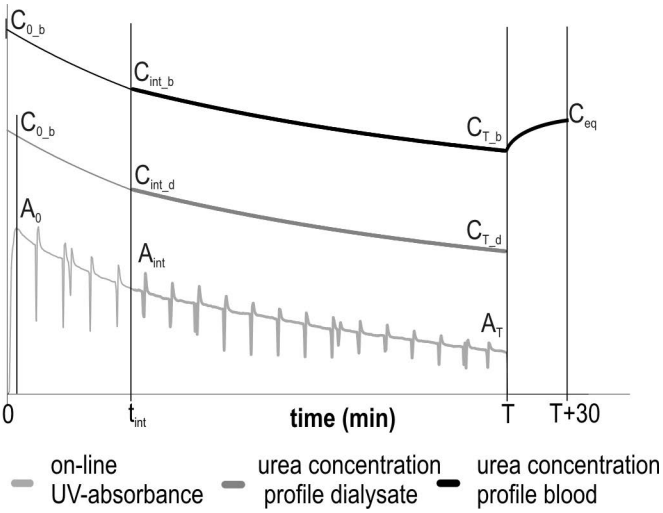

Figure 1. Schematic depiction of the blood, dialysate and UV absorbance values used for the calculation of C_{eq} [127] (with permission).

PDUR in Publication III was expressed both relative to C_T

$$R_1 = \frac{C_{eq} - C_T}{C_T} 100\% \tag{20}$$

as well as relative to the decrease in urea concentration during dialysis

$$R_2 = \frac{C_{eq} - C_T}{C_0 - C_T} 100\% \tag{21}$$

Calculations for R_1 and R_2 were based on based on urea concentration in blood and dialysate, and also UV absorbance in spent dialysate.

Forward stepwise regression was used to develop models for the assessment of creatinine concentration in dialysate (Publication I) and in blood (Publication II), and to determine the best wavelength for urea monitoring (Publication III). UV absorbance values in the collected spent dialysate samples at 210-330 nm were included in the analysis as independent variables. Regression analysis was also used to investigate the relationship between UV absorbance and the concentrations of uric acid and creatinine in spent dialysate (Publication I).

The systematic error (Publications I & III) was calculated as [138]

$$BIAS = \frac{\sum_{i=1}^{N} e_i}{N} \quad (22)$$

where N is the number of observations and e_i is the i-th residual.

The standard error of performance (Publications I & III) was calculated as [138]

$$SE = \sqrt{\frac{\sum_{i=1}^{N}(e_i - BIAS)^2}{N-1}} \quad (23)$$

The Student's t-test was used to compare the means of the calculated parameters and $p \leq 0.05$ was considered to be significant. Bland and Altman analysis [139] was used to examine individual differences in the values of the calculated parameters. Statistica 6.0 (Statsoft, Inc. for Windows) and Excel (versions 2000 and 2003 for Windows) were used for data analysis.

4.2 Optical measurement of creatinine in spent dialysate (Publication I)

Creatinine is regarded as a reference molecule for those low molecular weight water-soluble solutes that have a kinetic behaviour which is different from that of urea [44,56]. It also provides valuable information about the nutritional status of dialysis patients through the parameters CI and LBM. However, the standard biochemical methods for creatinine measurement may be affected by interference from other compounds; the necessary equipment is expensive and must be operated by professionals. Therefore, an application of the optical dialysis monitoring method would be essential in order to enable direct and easy assessment of creatinine concentration in spent dialysate. Therefore, the research for Publication I focused on developing a model for the estimation of creatinine concentration by using UV absorbance in spent dialysate.

The data of six studies (Table 1) was included in the analysis, which was split into a calibration set (covering 25% of the data), and a validation set (involving the remaining 75% of the data). The patients were adequately dialysed (spKt/V>1.2). A single wavelength (SW) model, based on UV absorbance at 299 nm, and a multi-wavelength (MW) model, based on UV absorbance at 263 nm, 299 nm and 317 nm, were developed using linear regression analysis. For the calibration set, better results were presented by the MW model than they were by the SW model. However, for the validation set the results were comparable for both models. Therefore, the SW model appears to be more stable, even though it did not provide as good a set of results as the MW model in case of the calibration set.

The most likely reason for the MW model not improving creatinine estimation accuracy is the fact that UV absorbance originating mainly from uric acid is employed in the assessment of creatinine concentration. An indication for this is the fact that UV absorbance at 299 nm is included in both of the models.

At wavelengths above 280 nm the strongest contribution arises from uric acid, as was revealed by the examination of creatinine and uric acid contributions to the measured UV absorbance. This was also indicated by an earlier HPLC study [140]. In addition, the correlation between uric acid concentration and creatinine concentration estimates by both models are higher than correlation between the estimates and creatinine concentration as determined in the laboratory. Furthermore, the correlation coefficients for the SW and MW models are in the same approximate range as the correlation coefficients for creatinine and uric acid concentrations in spent dialysate. Consequently, the correlation between creatinine and uric acid determines the accuracy of the creatinine concentration estimation through UV absorbance.

In order to assess the possibilities when it come to applying the models in dialysis monitoring, the creatinine removal ratio and the total amount of creatinine removed were calculated using creatinine concentration estimates from both models. RR_SW showed a better agreement with RRb than RR_MW. Apparently, the estimates of creatinine concentration in spent dialysate at the beginning and end of dialysis based on the MW model were not sufficiently accurate. For TRCr_SW and TRCr_MW calculation the creatinine concentration in TDC samples was estimated using both models. Both TRCr_SW and TRCr_MW were in good agreement with TRCr_lab.

The research for Publication I demonstrated that a good estimate of creatinine's removal pattern during dialysis is given by UV absorbance at 299 nm. Therefore it is possible to monitor creatinine optically even though measurements are taken for several chromophores, which are present in spent dialysate, and not solely for creatinine.

4.3 Possible application for the optical measurement of creatinine: lean body mass assessment (Publication II)

Dialysis patients have the risk of malnutrition due to several factors [64,65]. Lean body mass is one of the parameters that is recommended for the assessment of nutritional status [15]. However, the established creatinine kinetic approach is affected by shortcomings in standard biochemical methods for creatinine determination. One method that has been suggested as a quick, precise, and simple alternative is dual-energy x-ray absorptiometry [81]. However, in dialysis patients the measurements are influenced by the altered tissue hydration [16–18].Therefore the research presented in Publication II focused on examining the possibility of applying the optical monitoring method to assess LBM of dialysis patients.

An SW model was developed for the estimation of creatinine concentration in blood, which was based on UV absorbance in spent dialysate according to the results of Publication I. The estimate was used to calculate LBM by applying the method developed by Desmeules et al [14] (Equations 3-7). LBM was also

calculated based on blood creatinine concentration which was determined in the laboratory and determined by DXA measurement on an interdialytic day.

Average LBM_a was lower when compared to LBM_{blood}; however, the difference was not statistically significant ($p=0.06$). Possible reasons for the difference between the estimates include the fact that for the most part the UV absorbance of uric acid is used for the assessment of creatinine concentration and also the fact that dialysate samples were collected 10 minutes after the start of a dialysis session in contrast to the pre-dialysis blood samples, which were used to calculate LBM_{blood}.

Average LBM_{DXA} was significantly higher when compared to LBM_{blood} ($p \leq 0.05$). As the DXA scans were taken on an interdialytic day, the possible explanation for the difference between LBM_{DXA} and LBM_{blood} could be the altered fluid balance in dialysis patients. Previous studies have shown that in CAPD patients the peritoneal fluid is registered as lean mass [16] and DXA measurements overestimate LBM when compared to creatinine kinetics even when peritoneal fluid is drained from the abdomen before the measurement is carried out [17]. Moreover, in haemodialysis patients a significant decrease in LBM by DXA measurement was noted following dialysis therapy [18]. The hypothesis was additionally supported by the *in vitro* experiments that were carried out, and which showed that the amount of additional fluid in the mixture was closely followed by the shift in lean fraction mass.

The research for Publication II demonstrated the fact that the optical monitoring method may be applicable for the assessment of LBM in dialysis patients. In comparison with the reference method greater accuracy and precision were presented by LBM_a than were by LBM_{DXA}. Therefore the optical method could be preferable to DXA as it is not influenced by the patient's hydration status. The method described also offers the possibility of monitoring LBM trends in individual patients without the need for additional measurement procedures (e.g. DXA).

4.4 Urea rebound assessment (Publication III)

Urea Kt/V is considered to be a sensitive measure for the assessment of dialysis adequacy [19]. However, when immediate post-dialysis concentrations are employed in the calculation, Kt/V can be significantly overestimated due to urea rebound. Yet, waiting between 30 to 60 minutes after the completion of dialysis, which would supply a more accurate blood sample, would be inconvenient for both patients and dialysis facilities. Therefore the research for Publication III focused on examining the possibility of applying the optical monitoring method when it comes to assessing post-dialysis urea rebound in blood.

The data from two studies was divided into a calibration set (covering 30 dialysis sessions) and a validation set (covering 22 dialysis sessions). Firstly, C_{eq} was calculated according to the Smye algorithm [22] (Equations 8-9). Urea concentration in blood (C_{eq_b}) as well as in dialysate (C_{eq_d}) was employed. In

addition, a substitute value for C_{eq} was calculated by replacing urea concentrations in Equations 8 and 9 with UV absorbance values in spent dialysate samples at the wavelength 296 nm (C_{eq_a}). The slope of the urea concentration profile and UV absorbance profile was estimated using Equation 9 (i.e. for $C_{eq\_b\_60}$, $C_{eq\_d\_60}$, $C_{eq\_a\_60}$) and also obtained by line fitting (i.e. for $C_{eq\_b\_exp}$, $C_{eq\_d\_exp}$, $C_{eq\_a\_exp}$, $C_{eq\_a\_online}$). The calculated C_{eq} values were compared to urea concentration in the blood sample taken 30 minutes post-dialysis (C_{eq_30post}). Secondly, PDUR was calculated based on Equations 22 and 23, employing blood and dialysate urea concentrations as well as UV absorbance values in spent dialysate. The reason for using two PDUR estimates was the suggestion that while R_1 is a better approximation of the difference between spKt/V and eKt/V, R_2 is a better expression of the meaning of PDUR [94]. The PDUR estimates were compared to PDUR which was calculated based on C_{eq_30post}. For the validation set, in addition to reference values only $C_{eq\_a\_online}$ and corresponding PDUR estimates were calculated.

Both $C_{eq\_b\_60}$ and $C_{eq\_b\_exp}$ significantly overestimated C_{eq} ($p \leq 0.05$). Accordingly, the estimates of PDUR which were calculated based on these values also significantly overestimated PDUR when compared to the respective reference values. It has been shown previously that the actual equilibrated concentration is being slightly overestimated by the Smye algorithm [105]. Moreover, the results from Publication III indicated the algorithm's sensitivity to small errors in urea concentration measurement [101]. However, an improvement in results when more than one sample is used was demonstrated by the fact that the smallest systematic and standard error when compared to the reference was obtained for $C_{eq\_b\_exp}$. This is in compliance with the previous study showing that on-line monitoring allows those problems which are connected to the implementation of the Smye algorithm to be overcome [105].

Both $C_{eq\_d\_60}$ and $C_{eq\_d\_exp}$ significantly underestimated C_{eq} ($p \leq 0.05$). This can be explained by the fact that dialysate urea concentration is only a fraction when compared to the corresponding arterial urea concentration. Yet the result which shows that PDUR estimates which were calculated based on these values do not differ statistically from the reference values shows that blood urea concentration is proportionally followed by the respective dialysate concentration.

In addition to UV absorbance measurements in the collected spent dialysate samples, PDUR was also estimated based on on-line UV absorbance measurements in order to make full use of the possibilities offered by the optical monitoring method. There were no statistically significant differences between the PDUR estimates which were obtained and the corresponding reference values for the calibration set. The same applied to the validation set. Moreover, from all of the calculated PDUR estimates the smallest systematic and standard errors were presented by estimates which had been calculated based on UV absorbance. Therefore the assessment of PDUR by using UV absorbance in spent dialysate appears to be plausible.

The research for Publication III demonstrated that a good estimate of PDUR is given by UV absorbance at 296 nm. In comparison with the reference method, greater consistency and accuracy was presented by those estimates which were based on UV absorbance than was the case with estimates which were based on intradialytic blood samples. Additionally, the optical method offers the possibility of on-line monitoring which, due to its high sampling frequency, decreases the effect of possible measurement errors.

CONCLUSIONS

The studies presented in this thesis explored the possibilities of applying the optical dialysis monitoring method to the estimation of creatinine concentration in spent dialysate, the lean body mass of dialysis patients, and post-dialysis urea rebound. LBM monitoring would provide valuable information about the nutritional status of patients and the assessment of PDUR would offer more adequate information about treatment quality.

The results from Publications I-III can be summarised as follows:

- The removal pattern of creatinine during dialysis can be estimated using UV absorbance in spent dialysate at 299 nm.
- At wavelengths above 280 nm it is mainly uric acid which contributes to UV absorbance. Therefore the accuracy of creatinine concentration estimations depends upon the correlation between the two solutes.
- The optical dialysis monitoring method could be applicable to the assessment of LBM in dialysis patients. The advantages in the use of this technique include the fact that it is not influenced by the patient's hydration status and that it enables consistent monitoring of LBM trends.
- PDUR could be estimated by using UV absorbance in spent dialysate at 296 nm. The merits of this method include the fact that it eliminates the need to wait for the equilibrated blood sample and also that on-line monitoring would also decrease the effect of potential measurement errors.
- The optical dialysis monitoring method has the potential to allow a systematic review of LBM and PDUR efficiently and without adding any additional work load to that of medical personnel.

REFERENCES

[1] Gottschalk CW, Fellner SK. History of the science of dialysis. Am J Nephrol 1997;17:289–98. doi:10.1159/000169116.

[2] Fagette P. Hemodialysis 1912-1945: no medical technology: before its time: part I. ASAIO J 1999;45:238–49.

[3] Fagette P. Hemodialysis 1912-1945: no medical technology before its time: part II. ASAIO J 1999;45:379–91.

[4] Fresenius Medical Care. ESRD Patients in 2013. A Global Perspective. 2013.

[5] Wetmore JB, Collins AJ. Meeting the World's Need for Maintenance Dialysis. J Am Soc Nephrol 2015;26:2601–3. doi:10.1681/ASN.2015060660.

[6] Thomas B, Wulf S, Bikbov B, Perico N, Cortinovis M, Courville de Vaccaro K, et al. Maintenance Dialysis throughout the World in Years 1990 and 2010. J Am Soc Nephrol 2015;26:2621–33. doi:10.1681/ASN.2014101017.

[7] Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. Lancet 2015;385:1975–82. doi:10.1016/S0140-6736(14)61601-9.

[8] Pozzoni P, Del Vecchio L, Pontoriero G, Di Filippo S, Locatelli F. Long-term outcome in hemodialysis: morbidity and mortality. J Nephrol 2004;17 Suppl 8:S87-95.

[9] Collins AJ, Foley RN, Gilbertson DT, Chen S-C. The State of Chronic Kidney Disease, ESRD, and Morbidity and Mortality in the First Year of Dialysis. Clin J Am Soc Nephrol 2009;4 Suppl 1:S5–11. doi:10.2215/CJN.05980809.

[10] Sabatino A, Regolisti G, Karupaiah T, Sahathevan S, Sadu Singh BK, Khor BH, et al. Protein-energy wasting and nutritional supplementation in patients with end-stage renal disease on hemodialysis. Clin Nutr 2016. doi:10.1016/j.clnu.2016.06.007.

[11] Fouque D, Vennegoor M, Ter Wee P, Wanner C, Basci A, Canaud B, et al. EBPG Guideline on Nutrition. Nephrol Dial Transplant 2007;22 Suppl 2:ii45-ii87. doi:10.1093/ndt/gfm020.

[12] Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol Rev 2000;80:1107–213.

[13] Patel SS, Molnar MZ, Tayek JA, Ix JH, Noori N, Benner D, et al. Serum creatinine as a marker of muscle mass in chronic kidney disease: results of a cross-sectional study and review of literature. J Cachexia Sarcopenia Muscle 2013;4:19–29. doi:10.1007/s13539-012-0079-1.

[14] Desmeules S, Lévesque R, Jaussent I, Leray-Moragues H, Chalabi L, Canaud B. Creatinine index and lean body mass are excellent predictors of long-term survival in haemodiafiltration patients. Nephrol Dial Transplant 2004;19:1182–9. doi:10.1093/ndt/gfh016.

[15] Keshaviah PR, Nolph KD, Moore HL, Prowant B, Emerson PF, Meyer M, et al. Lean body mass estimation by creatinine kinetics. J Am Soc Nephrol 1994;4:1475–85.
[16] Bhatla B, Moore H, Emerson P, Keshaviah P, Prowant B, Nolph KD, et al. Lean body mass estimation by creatinine kinetics, bioimpedance, and dual energy x-ray absorptiometry in patients on continuous ambulatory peritoneal dialysis. ASAIO J 1995;41:M442-6.
[17] Nielsen PK, Ladefoged J, Olgaard K. Lean body mass by Dual Energy X-ray Absorptiometry (DEXA) and by urine and dialysate creatinine recovery in CAPD and pre-dialysis patients compared to normal subjects. Adv Perit Dial 1994;10:99–103.
[18] Horber FF, Thomi F, Casez JP, Fonteille J, Jaeger P. Impact of hydration status on body composition as measured by dual energy X-ray absorptiometry in normal volunteers and patients on haemodialysis. Br J Radiol 1992;65:895–900. doi:10.1259/0007-1285-65-778-895.
[19] National Kidney Foundation. KDOQI Clinical Practice Guideline for Hemodialysis Adequacy: 2015 Update. Am J Kidney Dis 2015;66:884–930. doi:10.1053/j.ajkd.2015.07.015.
[20] Depner TA. Uremic toxicity: urea and beyond. Semin Dial 2001;14:246–51.
[21] Depner T, Beck G, Daugirdas J, Kusek J, Eknoyan G. Lessons from the Hemodialysis (HEMO) Study: an improved measure of the actual hemodialysis dose. Am J Kidney Dis 1999;33:142–9.
[22] Smye SW, Evans JH, Will E, Brocklebank JT. Paediatric haemodialysis: estimation of treatment efficiency in the presence of urea rebound. Clin Phys Physiol Meas 1992;13:51–62.
[23] Tattersall JE, DeTakats D, Chamney P, Greenwood RN, Farrington K. The post-hemodialysis rebound: predicting and quantifying its effect on Kt/V. Kidney Int 1996;50:2094–102.
[24] Fridolin I, Magnusson M, Lindberg LG. On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: technique description. Int J Artif Organs 2002;25:748–61.
[25] Castellarnau A, Werner M, Günthner R, Jakob M. Real-time Kt/V determination by ultraviolet absorbance in spent dialysate: technique validation. Kidney Int 2010;78:920–5. doi:10.1038/ki.2010.216.
[26] Tortora GJ. Principles of human anatomy. 9th ed. John Wiley & Sons, Inc.; 2002.
[27] Schrier RW, Wang W, Poole B, Mitra A. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. J Clin Invest 2004;114:5–14. doi:10.1172/JCI22353.
[28] Lameire N, Van Biesen W, Vanholder R. Acute renal failure. Lancet 2005;365:417–30. doi:10.1016/S0140-6736(05)17831-3.
[29] Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute

Kidney Injury. Kidney Int Suppl 2012;2:1–138.
[30] El Nahas AM, Bello AK. Chronic kidney disease: the global challenge. Lancet 2005;365:331–40. doi:10.1016/S0140-6736(05)17789-7.
[31] Feest T. Epidemiology and causes of chronic renal failure. Medicine (Baltimore) 2007;35:438–41. doi:10.1016/j.mpmed.2007.05.006.
[32] Tomson C, Udayaraj U. Management of chronic kidney disease. Medicine (Baltimore) 2007;35:442–6. doi:10.1016/j.mpmed.2007.05.010.
[33] Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl 2013;3:1–150.
[34] ERA-EDTA. European Best Practice Guidelines for Haemodialysis I.3 When to start dialysis. Nephrol Dial Transplant 2002;17 Suppl 7:10–1. doi:10.1093/ndt/17.suppl_7.10.
[35] National Kidney Foundation. KDOQI guidelines. I Clinical practice guidelines for hemodialysis adequacy, Guideline 1. Initiation of dialysis 2006.
[36] Ott U, Steiner T, Schubert J, Wolf G. Nephrologie: Nierentransplantation. Med Klin 2007;102:219–29. doi:10.1007/s00063-007-1026-3.
[37] Neipp M, Jackobs S, Klempnauer J. Renal transplantation today. Langenbeck's Arch Surg 2009;394:1–16. doi:10.1007/s00423-008-0335-1.
[38] Ellam T, Wilkie M. Peritoneal dialysis. Medicine (Baltimore) 2007;35:466–9. doi:10.1016/j.mpmed.2007.05.011.
[39] Struijk DG. Peritoneal Dialysis in Western Countries. Kidney Dis 2015;1:157–64. doi:10.1159/000437286.
[40] Gokal R. Peritoneal dialysis in the 21st century: an analysis of current problems and future developments. J Am Soc Nephrol 2002;13 Suppl 1:S104-16.
[41] Sivalingam M, Farrington K. Haemodialysis. Medicine (Baltimore) 2007;35:461–5. doi:10.1016/j.mpmed.2007.05.005.
[42] ERA-EDTA. European Best Practice Guidelines for Haemodialysis II. 5 Dialysis schedules. Nephrol Dial Transplant 2002;17:25–31.
[43] Tanaka H, Sirich TL, Plummer NS, Weaver DS, Meyer TW. An Enlarged Profile of Uremic Solutes. PLoS One 2015;10:e0135657. doi:10.1371/journal.pone.0135657.
[44] Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, et al. Review on uremic toxins: classification, concentration, and interindividual variability. Kidney Int 2003;63:1934–43. doi:10.1046/j.1523-1755.2003.00924.x.
[45] Duranton F, Cohen G, De Smet R, Rodriguez M, Jankowski J, Vanholder R, et al. Normal and pathologic concentrations of uremic toxins. J Am Soc Nephrol 2012;23:1258–70. doi:10.1681/ASN.2011121175.

[46] Neirynck N, Vanholder R, Schepers E, Eloot S, Pletinck A, Glorieux G. An update on uremic toxins. Int Urol Nephrol 2013;45:139–50. doi:10.1007/s11255-012-0258-1.
[47] Vanholder R, De Smet R. Pathophysiologic effects of uremic retention solutes. J Am Soc Nephrol 1999;10:1815–23.
[48] Dhondt A, Vanholder R, Van Biesen W, Lameire N. The removal of uremic toxins. Kidney Int 2000;58:47–59. doi:10.1046/j.1523-1755.2000.07606.x.
[49] Duranton F, Depner TA, Argilés À. The Saga of Two Centuries of Urea: Nontoxic Toxin or Vice Versa? Semin Nephrol 2014;34:87–96. doi:10.1016/j.semnephrol.2014.02.002.
[50] Kalim S, Karumanchi SA, Thadhani RI, Berg AH. Protein carbamylation in kidney disease: pathogenesis and clinical implications. Am J Kidney Dis 2014;64:793–803. doi:10.1053/j.ajkd.2014.04.034.
[51] Velasquez MT, Ramezani A, Raj DS. Urea and protein carbamylation in ESRD: surrogate markers or partners in crime? Kidney Int 2015;87:1092–4. doi:10.1038/ki.2015.78.
[52] Verbrugge FH, Tang WHW, Hazen SL. Protein carbamylation and cardiovascular disease. Kidney Int 2015;88:474–8. doi:10.1038/ki.2015.166.
[53] Gajjala PR, Fliser D, Speer T, Jankowski V, Jankowski J. Emerging role of post-translational modifications in chronic kidney disease and cardiovascular disease. Nephrol Dial Transplant 2015;30:1814–24. doi:10.1093/ndt/gfv048.
[54] Kraus LM, Kraus AP. Carbamoylation of amino acids and proteins in uremia. Kidney Int Suppl 2001;78:S102–7. doi:10.1046/j.1523-1755.2001.59780102.x.
[55] Koeth RA, Kalantar-Zadeh K, Wang Z, Fu X, Tang WHW, Hazen SL. Protein carbamylation predicts mortality in ESRD. J Am Soc Nephrol 2013;24:853–61. doi:10.1681/ASN.2012030254.
[56] Eloot S, Torremans A, De Smet R, Marescau B, De Wachter D, De Deyn PP, et al. Kinetic behaviour of urea is different from that of other water-soluble compounds: the case of the guanidino compounds. Kidney Int 2005;67:1566–75. doi:10.1111/j.1523-1755.2005.00238.x.
[57] Eloot S, Torremans A, De Smet R, Marescau B, De Deyn PP, Verdonck P, et al. Complex compartmental behavior of small water-soluble uremic retention solutes: evaluation by direct measurements in plasma and erythrocytes. Am J Kidney Dis 2007;50:279–88. doi:10.1053/j.ajkd.2007.05.009.
[58] ERA-EDTA. European Best Practice Guidelines for Haemodialysis II . 1 Haemodialysis dose quantification : small solutes. Nephrol Dial Transplant 2002;17 Suppl 7:17–21.
[59] Depner TA. History of Dialysis Quantitation. Semin Dial 1999;12 Suppl 1:S14–9. doi:10.1046/j.1525-139X.1999.90216.x.

[60] Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. J Am Soc Nephrol 1993;4:1205–13.
[61] Leypoldt JK. Urea standard Kt/V(urea) for assessing dialysis treatment adequacy. Hemodial Int 2004;8:193–7. doi:10.1111/j.1492-7535.2004.01095.x.
[62] Schneditz D, Daugirdas JT. Compartment effects in hemodialysis. Semin Dial 2001;14:271–7. doi:sdi00066 [pii].
[63] Daugirdas JT, Schneditz D. Overestimation of hemodialysis dose depends on dialysis efficiency by regional blood flow but not by conventional two pool urea kinetic analysis. ASAIO J 1995;41:M719-24.
[64] Laville M, Fouque D. Nutritional aspects in hemodialysis. Kidney Int Suppl 2000;76:S133–9.
[65] Locatelli F, Fouque D, Heimburger O, Drüeke TB, Cannata-Andía JB, Hörl WH, et al. Nutritional status in dialysis patients: a European consensus. Nephrol Dial Transplant 2002;17:563–72.
[66] Ikizler TA. Protein and energy intake in advanced chronic kidney disease: how much is too much? Semin Dial 2007;20:5–11. doi:10.1111/j.1525-139X.2007.00231.x.
[67] Lim VS, Kopple JD. Protein metabolism in patients with chronic renal failure: role of uremia and dialysis. Kidney Int 2000;58:1–10. doi:10.1046/j.1523-1755.2000.00135.x.
[68] Ravel VA, Molnar MZ, Streja E, Kim JC, Victoroff A, Jing J, et al. Low protein nitrogen appearance as a surrogate of low dietary protein intake is associated with higher all-cause mortality in maintenance hemodialysis patients. J Nutr 2013;143:1084–92. doi:10.3945/jn.112.169722.
[69] Taylor AJ, Vadgama P. Analytical reviews in clinical biochemistry: the estimation of urea. Ann Clin Biochem 1992;29 (Pt 3):245–64.
[70] Barker SB. The direct colorimetric determination of urea in blood and urea. J Biol Chem 1944;152:453–63.
[71] Jung D, Biggs H, Erikson J, Ledyard PU. New Colorimetric Reaction for End-Point, Continuous-Flow, and Kinetic Measurement of Urea. Clin Chem 1975;21:1136–40.
[72] De Deyn PP, Vanholder R, Eloot S, Glorieux G. Guanidino compounds as uremic (neuro)toxins. Semin Dial 2009;22:340–5. doi:10.1111/j.1525-139X.2009.00577.x.
[73] Proctor DN, O'Brien PC, Atkinson EJ, Nair KS. Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. Am J Physiol 1999;277:E489-95.
[74] Gerber LM, Mann SJ. Development of a model to estimate 24-hour urinary creatinine excretion. J Clin Hypertens (Greenwich) 2014;16:367–71. doi:10.1111/jch.12294.
[75] Schutte JE, Longhurst JC, Gaffney FA, Bastian BC, Blomqvist CG. Total plasma creatinine: an accurate measure of total striated muscle mass. J

Appl Physiol 1981;51:762–6.
[76] Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem 1992;38:1933–53.
[77] Theodorou DJ, Theodorou SJ. Dual-energy X-ray absorptiometry in clinical practice: application and interpretation of scans beyond the numbers. Clin Imaging 2002;26:43–9.
[78] Albanese C V, Diessel E, Genant HK. Clinical applications of body composition measurements using DXA. J Clin Densitom 2003;6:75–85.
[79] Toombs RJ, Ducher G, Shepherd JA, De Souza MJ. The impact of recent technological advances on the trueness and precision of DXA to assess body composition. Obesity 2012;20:30–9. doi:10.1038/oby.2011.211.
[80] Plank LD. Dual-energy X-ray absorptiometry and body composition. Curr Opin Clin Nutr Metab Care 2005;8:305–9.
[81] Andreoli A, Scalzo G, Masala S, Tarantino U, Guglielmi G. Body composition assessment by dual-energy X-ray absorptiometry (DXA). Radiol Med 2009;114:286–300. doi:10.1007/s11547-009-0369-7.
[82] Pietrobelli A, Formica C, Wang Z, Heymsfield SB. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. Am J Physiol 1996;271:E941-51.
[83] Canaud B, Garred LJ, Argiles A, Flavier JL, Bouloux C, Mion C. Creatinine kinetic modelling: a simple and reliable tool for the assessment of protein nutritional status in haemodialysis patients. Nephrol Dial Transplant 1995;10:1405–10.
[84] Canaud B, Leblanc M, Garred LJ, Bosc JY, Argilés A, Mion C. Protein catabolic rate over lean body mass ratio: a more rational approach to normalize the protein catabolic rate in dialysis patients. Am J Kidney Dis 1997;30:672–9.
[85] Fesler P, Mimran A. Estimation of glomerular filtration rate: what are the pitfalls? Curr Hypertens Rep 2011;13:116–21. doi:10.1007/s11906-010-0176-5.
[86] ERA-EDTA. European Best Practice Guidelines for Haemodialysis I.1 Measurement of renal function. Nephrol Dial Transplant 2002;17 Suppl 7:7–9. doi:10.1093/ndt/17.suppl_7.7.
[87] Spencer K. Analytical reviews in clinical biochemistry: the estimation of creatinine. Ann Clin Biochem 1986;23:1–25.
[88] Peake M, Whiting M. Measurement of serum creatinine--current status and future goals. Clin Biochem Rev 2006;27:173–84.
[89] Butler AR. The Jaffé reaction. Part II. A kinetic study of the Janovsky complexes formed from creatinine(2-imino-1-methylimazolidin-4-one) and acetone. J Chem Soc, Perkin Trans 2 1975:853–7. doi:10.1039/P29750000853.
[90] Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by the Jaffe reaction. J Biol Chem 1945:581–91.

[91] Husdan H, Rapoport A. Estimation of Creatinine by the Jaffe Reaction. Clin Chem 1968;14:222–38.
[92] Weber JA, van Zanten AP. Interferences in current methods for measurements of creatinine. Clin Chem 1991;37:695–700.
[93] Artiss JD, Karcher RE, Collins SL, Zak B. Application and evaluation of a new cold-stable kinetic Jaffé reagent to the Hitachi 747 for the determination of serum creatinine. Microchem J 2000;65:277–82. doi:10.1016/S0026-265X(00)00125-9.
[94] Alloatti S, Molino A, Manes M, Bosticardo GM. Urea rebound and effectively delivered dialysis dose. Nephrol Dial Transplant 1998;13 Suppl 6:25–30.
[95] National Kidney Foundation. KDOQI Guidelines I. Clinical practice guidelines for vascular access. Guideline 4. Detection of access dysfunction: monitoring, surveillance, and diagnostic testing. http://www2.kidney.org/professionals/KDOQI/guideline_upHD_PD_VA/va_guide4.htm 2006.
[96] ERA-EDTA. European Best Practice Guidelines for Haemodialysis II. 4 Monitoring of treatment. Nephrol Dial Transplant Transplant 2002;17 Suppl 7:24–5.
[97] National Kidney Foundation. KDOQI guidelines. I Clinical practice guidelines for hemodialysis adequacy, Guideline 3. Methods for postdialyis blood sampling 2006.
[98] Descombes E, Perriard F, Fellay G. Diffusion kinetics of urea, creatinine and uric acid in blood during hemodialysis. Clinical implications. Clin Nephrol 1993;40:286–95.
[99] Schneditz D, Yang Y, Christopoulos G, Kellner J. Rate of creatinine equilibration in whole blood. Hemodial Int 2009;13:215–21. doi:10.1111/j.1542-4758.2009.00351.x.
[100] Schneditz D, Platzer D, Daugirdas JT. A diffusion-adjusted regional blood flow model to predict solute kinetics during haemodialysis. Nephrol Dial Transplant 2009;24:2218–24. doi:10.1093/ndt/gfp023.
[101] Gotch F, Keen M. Kinetic modeling in hemodialysis. In: Nissenson A, Fine R, editors. Clin. Dial., New York: McGraw-Hill; 2005, p. 153–202.
[102] Smye SW, Lindley EJ, Will EJ. Simulating the effect of exercise on urea clearance in hemodialysis. J Am Soc Nephrol 1998;9:128–32.
[103] Kong CH, Tattersall JE, Greenwood RN, Farrington K. The effect of exercise during haemodialysis on solute removal. Nephrol Dial Transplant 1999;14:2927–31.
[104] Schneditz D, Fariyike B, Osheroff R, Levin NW. Is intercompartmental urea clearance during hemodialysis a perfusion term? A comparison of two pool urea kinetic models. J Am Soc Nephrol 1995;6:1360–70.
[105] Garred LJ, Canaud B, Bosc JY, Tetta C. Urea rebound and delivered Kt/V determination with a continuous urea sensor. Nephrol Dial Transplant 1997;12:535–42. doi:10.1093/ndt/12.3.535.

[106] Smye SW, Tattersall JE, Will EJ. Modeling the postdialysis rebound: the reconciliation of current formulas. ASAIO J 1999;45:562–7.
[107] Tilley R. Colour and the optical properties of materials. John Wiley & Sons, Ltd; 2000.
[108] Welch AJ, van Gemert MJC. Optical-thermal response of laser-irradiated tissue. Springer; 1995.
[109] Uhlin F, Fridolin I. Optical monitoring of dialysis dose. In: Azar AT, editor. Model. Control Dial. Syst. Vol. 2 Biofeedback Syst. soft Comput. Tech. Dial., Springer; 2013, p. 867–928.
[110] Splinter R, Hooper BA. An Introduction to Biomedical Optics. Taylor & Francis; 2007.
[111] Schmidt W. Optical Spectroscopy in Chemistry and Life Sciences. Wiley-VCH Verlag GmbH & Co. KGaA; 2005.
[112] Fridolin I. Photon propagation in tissue and in biological fluids applied for vascular imaging and haemodialysis monitoring. Linköping University; 2003.
[113] Gál G, Gróf J. Continuous UV photometric monitoring of the efficiency of hemodialysis. Int J Artif Organs 1980;3:338–41.
[114] Falkvall T, Sandberg L-O, Fridolin I, Lindberg L-G. Method for determining waste products in the dialysis liquid in dialysis treatment. WO1999062574, 1999.
[115] Fridolin I, Magnusson M, Lindberg L-G. Measurement of solutes in dialysate using UV absorption. In: Priezzhev A V., Cote GL, editors. Proc. SPIE 4263, Opt. Diagnostics Sens. Biol. Fluids Glucose Cholest. Monit., International Society for Optics and Photonics; 2001, p. 40–7. doi:10.1117/12.429345.
[116] Fridolin I, Lindberg L-G. On-line monitoring of solutes in dialysate using wavelength-dependent absorption of ultraviolet radiation. Med Biol Eng Comput 2003;41:263–70. doi:10.1007/BF02348430.
[117] Uhlin F, Fridolin I, Lindberg L-G, Magnusson M. Estimation of delivered dialysis dose by on-line monitoring of the ultraviolet absorbance in the spent dialysate. Am J Kidney Dis 2003;41:1026–36.
[118] Uhlin F, Fridolin I, Lindberg L-G, Magnusson M. Estimating total urea removal and protein catabolic rate by monitoring UV absorbance in spent dialysate. Nephrol Dial Transplant 2005;20:2458–64. doi:10.1093/ndt/gfi026.
[119] Tomson R, Fridolin I, Uhlin F, Holmar J, Lauri K, Luman. Development of the model for the optical monitoring of urea in spent dialysate. Proc. 13th Bienn. Balt. Electron. Conf., IEEE; 2012, p. 179–82.
[120] Tomson R, Uhlin F, Holmar J, Lauri K, Luman M, Fridolin I. Development of a method for optical monitoring of creatinine in the spent dialysate. Est J Eng 2011;17:140–50. doi:10.3176/eng.2011.2.04.
[121] Tomson R, Fridolin I, Uhlin F, Holmar J, Lauri K, Luman M. Optical measurement of creatinine in spent dialysate. Clin Nephrol 2013;79:107–

17. doi:10.5414/CN107338.
[122] Jerotskaja J, Uhlin F, Fridolin I, Lauri K, Luman M, Fernström A. Optical online monitoring of uric acid removal during dialysis. Blood Purif 2010;29:69–74. doi:10.1159/000264269.
[123] Holmar J, Fridolin I, Uhlin F, Lauri K, Luman M. Optical method for cardiovascular risk marker uric acid removal assessment during dialysis. ScientificWorldJournal 2012;2012:506486. doi:10.1100/2012/506486.
[124] Arund J, Tanner R, Uhlin F, Fridolin I. Do Only Small Uremic Toxins, Chromophores, Contribute to the Online Dialysis Dose Monitoring by UV Absorbance? Toxins (Basel) 2012;4:849–61. doi:10.3390/toxins4100849.
[125] Uhlin F, Fridolin I, Magnusson M, Lindberg L-G. Dialysis dose (Kt/V) and clearance variation sensitivity using measurement of ultraviolet-absorbance (on-line), blood urea, dialysate urea and ionic dialysance. Nephrol Dial Transplant 2006;21:2225–31. doi:10.1093/ndt/gfl147.
[126] Tomson R, Uhlin F, Fridolin I. Optical urea rebound estimation during dialysis. IFMBE Proc., vol. 48, 2015. doi:10.1007/978-3-319-12967-9_30.
[127] Tomson R, Uhlin F, Fridolin I. Urea rebound assessment based on UV absorbance in spent dialysate. ASAIO J 2014;60. doi:10.1097/MAT.0000000000000091.
[128] Uhlin F, Fridolin I, Magnusson M, Lindberg L-G. Ultra violet absorbance on-line measurement utilized to monitor clinical event during haemodialysis. J Ren Care 2006;32:132–6. doi:10.1111/j.1755-6686.2006.tb00005.x.
[129] Fridolin I, Lauri K, Jerotskaja J, Luman M. Nutrition estimation of dialysis patients by on-line monitoring and kinetic modelling. Est J Eng 2008;14:177–88. doi:10.3176/eng.2008.2.07.
[130] Luman M, Jerotskaja J, Lauri K, Fridolin I. Dialysis dose and nutrition assessment by optical on-line dialysis adequacy monitor. Clin Nephrol 2009;72:303–11.
[131] Tomson R, Fridolin I, Luman M. Lean body mass assessment based on UV absorbance in spent dialysate and dual-energy x-ray absorptiometry. Int J Artif Organs 2015;38. doi:10.5301/ijao.5000415.
[132] Tomson R, Fridolin I, Luman M. Lean body mass assessment based on UV-absorbance in spent dialysate. Proc. Bienn. Balt. Electron. Conf. BEC, 2015. doi:10.1109/BEC.2014.7320587.
[133] Vasilevskii AM, Konoplev GA, Kornilov N V. Study of the absorption spectra of albumin and uric acid in the UV region. J Opt Technol 2001;68:928–30. doi:10.1364/JOT.68.000928.
[134] Olesberg JT, Arnold MA, Flanigan MJ. Online measurement of urea concentration in spent dialysate during hemodialysis. Clin Chem 2004;50:175–81. doi:10.1373/clinchem.2003.025569.
[135] Umimoto K, Kanaya Y, Kawanishi H, Kawai N. Measuring of uremic

substances in dialysate by visible ultraviolet spectroscopy. IFMBE Proc., 2009, p. 42–5. doi:10.1007/978-3-642-03885-3_12.
[136] B.Braun Avitum AG. Adimea real-time monitoring process. 2016 n.d. https://www.bbraun.com/en/products/b0/adimea.html.
[137] NIKKISO Europe GmbH. Kt/V messung. Dialysis Dose Monitor. Die verabreichte Dialysedosis messen. 2013 n.d. http://www.nikkiso-europe.eu/fileadmin/user_upload/Downloads/Produkte/Broschueren/Deutsch/ddm_deutsch_ausgabe2013-03_vers04_web.pdf.
[138] Esbensen K. Multivariate data analysis - in practice. CAMO Process AS; 2009.
[139] Bland J, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10. doi:10.1016/j.ijnurstu.2009.10.001.
[140] Lauri K, Tanner R, Jerotskaja J, Luman M, Fridolin I. HPLC study of uremic fluids related to optical dialysis adequacy monitoring. Int J Artif Organs 2010;33:96–104.

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KOKKUVÕTE

Hoolimata neeruasendusravi märkimisväärsest arengust ei ole patsientide haigestumus ja suremus sellega võrdeliselt vähenenud. Seega on edasine uurimis- ja arendustöö vajalik, et tõsta ravikvaliteeti, patsientide elukvaliteeti ja elumust.

Kuna alatoitumus on üks teguritest, mida seostatakse dialüüsipatsientide haigestumuse ja suremusega, soovitatakse regulaarselt läbi viia toitumisseisundi hindamist. Ühe sobiva parameetrina on välja toodud kreatiniinil põhjal määratav lihasmass. Kahjuks on hetkel kasutusel olevad meetodid koormavad, kuna eeldavad uriini ja heitdialüsaadi kogumist, või on mõjutatud vedelikuhulgast patsientide kudedes.

Uureal põhinev Kt/V on enimkasutatud neeruasendusravi kvaliteediparameeter. Täpse Kt/V väärtuse saamiseks on vaja arvesse võtta ka dialüüsijärgset uurea tagasilöögi efekti, kasutades arvutustes tasakaalustatud uurea kontsentratsiooni vahetult dialüüsijärgse uurea kontsentratsiooni asemel. Ent see lähenemine on koormav nii patsientidele kui ka neeruasendusravi keskustele, sest dialüüsijärgse vereproovi võtmiseks on vaja oodata kuni tund aega.

Neeruasendusravi optiline monitooringu meetod omab potentsiaali pakkuda lahendus mõlemale välja toodud probleemile.

Käesoleva töö eesmärk oli uurida neeruasendusravi optilise monitooringu meetodi rakendamise võimalusi uureal ja kreatiniinil põhinevate parameetrite hindamiseks patsiendi lihasmassi ja uurea tagasilöögi efekti näitel.

Töö esimene osa annab ülevaate neerude funktsioonidest, neerupuudulikkusest ja neeruasendusravi võimalustest.

Teine osa keskendub kahele väikese molekulmassiga ainele, uureale ja kreatiniinile, ning dialüüsijärgsele tagasilöögi efektile.

Kolmandas osas käsitletakse neeruasendusravi optilise monitooringu põhimõtteid.

Neljas osa keskendub autori eksperimentaalsele uurimistööle. Antakse ülevaade Tallinnas ja Linköpingis läbi viidud kliinilistest uuringutest. Kogutud optiliste ja biokeemiliste andmete põhjal töötati välja mudel kreatiini kontsentratsiooni hindamiseks dialüsaadis. Uuriti võimalust rakendada neeruasendusravi optilist monitooringu meetodit patsientide lihasmassi muutuste ja dialüüsijärgse uurea tagasilöögi efekti hindamiseks.

Töö peamised tulemused on:

- Kreatiniini eemaldamist protseduuri ajal on võimalik hinnata kasutades UV-kiirguse sumbuvust heitdialüsaadis lainepikkusel 299 nm.
- Neeruasendusravi optilist monitooringu meetodit on võimalik rakendada patsientide lihasmassi hindamiseks. Meetodi eelistena võib välja tuua, et mõõtetulemus ei ole mõjutatud vedelikuhulgast patsiendi kudedes, ja et see võimaldab järjepidevat lihasmassi muutuste jälgimist.

- Dialüüsijärgset tagasilöögi efekti on võimalik hinnata kasutades UV-kiirguse neelduvust heitdialüsaadis lainepikkusel 296 nm. Meetodi eelistena võib välja tuua, et sel juhul ei ole vaja oodata kuni tund aega dialüüsijärgse vereproovi võtmiseks, ja et reaalajas toimuv monitooring vähendab võimalikke mõõtevigade mõjusid.
- Neeruasendusravi optiline monitooringu meetod omab potentsiaali võimaldada efektiivset ja järjepidevat lihasmassi ning uurea tagasilöögi efekti jälgimist, meditsiinipersonalile töökoormust lisamata.

ABSTRACT

Despite the advances in the haemodialysis treatment, the trends in patient morbidity and mortality have not improved accordingly. Therefore further development is required in order to enhance treatment quality, the quality of life of patients, and their survival rates.

As protein-energy malnutrition is one the factors which is associated with morbidity and mortality in dialysis patients, regular assessment of the nutritional status is recommended. Lean body mass, a creatinine-based parameter, is suggested as being one of the suitable measures for this purpose. However, the current methods are cumbersome due to the necessity for urine and dialysate collection or they are affected by the altered tissue hydration in dialysis patients.

Urea-based Kt/V is the most used index for dialysis adequacy. In order to obtain an accurate estimate of Kt/V it is necessary to take into account the post-dialysis urea rebound and to use the equilibrated urea concentration in the calculations instead of the more convenient immediate post-dialysis urea concentration. However, this approach is cumbersome both for patients and dialysis facilities as it involves waiting up to an hour so that the post-dialysis blood sample can be taken.

The optical dialysis monitoring method has the potential to offer a solution to both of the aforementioned problems.

The aim of the thesis was to examine the possibility of using the optical dialysis monitoring method to assess urea- and creatinine-based parameters via the example of lean body mass and urea rebound calculation.

Section I summarises the functions of the kidneys, renal failure, and renal replacement therapy options.

Section II focuses on two low molecular weight water-soluble molecules, urea and creatinine, and the post-dialysis rebound of solutes.

Section III reviews the principles of optical dialysis monitoring.

Section IV is dedicated to the results of the author's experimental studies. Clinical studies are described that were carried out in Tallinn, Estonia and Linköping, Sweden. A model was developed for the estimation of creatinine concentration in dialysate based on the optical and biochemical data that was collected during the course of the studies. The possibility of monitoring lean body mass trends in individual dialysis patients by applying the optical dialysis monitoring technique was examined. Also, the feasibility of post-dialysis urea rebound assessment using the optical dialysis monitoring method was investigated.

The main results of the thesis are:

- The removal pattern of creatinine during dialysis can be estimated using UV absorbance in spent dialysate at 299 nm.
- The optical dialysis monitoring method could be applicable for the assessment of lean body mass (LBM) in dialysis patients. The advantages of this technique are that it is not influenced by the patient's

hydration status and that it permits consistent monitoring of LBM trends.

- Post-dialysis urea rebound (PDUR) could be estimated by using UV absorbance in spent dialysate at 296 nm. The merits of this method are that it eliminates the need to wait for the equilibrated blood sample and that on-line monitoring will also decrease the effect of potential measurement errors.
- The optical monitoring method has the potential to enable the systematic monitoring of LBM and PDUR efficiently and without adding any additional work load to that of medical personnel.

PUBLICATIONS

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Optical measurement of creatinine in spent dialysate

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Key words
hemodialysis monitoring – creatinine – UV-absorption

Abstract. Aim: The aim of the study was to develop an optical method for the estimation of creatinine (Cr) removal during dialysis using UV-absorbance. Material and methods: 29 hemodialysis patients on chronic 3-times-a-week hemodialysis were studied in 6 separate studies. Double-beam spectrophotometer was used for the determination of UV-absorbance in the collected spent dialysate samples. A single wavelength (SW) and a multi-wavelength (MW) model were developed using stepwise regression utilizing Cr values from the laboratory as the dependent parameter. The reduction ratio (RR) and total removed Cr (TRCr) were estimated. Results: For blood-Cr RRb (mean ± SD) was 60.9 ± 5.0% (calibration set) and 58.1 ± 6.0% (validation set), for SW UV-absorbance RR_SW was 61.5 ± 5.9% and 57.3 ± 6.0%, and for MW UV-absorbance RR_MW was 65.8 ± 5.8% and 61.7 ± 6.4%, respectively. RR_SW and RRb were not statistically different. RR_MW was higher compared to RRb ($p < 0.05$). TRCr_lab was 13.8 ± 3.8 mmol, TRCr_SW 14.5 ± 2.5 mmol and TRCr_MW 13.8 ± 2.6 mmol, being not statistically different. Conclusion: In summary, creatinine removal during dialysis can be estimated as reduction ratio and total removed creatinine with the UV-absorbance technique.

Introduction

One of the substances retained in uremic patients and eliminated from blood by hemodialysis (HD) is creatinine. It is a breakdown product of creatine phosphate in muscle cells, and the concentration in blood primarily depends on muscle mass. Creatinine is usually produced at a constant rate, removed from the plasma by glomerular filtration and excreted in the urine without any appreciable re-absorption by the tubules. Typically 7 – 10% of creatinine in the urine is derived from tubular secretion. Creatinine is widely used to assess kidney function, as it is endogenous and freely filtered at the glomerulus, and is expressed either as plasma concentration or renal clearance. Elevated levels of plasma creatinine are associated with impaired renal function. Increase in serum creatinine is the result of uremic retention, but can also be a consequence of muscle breakdown [1]. A higher level of serum creatinine has been proven to be one independent significant predictor of long-term survival in incident dialysis patients [2]. Creatinine is considered to be a non-toxic reference molecule for the removal of water-soluble low-molecular-weight uremic retention solutes whose kinetic behaviour may differ from that of urea [3, 4], the most commonly used marker in dialysis treatment.

Creatinine based parameters, creatinine index (CI) and lean body mass (LBM), have been suggested as excellent predictors of long-term survival in dialysis patients [5]. It has also been shown that LBM has an association with increased relative risk for having cardiovascular disease [6]. One of the advantages of creatinine-based indices is that they reflect somatic protein metabolism [5]. In cases of nutritional assessment it has been suggested that LBM is better suited for normalizing the protein catabolic rate (PCR) than the dry body weight, as its variability is usually much smaller than that of body weight [7]. CI has also been found to correlate strongly with PCR, which indicates a strong connection between patient muscle mass and dietary protein intake [8].

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Table 1. Number of dialysis sessions, number and age of studied patients.

Study	No of sessions	No of patients	Age, y
1	5	5	56 – 80
2	44	6	20 – 79
3	40	10	21 – 81
4	19	7	22 – 77
5	30	9	23 – 78
6	30	10	44 – 80

One of the widely accepted ways to determine creatinine is the Jaffé method, which utilizes picric acid for the Jaffé Reaction to test for creatinine [9]. Another way for the determination of creatinine is the enzymatic colourimetric method, which largely eliminates interferences known to the Jaffé method [10].

The demerits of the above mentioned methods include: 1) some compounds similar to creatinine contained in the sample of biological fluid may affect test accuracy; 2) the operation is complex, needs lots of agents that are hard to keep, and should be operated by professionals; 3) the sample must be de-protein pre-treated; and 4) the necessary equipment is expensive.

There is a need for a new method that performs quantitative concentration measurements of creatinine in biological fluid (e.g., in spent dialysate) directly and easily avoiding the disadvantages caused by the analysis in a laboratory. This would make estimating the parameter LBM suitable for an automatic and time-efficient way to review the muscle mass and protein nutritional status in HD patients.

An optical method, utilizing UV-absorbance, has been proposed for the monitoring of dialysis adequacy [11, 12]. This method has the potential to be able to monitor several compounds – uremic toxins in spent dialysate [13, 14, 15]. An earlier HPLC study has indicated that the main solute responsible for UV-absorbance in spent dialysate at 280 nm is a low-molecular-weight water-soluble non-protein-bound compound, uric acid [16]. A proposed development of the UV-method, which relies on the Beer-Lambert law and utilizes the millimolar extinction coefficients of the components in spent dialysate has been presented [17, 18]. The determination of urea, phosphate, creatinine and uric acid concentrations is described. However, the example is given for a small number of dialysis sessions, which is a serious limitation, and it cannot be applied for general use. Moreover, because of several unknown chromophores in spent dialysate the concentration measurement of creatinine and uric acid is hardly applicable using the Beer-Lambert law.

An optical method for quantitative concentration measurements of creatinine in biological fluids would give an additional value for the real-time, automatic monitoring of dialysis patients. An earlier study has indicated that the multi-wavelength UV-absorbance method may improve the measurement accuracy for optical creatinine concentration estimation in spent dialysate in terms of relative error compared to the algorithm based on the single wavelength approach [19]. This study was undertaken to further explore the effects of the multi-wavelength UV-absorbance method and the effect of the contribution of uric acid to the UV-absorbance signal on the accuracy of measurement for optical creatinine concentration estimation in spent dialysate.

The aim of the study was to develop a method suitable for the estimation of creatinine concentration during dialysis through UV-absorbance.

Subjects and methods

Subjects

After approval of the protocol by the Regional Ethics Committee, Linköping, Sweden and by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia a total of 29 HD patients on chronic 3-times-a-week HD were studied in six separate studies at the Department of Nephrology, University Hospital, Linköping (Table 1) (Studies 1 – 5) and at the Department of Dialysis and Nephrology, North Estonia Medical Center, Estonia (Table 1) (Study 6). Informed consent was obtained from all participating patients. The number of dialysis sessions, number of participating patients and the age of the patients for the studies are presented in Table 1. Treatment durations ranged from 240 to 300 min and the dialysate flow was fixed at 500 ml/min.

Table 2. Data of the studies

Study	Dialyser	Area m^2	n	Dialysis machine	Blood flow ml/min
1	GFS-16 F50 F6	1.7 1 1.3	3 1 1	AK200	250 – 300
2	F50 F6 HPS	1 1.3	22 22	AK200	146 – 350
3	AF180	1.8	40	AK200 Fresenius 4008H	230 – 300
4	AF180 Polyflux17S	1.8 1.7	7 12	AK200 Fresenius 4008H	300 – 350
5	Polyflux17L TCA150G Nephral300	1.7 1.5 1.3	18 3 9	AK200 Fresenius 4008H	200 – 350
6	F8 F10 FX80	1.8 2.2 1.8	14 3 13	Fresenius 4008H	245 – 350

The dialysers used in the studies, the effective membrane areas of the dialysers, number of sessions when the particular dialyser was used, the type of dialysis machines used and blood flows for the studies are presented in Table 2.

Sampling and laboratory analysis

For all studies samples of blood and spent dialysate were taken at discrete times for analysis (Table 3). The numbers for "Sampling time" correspond to minutes after the start of HD. The dialysate samples were taken at 255, 270 and 300 min when the duration of the session was long enough.

Total dialysate collection (TDC) was performed in Studies 3 – 5. TDC started when blood filled the dialyzer and ended when blood was returned to the patient at the end of the dialysis. All spent dialysate was collected in a tank equipped with a scale. After its weight was recorded and the collected spent dialysate was carefully stirred, a TDC sample was taken.

Blood and dialysate samples were sent to the laboratory for analysis within 2 – 4 h. Laboratory's standard sampling procedures were followed. The concentration of creatinine, uric acid and urea were determined at the Clinical Chemistry Laboratory at the Linköping University Hospital (Studies 1 – 5) and at the Clinical Chemistry Laboratory at the North Estonia Medical Centre (Study 6) using standardised methods. The two-point, fixed-time kinetic Jaffé method on an ADVIA 1800 automated analyser (Siemens, Deerfield, IL, USA) was used in Linköping to determine creatinine concentration. In Tallinn the Hitachi 912 technology was implemented that uses the kinetic potentiometric method of fixation of the end-point of the Jaffé reaction. The accuracy of the methods for the determination of creatinine, uric acid and urea in dialysate was ± 5%.

A double-beam spectrophotometer (in Linköping UVIKON 943, Konotron, Italy; in Tallinn Shimatsu UV-2401 PC, Japan) was used for the determination of UV-absorbance in the collected spent dialysate samples. Spectrophotometric analysis over a wavelength range of 190 – 380 nm was performed using an optical cuvette with an optical path length of 1 cm. Pure dialysate was tested for UV-absorbance showing an absorbance value of approximately zero. Pure dialysate was collected before the start of each dialysis session when the dialysis machine was prepared and the conductivity was stable. The collected sample was used as reference solution during the UV-scanning of spent dialysate, and placed in the reference holder of the spectrophotometer. This means that the measured absorbance expresses the absorbance of additional solutes that the dialysate

Table 3. Discrete sampling of spent dialysate

Study	Sampling time, min	
	Dialysate	Blood
1	2, 4, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240	0, 4, 10, 20, 30, 45, 60, 90,120, 150, 180, 210, 240
2	2, 4, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300	0, 4, 10, 20, 30, 60, 120, 180, 240, 270, 300
3	5, 15, 30, 60, 90, 120, 180, 240, 270, 300	0, 15, 60, 180, 240, 270, 300
4	5, 15, 30, 60, 90, 120, 180, 240, 255, 270, 300	0, 15, 60, 180, 240, 255, 270, 300
5	5, 60, 120, 180, 240, 255, 270	0, 60, 120, 180, 240, 255, 270
6	10, 60, 120, 180, 240	0, 240

contains after passing the dialyzer. Some of the measured values (absorbance or concentration) were excluded from data before analysis. The exclusion criteria were incorrect or illogical values of the measured concentration or absorption, e.g. sampling coexisting with self-tests of the dialysis machine.

Data analysis

Stepwise regression, i.e., forward stepwise, was used to obtain a single wavelength (SW) and a multi-wavelength (MW) model for the assessment of creatinine concentration through UV-absorbance. Independent variables included were UV-absorbance values at the wavelengths 210 – 330 nm. Stepwise regression was chosen because it was presumed that all assumptions were fulfilled, i.e., independent variables are linearly independent, and that creatinine concentration is related to UV-absorbance. The data was divided into a calibration set (25% of the material, i.e. 41 dialysis sessions) and a validation set (75% of the material, i.e., 123 dialysis sessions).

Systematic error was calculated for the two models as follows

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

where e_i is the residual and N is the number of observations [20].

Standard error of performance, corrected for BIAS was calculated for the two models as follows [20]

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

In order to explain the origin and behaviour of the models between UV-absorbance and creatinine concentrations in spent dialysate, a regression analysis was done for uric acid concentrations in spent dialysate in respect to creatinine and UV-absorbance in the spent dialysate.

The reduction ratio (RR) of creatinine calculated from the concentration at the start of HD (C_{start}) and concentration at the end of HD (C_{end}) is as follows

$$RR = \frac{C_{start} - C_{end}}{C_{start}} 100\% \quad (3)$$

The RR was calculated based on creatinine concentration in blood (RRb) as well as creatinine concentration in dialysate estimated by the SW model (RR_SW) and the MW model (RR_MW). In case of blood creatinine, concentrations from the sample drawn before the start of the treatment was utilized as C_{start}. In case of creatinine concentration in dialysate estimated by the SW and the MW model the values 4, 5 and 10 min after the start of HD were utilized as C_{start}. The student's t-test was used to compare means for RRb, RR_SW and RR_MW and $p \leq 0.05$ was considered significant. Individual differences in RR_SW and RR_MW compared to RRb values were also examined using Bland and Altman analysis [21].

Urea reduction ratio was calculated as follows

$$URR = \frac{C_0 - C_t}{C_0} 100\% \quad (4)$$

where C_0 and C_t are the blood urea concentrations before and at the end of the dialysis respectively, measured in mmol/l.

The single-pool Kt/V (spKt/V) was calculated according to [22] as

$$spKt/V = -\ln\left(\frac{C_t}{C_0} - 0.008T\right) + \left(4 - 3.5\frac{C_t}{C_0}\right)\frac{dBW}{BW} \quad (5)$$

where T is treatment time in hours, dBW intradialytic weight loss in kilograms and BW end-session body weight in kilograms.

The total removed creatinine (TRCr) was calculated as

$$TRCr = C_{total} W \quad (6)$$

where C_{total} is creatinine concentration in the TDC sample in μmol/l and W the collected weight in kilograms, assuming that, for dialysate, 1 kg = 1 l. The TRCr was calculated based on the creatinine concentration in the TDC sample determined by the laboratory (TRCr_lab) as well as creatinine concentration estimated by the SW model (TRCr_SW) and the MW model (TRCr_MW).

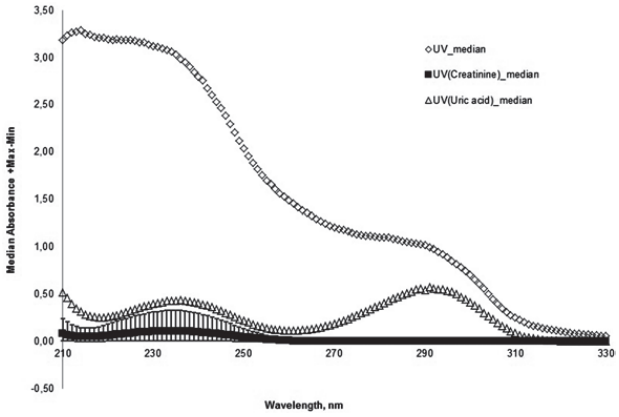

Figure 1. The median values of the measured UV-absorbance for the total material and contribution of creatinine and uric acid.

For the analysis Statistica 6.0 (Statsoft, Inc. for Windows) and Excel (version 2000 for Windows) were used.

Results

Figure 1 presents the median values of the measured UV-absorbance for the total material and contribution of creatinine and uric acid to the UV-absorbance signal at each wavelength. The contributions were calculated using millimolar extinction coefficients and concentrations obtained from the laboratory for the respective solute. Min. and max. values for creatinine are also shown, indicating that a stronger contribution arises from uric acid at all wavelengths. The major part of the total UV-absorbance is originating from other solutes than creatinine and uric acid at the wavelengths below 280 nm.

Figures 2A and 2B show the scatter plot of the creatinine concentration predicted by the SW model, based on the wavelength of 299 nm (correlation max. for creatinine), for: A) the calibration set, and B) the validation set.

Figure 3 presents the correlation coefficient r values between UV-absorbance over the wavelength range 210 – 330 nm and creatinine concentration estimated by the SW model for the calibration and validation set. It shows that the highest correlation between UV-absorbance and creatinine concentration estimated by the SW model is around 300 nm.

The final MW model included the wavelengths 263 nm, 299 nm and 317 nm. Figures 4A and 4B show the scatter plot of the creatinine concentration predicted by the MW model for: A) the calibration set, and B) the validation set.

The number of cases, the correlation coefficients, coefficients of determination, the systematic errors and standard errors of the two models for the calibration and validation set are presented in Table 4. For the calibration set the higher r value and R^2 value were obtained with the MW model compared to the SW model. For the validation set the r value and R^2 value were also somewhat higher with the MW model. BIAS was low for both models in the case of the calibration set. For the validation set the BIAS values for the SW and MW models were not statistically different ($p = 0.06$). SE was lower for the calibration set when the MW model was applied ($p < 0.05$). In case of the validation

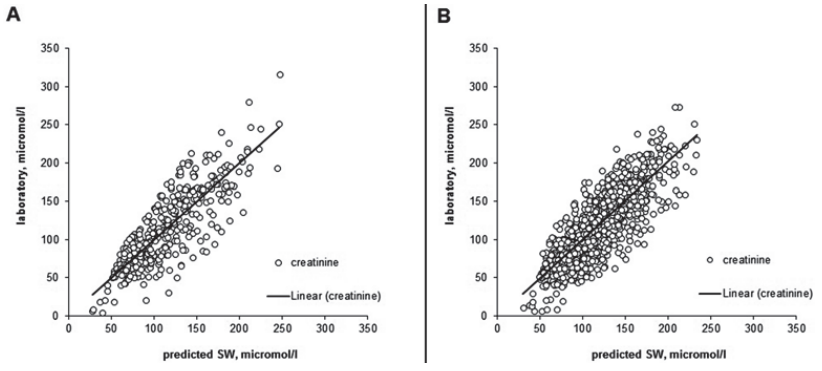

Figure 2. Creatinine concentration determined at the laboratory and the creatinine concentration predicted by the SW model: A) for the calibration set, and B) for the validation set.

set SE values were similar for both models ($p = 0.35$).

The numbers of cases, the correlation coefficients and coefficients of determination between the concentrations of uric acid and creatinine in spent dialysate obtained from the laboratory, estimated by the SW and the MW model for the calibration and validation set are presented in Table 5. Data on uric acid concentration was not available for all HD treatments; therefore, the number of cases is smaller for both sets. For both the calibration and validation set, the highest R^2 value was found for the uric acid concentration in spent dialysate and creatinine concentration estimated by the SW model.

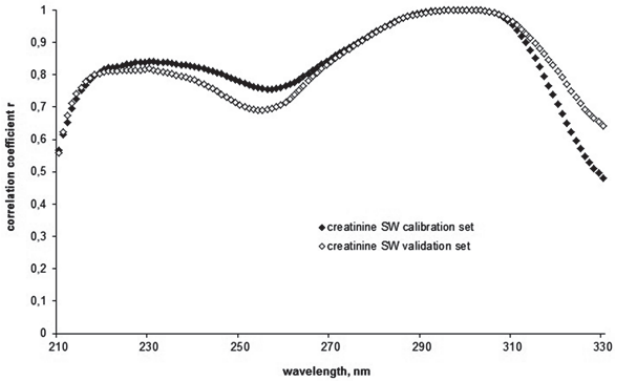

Figure 3. Correlation coefficient values between UV-absorbance and creatinine concentration estimated by the SW model for the calibration and validation set.

The average RRb of creatinine was 60.9 ± 5.0% and 58.1 ± 6.0% for the calibration and validation set, respectively. Average RR_SW was 61.5 ± 5.9% and 57.3 ± 6.0% for the calibration and validation set, respectively. Average RR_MW was 65.8 ± 5.8% and 61.7 ± 6.4% for the calibration and validation set, respectively. RR_SW and RRb were not statistically different ($p = 0.47$ and $p = 0.10$ for the calibration and validation set, respectively). RR_MW was significantly higher compared to RRb ($p \leq 0.05$) for both the calibration and validation set. Figure 5 shows the Bland-Altman plot of the differences between RRb and RR_SW for: A) the calibration set, and B) the validation set. The mean difference between RRb and RR_SW was –0.6 ± 4.6% for the calibration set and 0.8 ± 4.4% for the calibration set.

Figure 6 shows the Bland-Altman plot of the differences between RRb and RR_MW for: A) the calibration set, and B) the validation set. The mean difference between RRb

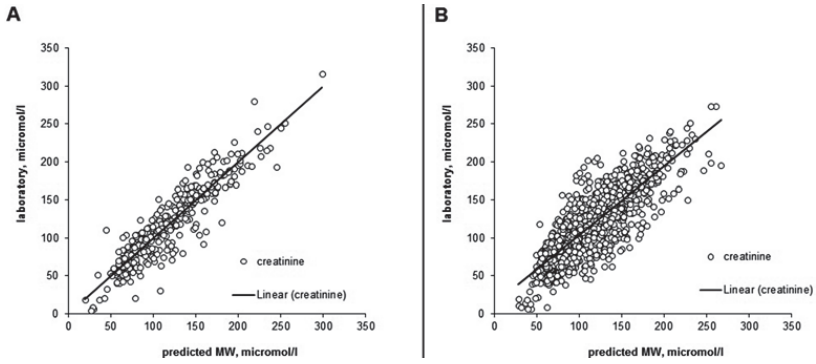

Figure 4. Creatinine concentration determined at the laboratory and the creatinine concentration predicted by the MW model: A) for the calibration set, and B) for the validation set.

Table 4. Number of cases, correlation coefficients, coefficients of determination, systematic errors and standard errors of SW and MW model for the calibration and validation set.

Model	Set	N	r	R^2	BIAS µmol/l	SE µmol/l
single wavelength	calibration	352	0.828	0.686	0.00	28.56
	validation	1,016	0.833	0.694	0.70	25.76
multi wavelength	calibration	352	0.925	0.856	0.00	19.32
	validation	1,016	0.835	0.698	2.90	25.88

Table 5. Numbers of cases, correlation coefficients and coefficients of determination between concentration of uric acid and creatinine in spent dialysate obtained from the laboratory, estimated by the SW and MW model for the calibration and validation set.

Creatinine	Set	N	r	R^2
laboratory	calibration	184	0.856	0.733
	validation	550	0.804	0.646
SW estimation	calibration	184	0.953	0.909
	validation	550	0.947	0.897
MW estimation	calibration	184	0.944	0.892
	validation	550	0.929	0.864

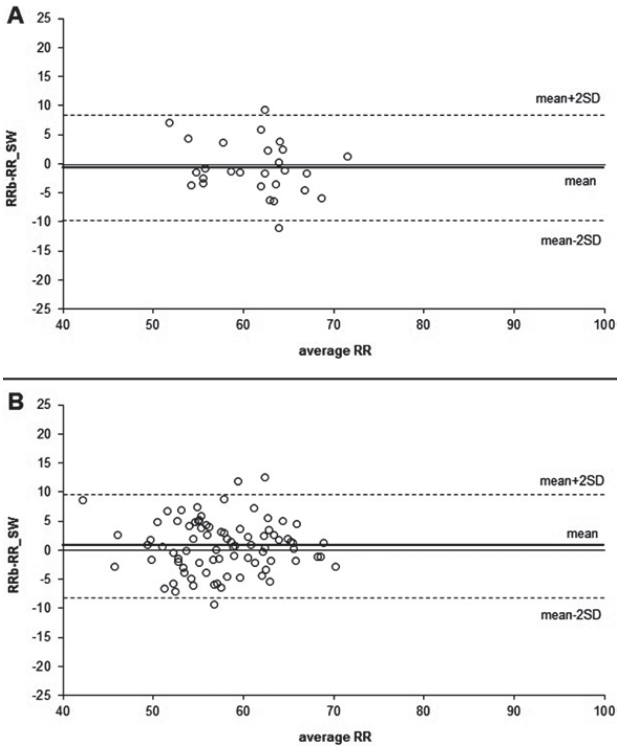

Figure 5. Bland-Altman plot of the differences between RRb and RR_SW for: A) the calibration set, and B) the validation set.

and RR_MW was –4.9 ± 4.8% for the calibration set and –3.6 ± 4.8% for the validation set.

Average URR was 71.3 ± 4.7% and 68.6 ± 5.7% for the calibration and validation set, corresponding to the spKt/V values 1.5 ± 0.2 and 1.4 ± 0.2, respectively. URR was significantly higher compared to RRb of creatinine ($p \leq 0.05$) for both the calibration and validation set.

Average TRCr_lab was 13.8 ± 3.8 mmol, TRCr_SW was 14.5 ± 2.5 mmol and TRCr_MW was 13.8 ± 2.6 mmol for studies 3-5. TRCr_SW and TRCr_MW were not statistically different from TRCr_lab ($p = 0.10$ and $p = 0.94$, respectively) (Figure 7).

Discussion

The present study investigated the effects of the single and multi-wavelength UV-absorbance method and the contribution of uric acid to the UV-absorbance signal on the measurement accuracy for optical creatinine concentration estimation in spent dialysate.

The results indicated that: (i) the MW algorithm does not significantly improve creatinine estimation accuracy compared to the SW algorithm; (ii) UV-absorbance originating from uric acid is used to assess creatinine concentration in spent dialysate; (iii) it is possible to monitor creatinine even when the technique does not solely measure creatinine but several UV chromophores in spent dialysate.

Utilizing regression, analysis a SW and a MW model for the assessment of creatinine concentration through UV-absorbance was obtained. In the calibration set, a higher R^2 value was obtained. In the case of the MW model for the validation set, R^2 was only slightly higher for the MW model than for the SW model (Table 4). SE was lower when the MW model was applied in the case of the calibration set ($p < 0.05$); however, for the validation set, the SE values were similar for both models ($p = 0.35$). Also, BIAS was not statistically different when the MW model was applied to the validation set ($p = 0.06$). Thus, although the MW model presented better results in the case of the calibration set, the validation set's outcome did not significantly differ from the results presented by the SW model.

As the strongest contribution to UV-absorbance arises from uric acid at wavelengths above 280 nm (Figure 1), the correlation between creatinine concentration in spent dialysate and creatinine concentration estimated by a model utilizing one or several wavelengths depends on the correlation between uric acid and creatinine. This can be seen by the fact that the R^2 value for the concentration of uric acid and creatinine in spent dialysate (Table 5) is approximately in the

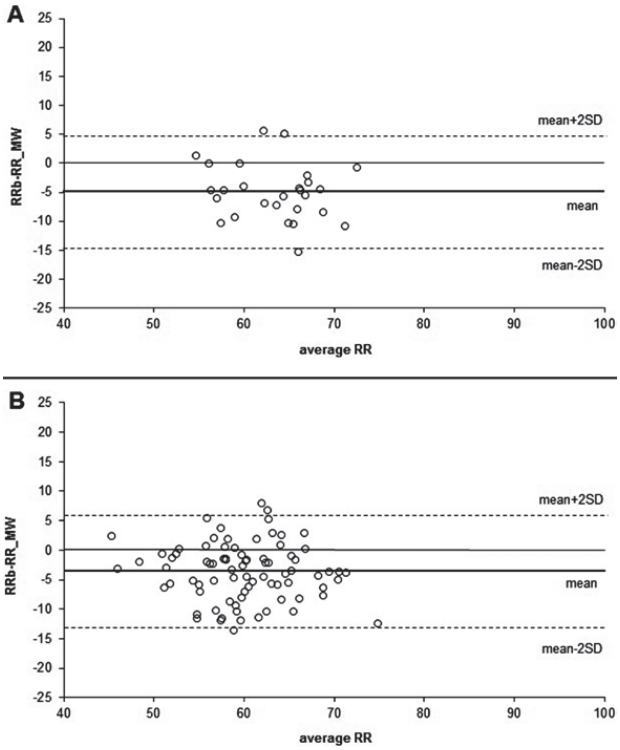

Figure 6. Bland-Altman plot of the differences between RRb and RR_MW for: A) the calibration set, and B) the validation set.

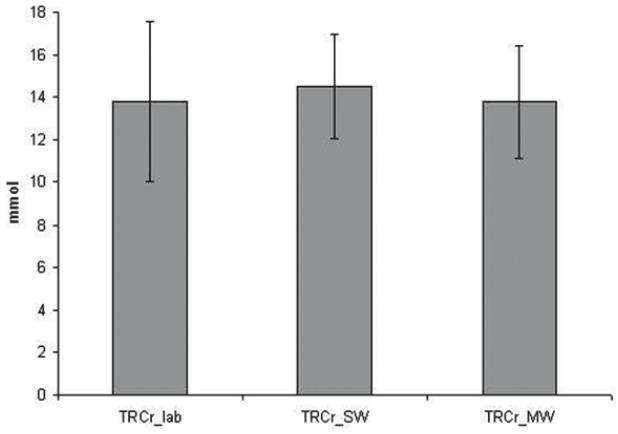

Figure 7. Mean values of TRCr_lab, TRCr_SW and TRCr_MW for Studies 3 – 5.

same range as R^2 values for the SW and MW model estimating creatinine concentration (Table 4). It appears that the UV-absorbance originating from uric acid is used to assess creatinine concentration. This is indicated by the fact that both the SW and the MW model included UV-absorbance at wavelength 299 nm. It is also explained by the outcome that the R^2 values between uric acid and creatinine concentration estimated by the SW and MW models are higher than the R^2 values of the models for creatinine concentration obtained from the laboratory (Table 5). This is the most likely reason MW algorithms do not significantly improve creatinine estimation accuracy. The previous hypothesis is supported by the fact that the highest correlation between UV-absorbance and creatinine concentration estimated by the SW model is around 300 nm (Figure 3), which coincides with the region where the strongest contribution to UV-absorbance arises from uric acid.

A comparison between creatinine and urea kinetics by utilising the parameters RRb (for creatinine) and URR (for urea) was made. URR was significantly higher compared to RRb of creatinine ($p \leq 0.05$) for both the calibration and validation set. Moreover, linear regression indicated a rather similar but still somewhat different removal pattern for creatinine compared to urea ($R^2 = 0.7107$ for calibration group and $R^2 = 0.6484$ for validation group). This motivates individual creatinine monitoring during dialysis. The corresponding spKt/V values (> 1.2) showed that the patients were adequately dialyzed.

RRb and RR_SW show a better agreement than RRb and RR_MW (Figure 5, 6). It appears that the MW model that was developed based on creatinine concentrations at different time points during dialysis is not able to estimate creatinine concentration at the start and end of the dialysis accurately. The accuracy of RR_SW would be further improved if creatinine concentration values in dialysate 4 – 5 min after the start of the dialysis session were solely utilized as C_{start}, as the difference between RR_SW calculated based on 10-min values and RRb increases. The reason for this is due to the larger time difference between taking the blood and the dialysate sample. This is confirmed by the outcome that the mean difference between RR_SW was calculated based on 4 – 5-min values and RRb was –0.6 ± 4.6% and 0.5 ± 4.2% for the calibration and validation set, respectively, whereas the mean difference between RR_SW calculated based on

10-min values and RRb was 2.9 ± 5.1% and 3.8 ± 4.7% for the calibration and validation set, respectively.

The difference between the RR obtained utilizing blood creatinine and the RR calculated based on creatinine concentration in dialysate estimated through UV-absorbance could be explained by the specific removal characteristics of creatinine compared to the net removal of the UV-absorbing chromophores contributing to the optically measured signal in spent dialysate. Despite the fact that the online UV-absorbance at 280 nm was closest to the removal of small water-soluble non-protein bound solutes like urea, creatinine and uric acid [16, 23], the overall removal pattern is far more complex because each uremic solute still has a distinctive distribution volume in the body and removal rate during dialysis. However, the study demonstrates that UV-absorbance at the wavelengths included in the models gives a good estimate of the removal pattern of the small water-soluble non-protein-bound solute creatinine during dialysis. This makes it possible to monitor creatinine even when the technique does not measure solely creatinine but several UV chromophores in spent dialysate.

To explore the possibility of assessing the total amount of creatinine removed during dialysis the SW and MW model were applied to estimate the creatinine concentration in the TDC samples collected in Studies 3 – 5. The estimates were used to calculate TRCr_SW and TRCr_MW. Both TRCr_SW and TRCr_MW show a good agreement with TRCr_lab (Figure 7). The results show that it is possible to estimate the total removed creatinine using UV-absorbance, which together with the relative estimate including creatinine concentration in the beginning and after the dialysis treatment offer a possibility to non-invasively evaluate CI and LBM for dialysis patients.

Patient-dependent parameters were not taken into account in the model build-up. However, in a previous study the following two parameters: “weight after dialysis” and “diabetic status of the patient”, were incorporated in the final models for the estimation of creatinine concentration [24]. Urea clearance of the dialyzer, time, when the dialysate sample was taken, the patients systolic and diastolic blood pressure after dialysis were also considered as parameters, but not included in the final models. The fact that the parameter weight after dialysis was included in the final models indicates that the relationship between creatinine and UV-absorbance is related to the patients’ muscle mass where creatinine is actually produced. This influence seems to be more complex than can be predicted by stepwise regression analysis assuming a linear relationship between independent and dependent parameters. The diabetic status of the patients also played a significant role that probably points out a specific metabolic behavior affecting the correlation between UV-absorbance and creatinine concentration in spent dialysate. One possible cause for this may be the altered tissue blood flow, tissue oxygenation and metabolic rate that hemodialysis only brings about in patients with diabetes [25]. Therefore, adding patient-dependent parameters to the material may improve the correlation between creatinine concentration in spent dialysate and UV-absorbance.

In summary, although in the calibration set, the MW model presented better results than the SW model, in the case of the validation set, the outcomes did not differ significantly. It appears that while the SW model did not give as good results for the calibration set as the MW model, it is more stable when used to estimate creatinine concentration in case of the validation set. One of the reasons for this could be that UV-absorbance originating from uric acid is used to assess creatinine concentration in spent dialysate. Further exploration of the effect of patient-dependent parameters on the measurement accuracy for optical creatinine concentration estimation in spent dialysate will be an issue of further studies. Also, the optical technique of estimating creatinine concentration in spent dialysate from on-line UV-absorbance measurements presented in this paper makes it possible to assess post dialysis rebound without the need for blood samples. Algorithms for anticipating post dialysis rebound of solutes have been previously developed [26, 27]. However, this issue is too comprehensive for this paper and will be explored in further studies, like the possibility to estimate CI and LBM for dialysis patients.

Conclusion

The results show that it is possible to estimate the creatinine concentration removed during dialysis using UV-absorbance. The merits of the described method are that it does not need blood samples, disposables or chemicals, and is fast. In the future the calculation of the dialysis dose and nutrition parameters like LBM will be investigated further and in relation to other parameters.

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References

[1] *Yavuz A, Tetta C, Ersoy FF, D'intini V, Ratanarat R, De Cal M, Bonello M, Bordoni V, Salvatori G, Andrikos E, Yakupoglu G, Levin NW, Ronco C.* Uremic toxins: a new focus on an old subject. Semin Dial. 2005; *18:* 203-211. doi:10.1111/j.1525-139X.2005.18313.x PubMed

[2] *Basile C, Vernaglione L, Lomonte C, Bellizzi V, Libutti P, Teutonico A, Di Iorio B.* Comparison of alternative methods for scaling dialysis dose. Nephrol Dial Transplant. 2010; *25:* 1232-1239. doi:10.1093/ndt/gfp603 PubMed

[3] *Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jörres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, et al; European Uremic Toxin Work Group (EUTox).* Review on uremic toxins: classification, concentration, and interindividual variability. Kidney Int. 2003; *63:* 1934-1943. doi:10.1046/j.1523-1755.2003.00924.x PubMed

[4] *Eloot S, Torremans A, De Smet R, Marescau B, De Wachter D, De Deyn PP, Lameire N, Verdonck P, Vanholder R.* Kinetic behavior of urea is different from that of other water-soluble compounds: the case of the guanidino compounds. Kidney Int. 2005; *67:* 1566-1575. doi:10.1111/j.1523-1755.2005.00238.x PubMed

[5] *Desmeules S, Lévesque R, Jaussent I, Leray-Moragues H, Chalabi L, Canaud B.* Creatinine index and lean body mass are excellent predictors of long-term survival in haemodiafiltration patients. Nephrol Dial Transplant. 2004; *19:* 1182-1189. doi:10.1093/ndt/gfh016 PubMed

[6] *Terrier N, Senécal L, Dupuy AM, Jaussent I, Delcourt C, Leray H, Rafaelsen S, Bosc JY, Maurice F, Canaud B, Cristol JP.* Association between novel indices of malnutrition-inflammation complex syndrome and cardiovascular disease in hemodialysis patients. Hemodial Int. 2005; *9:* 159-168. doi:10.1111/j.1492-7535.2005.01127.x PubMed

[7] *Canaud B, Leblanc M, Garred LJ, Bosc JY, Argilés A, Mion C.* Protein catabolic rate over lean body mass ratio: a more rational approach to normalize the protein catabolic rate in dialysis patients. Am J Kidney Dis. 1997; *30:* 672-679. doi:10.1016/S0272-6386(97)90492-3 PubMed

[8] *Canaud B, Garred LJ, Argiles A, Flavier JL, Bouloux C, Mion C.* Creatinine kinetic modelling: a simple and reliable tool for the assessment of protein nutritional status in haemodialysis patients. Nephrol Dial Transplant. 1995; *10:* 1405-1410. PubMed

[9] *Artiss JD, Karcher RE, Collins SL, Zak B.* Application and evaluation of a new cold-stable kinetic Jaffe reagent to the Hitachi 747 for the determination of serum creatinine. Microchem J. 2000; *65:* 277-282. doi:10.1016/S0026-265X(00)00125-9

[10] *Perrone RD, Madias NE, Levey AS.* Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem. 1992; *38:* 1933-1953. PubMed

[11] *Fridolin I, Magnusson M, Lindberg L-G.* On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: technique description. Int J Artif Organs. 2002; *25:* 748-761. PubMed

[12] *Castellarnau A, Werner M, Günthner R, Jakob M.* Real-time Kt/V determination by ultraviolet absorbance in spent dialysate: technique validation. Kidney Int. 2010; *78:* 920-925. doi:10.1038/ki.2010.216 PubMed

[13] *Fridolin I, Lindberg L-G.* On-line monitoring of solutes in dialysate using wavelength-dependent absorption of ultraviolet radiation. Med Biol Eng Comput. 2003; *41:* 263-270. doi:10.1007/BF02348430 PubMed

[14] *Jerotskaja J, Lauri K, Tanner R, Luman M, Fridolin I.* Optical dialysis adequacy sensor: wavelength dependence of the ultra violet absorbance in the spent dialysate to the removed solutes. 29th Annual International Conference of the IEEE EMBS, Lyon: EMBS; 2007.

[15] *Umimoto K, Kanaya Y, Kavanishi H, Kawai N.* Measuring of uremic substances in dialysate by visible ultraviolet spectroscopy. World Congress on Medical Physics and Biomedical Engineering 2009, Munich: Springer; 2009.

[16] *Lauri K, Tanner R, Jerotskaja J, Luman M, Fridolin I.* HPLC study of uremic fluids related to optical dialysis adequacy monitoring. Int J Artif Organs. 2010; *33:* 96-104. PubMed

[17] *Vasilevskij AM, Knopolev GA.* Optiko-eletronnaya informatsionno-izmeritelnaya sistema spektralnogo analiza sostava polikompnentnyh sred po dominiruyushchej komponente. Upravlenie v meditsine i biologii. 2003: 40-46.

[18] *Vasilevskij AM.* Informatsionno-izmeritelnaya sistema monitoringa seansa gemodializa po spektram ekstinkzij v uf-oblasti spectra. Upravlenie v meditsine i biologii. 2006: 48-54.

[19] *Fridolin I, Jerotskaja J, Lauri K, Uhlin F, Luman M.* A new optical method for measuring creatinine concentration during dialysis. 12th Mediterranean Conference on Medical and Biological Engineering and Computing, MEDICON 2010, Chalkidiki: Springer; 2010.

[20] *Esbensen KH.* Multivariate data analysis – in practice. CAMO Process AS; 2009.

[21] *Bland JM, Altman DG.* Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986; *1:* 307-310. doi:10.1016/S0140-6736(86)90837-8 PubMed

[22] *European Best Practice Guidelines for Haemodialysis, European Renal Association – European Dialysis and Transplant Association (ERA-EDTA). Section II.* Haemodialysis adequacy. Nephrol Dial Transplant. 2002; *17 (Suppl 7):* 16-31.

[23] *Lauri K, Arund J, Tanner R, Jerotskaja J, Luman M, Fridolin I.* Behaviour of uremic toxins and UV absorbance in respect to low and high flux dialyzers. Estonian J Eng. 2010; *16:* 95-106. doi:10.3176/eng.2010.1.09

[24] *Tomson R, Fridolin I, Uhlin F, Jerotskaja J, Lauri K, Luman M.* Development of the model for the optical multiwavelength monitoring of creatinine in the spent dialysate. 12th Biennial Baltic Electronics Conference, BEC 2010, Tallinn.

[25] *De Blasi RA, Luciani R, Punzo G, Arcioni R, Romano R, Boezi M, Menè P.* Microcirculatory changes and skeletal muscle oxygenation measured at rest by non-infrared spectroscopy in patients with and without diabetes undergoing haemodialysis. Crit Care. 2009; *13 (Suppl 5):* S9. doi:10.1186/cc8007 PubMed

[26] *Smye SW, Evans JH, Will E, Brocklebank JT.* Paediatric haemodialysis: estimation of treatment efficiency in the presence of urea rebound. Clin Phys Physiol Meas. 1992; *13:* 51-62. doi:10.1088/0143-0815/13/1/005 PubMed

[27] *Daugirdas JT, Schneditz D.* Overestimation of hemodialysis dose depends on dialysis efficiency by regional blood flow but not by conventional two pool urea kinetic analysis. ASAIO J. 1995; *41:* M719-M724. doi:10.1097/00002480-199507000-00107 PubMed

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ORIGINAL ARTICLE

Lean body mass assessment based on UV absorbance in spent dialysate and dual-energy x-ray absorptiometry

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ABSTRACT

Purpose: The aim of the study was to explore the possibility of assessing lean body mass (LBM) based on UV absorbance measurements in spent dialysate.

Methods: 9 patients on chronic three-times-a-week HD (4 female, 5 male, mean age 58.8 ± 8.6 years) were studied. Blood and spent dialysate samples were collected for 3 consecutive hemodialysis (HD) sessions from every patient. A double-beam spectrophotometer was used for the determination of UV absorbance in the collected spent dialysate samples. Dual-energy x-ray absorptiometry (DXA) scans were performed on an interdialytic day. LBM was calculated based on creatinine concentration in blood (LBM_{blood}) and UV absorbance in spent dialysate (LBM_a) and assessed by DXA (LBM_{DXA}). Also, *in vitro* experiments were carried out to investigate the effect of tissue hydration on DXA measurements.

Results: Although LBM_a was slightly lower compared to LBM_{blood}, the estimates based on UV absorbance in spent dialysate presented greater accuracy and precision compared to LBM_{DXA}. The significant difference between LBM_{blood} and LBM_{DXA} was with high probability caused by the altered tissue hydration of HD patents.

Conclusions: In summary, the results show that it is possible to asses LBM based on UV absorbance in spent dialysate.

Keywords: Creatinine, Dual-energy x-ray absorptiometry, Hemodialysis monitoring, Lean body mass, UV-absorption

Introduction

Hemodialysis patients are at risk of malnutrition due to several factors (1, 2). A number of tools have been recommended for diagnosing malnutrition, including measuring serum albumin, serum prealbumin and normalized protein nitrogen appearance (nPNA), body mass index (BMI) and dietary assessment (3). It has been suggested that since creatinine index (CI) and lean body mass (LBM) reflect somatic proteins these indices should be also included in the panel (4). LBM is expected to be a stable and reliable measure of nutritional status (5).

Body composition can be assessed by a number of methods, e.g. underwater weighing, air-displacement plethysmography, dilution techniques, whole body counting and neutron activation analysis (6), which also have shortcomings being costly, time-consuming and cumbersome. Dual-energy x-ray absorptiometry (DXA) has been proposed as a simple, quick and precise method for determining body composition (7). However, it has been shown that in dialysis patients LBM estimated by DXA measurements is influenced by the altered tissue hydration (8-10). Also, bioelectrical impedance analysis (6) is a frequently used method for body composition analysis. Its advantages are ease of operation and portability. However, it has been shown that in dialysis patients this method is also influenced by the excess body water (8).

The creatinine kinetic approach to LBM estimation is based on the measurement of creatinine excretion (5). As creatinine excretion is dependent upon the patient's muscle mass, the advantage of creatinine kinetics is that LBM is calculated as if hydration status was normal relative to muscle mass (8). However, as the traditional creatinine kinetic approach requires collecting blood and dialysate samples, a method that enables the evaluation of LBM and CI non-invasively would be advantageous.

This study was undertaken to explore the possibility of assessing LBM based on UV absorbance measurements in spent dialysate. The optical method utilising UV absorbance for the monitoring of dialysis adequacy has been proposed earlier (11) and it has the potential to be able to monitor several uremic toxins in spent dialysate (12, 13). An application of this method

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that performs quantitative concentration measurements of creatinine in biological fluid and estimates LBM would enable reviewing muscle mass and protein nutritional status in HD patients in an automatic and time-efficient manner.

The aim of the study was to examine if LBM can be assessed by utilizing the UV absorbance method and also to validate the accuracy of the proposed method and of the DXA measurements compared to the blood-based method.

Methods

Subjects

After approval of the protocol by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia, 9 patients during 27 dialysis sessions were studied at the Center of Nephrology, North Estonian Medical Center. An informed consent was obtained from all participating patients.

Three patients on chronic 3-times-a-week HD, 2 female and 1 male, were followed during a total of 9 HD sessions. A low-flux membrane (FX10, Fresenius Medical Care, Bad Homburg, Germany), with an effective membrane area of 1.8 m^2, was used. Six patients on chronic 3-times-a-week HDF, 2 female and 4 male, were followed during a total of 19 HDF sessions. A high-flux membrane (FX80, Fresenius Medical Care, Germany), with an effective membrane area of 1.8 m^2, was used. The mean age of the patients was 58.8 ± 8.6 years. Treatment durations ranged from 180 to 240 min. The dialysate flow was fixed at 500 ml/min and the blood flow varied between 250 to 300 ml/min. The type of dialysis machine used was Fresenius 5008.

In order to examine the body composition of the patients DXA measurements as whole body scans were performed on an interdialytic day utilizing the Lunar iDXA system (GE Healthcare, Hatfield, UK).

Sampling and laboratory analysis

Blood samples were taken before the start of the dialysis and at the end of the treatment. Spent dialysate samples of were taken 10 min after the start of the dialysis and at the end of the treatment. Blood and dialysate samples were sent to the laboratory for analysis within 2 to 4 h, following standard procedures. The concentration of creatinine was determined at the Clinical Chemistry Laboratory at the North Estonia Medical Center using standardized methods. The accuracy of the methods for the determination of creatinine was ±5%.

A double-beam spectrophotometer (Shimadzu UV-2401 PC, Shimadzu, Kyoto, Japan) was used for the determination of UV absorbance in the collected spent dialysate samples. Spectrophotometric analysis over a wavelength range of 190 nm to 380 nm was performed using an optical cuvette with an optical path length of 5 mm. Pure dialysate was tested for UV absorbance showing an absorbance value of approximately zero. Pure dialysate was collected before the start of each dialysis treatment when the dialysis machine was prepared and the conductivity was stable. The collected sample was used as reference solution during the UV scanning of spent dialysate, and placed in the reference holder of the spectrophotometer. This way of measuring the absorbance of a spent dialysate sample expresses the absorbance of additional solutes from the blood that the dialysate contains after passing the dialyzer when the patient is connected to the dialysis machine. Some of the measured values (absorbance or concentration) were excluded from data before analysis. The exclusion criteria were incorrect or illogical values of the measured concentration of absorption, e.g. sampling coexisting with self-tests of the dialysis machine.

Data analysis

In order to estimate creatinine concentration in blood through UV absorbance in spent dialysate, regression analysis was performed to obtain a single wavelength (SW) model as published earlier (12).

LBM was calculated according to the method developed by (4)

$$LBM(kg) = 0.029 \times Cl \times BW_{post} + 7.38 \quad \text{[Eq. 1]}$$

$$Cl(mg/kg/day) = 162.7 \times G_{Cr}/BW_{post} + 0.00429 \times TAC_{Cr}lm \quad \text{[Eq. 2]}$$

$$G_{Cr}male = 0.8 + (BW_{pre} \times C_{pre} - BW_{post} \times C_{post}) \times (1 - R_{Cr}) / \{-\ln(R_{Cr}) \times BMI \times 152\} \quad \text{[Eq. 3a]}$$

$$G_{Cr}female = 0.8 + (BW_{pre} \times C_{pre} - BW_{post} \times C_{post}) \times (1 - R_{Cr}) / \{-\ln(R_{Cr}) \times BMI \times 172.7\} \quad \text{[Eq. 3b]}$$

$$TAC_{Cr}lm = C_{pre} \times (1 - R_{Cr}) / -\ln(R_{Cr}) \quad \text{[Eq. 4]}$$

$$R_{Cr} = C_{pre}/C_{post} \quad \text{[Eq. 5]}$$

where C_{pre} and C_{post} are the creatinine concentration in the blood in µmol/l before and at the end of the dialysis, respectively; BW_{pre} and BW_{post} are body weight in kilograms before and after the treatment, respectively; G_{Cr} is creatinine generation rate in mg/day; and TAC_{Cr} lm is the logarithmic mean-based, time-averaged creatinine concentration in µmol/l. Body mass index (BMI), in kg/m^2, was calculated from BW_{post} and height. LBM was calculated based on creatinine concentration in blood determined by the laboratory (LBM_{blood}) as well as the creatinine concentration estimated by the SW model (LBM_a).

Student's *t* test for dependent samples was used to compare means for LBM_{blood}, LBM_a and LBM_{DXA}, and $p \leq .05$ was considered significant. Individual differences in LBM_a and LBM_{DXA} compared to LBM_{blood} were also examined using Bland and Altman analysis (14).

For the analysis Excel 2003 (Microsoft, USA) was used.

In vitro experiments

To investigate the effect of tissue hydration on DXA measurements *in vitro* experiments were carried out. Minced pork (Rakvere Meat Processing Plant, Rakvere, Estonia), fat 28 g and protein 16 g per 100 g raw or unprocessed meat, was obtained from a local supermarket. NaCl 0.9% solution (B. Braun, Melsungen, Germany) was used as the excess fluid. Four mixtures with a different amount of added fluid (Tab. I) were prepared. The components were weighted on a Soehnle Siena digital kitchen scale (Leifheit, Nassau, Germany), thoroughly mixed and scanned in a 2-L PMP beaker (LP Italiana, Milan, Italy), weight 169 g. All DXA scans were run in triplicate and the results were averaged.

Results

Average LBM_{blood} was 44.6 ± 8.3 kg, LBM_a was 41.6 ± 7.9 kg and LBM_{DXA} was 53.2 ± 9.4 kg (Fig. 1). LBM_a was not statistically different from LBM_{blood} ($p = .06$), whereas LBM_{DXA} was significantly higher compared to LBM_{blood} ($p<.05$). The overall reproducibility of LBM_{blood} was investigated by calculating the mean value of the 3 dialysis sessions for every patient and comparing the individual LBM_{blood} values to it. The mean variability was below 7%.

Figure 2 shows the Bland-Altman plot of the differences between LBM_{blood} and LBM_a. The mean difference between LBM_{blood} and LBM_a was 3.0 ± 8.0 kg.

Figure 3 shows the Bland-Altman plot of the differences between LBM_{blood} and LBM_{DXA}. The mean difference between LBM_{blood} and LBM_{DXA} was -8.6 ± 9.6 kg.

The results of the *in vitro* experiments are presented in Figure 4. The results are presented as mean ± SD. However, as SD is small compared to the mean, it is not visible on the figure. The tissue mass determined by DXA followed closely the phantom mass by scale. The fat fraction mass measured by DXA ranged from 477 ± 4 g (III mixture) to 487 ± 12 g (II mixture). The lean fraction mass measured by DXA increased from 887 ± 1 g in the I mixture to 1192 ± 2 g in the IV mixture (Fig. 4A). The change in the lean fraction mass followed closely the amount of NaCl 0.9% solution added to the mixture (Fig. 4B).

Discussion

The present study investigated the possibility of assessing LBM by utilizing the UV absorbance method and the method's accuracy compared to the blood-based method and DXA measurement.

The results indicate that: (i) it is possible to assess LBM in hemodialysis patients based on creatinine concentration estimated by the SW UV model; (ii) in comparison to the blood

TABLE I - Composition of the mixtures

Mixture	I	II	III	IV
Minced pork, g	1200	1200	1200	1200
NaCl 0.9%, mL	-	65	135	305
Ratio tissue: fluid	100:0	95:5	90:10	80:20

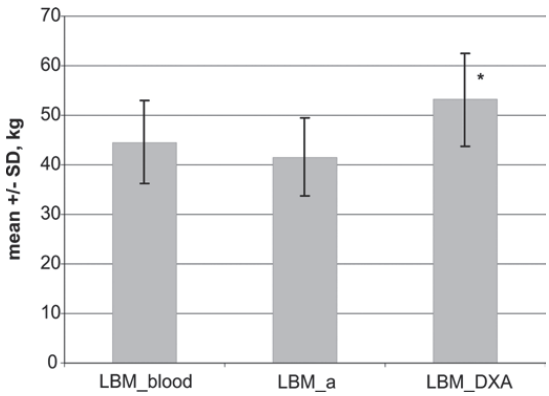

Fig. 1 - Average values and standard deviations (mean ± SD) of LBM_{blood}, LBM_a, LBM_{DXA} (N = 27).

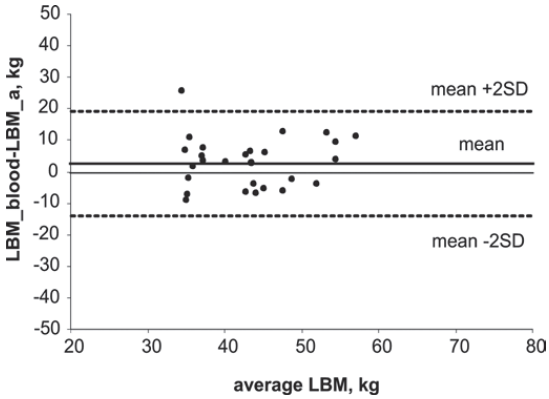

Fig. 2 - Bland-Altman plot of the differences between LBM_{blood} and LBM_a (N = 27).

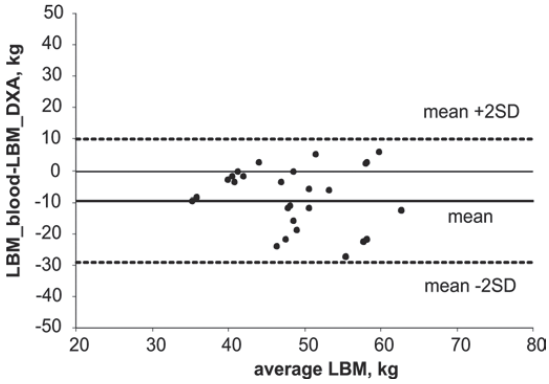

Fig. 3 - Bland-Altman plot of the differences between LBM_{blood} and LBM_{DXA} (N = 27).

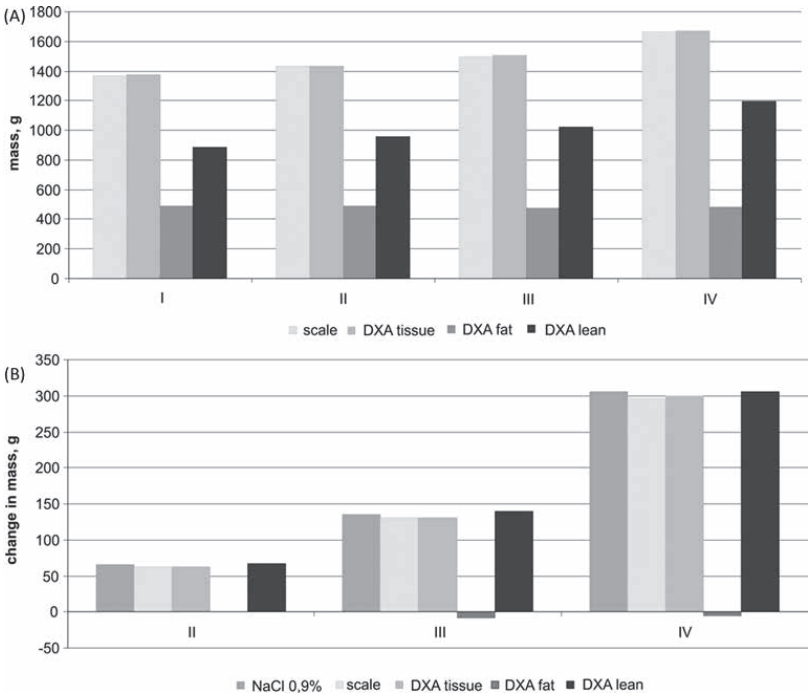

Fig. 4 - A) Phantom mass according to scale (including beaker) and tissue, fat and lean fraction mass determined by DXA; **B)** change in the phantom, tissue, fat and lean fraction mass with respect to the amount of added fluid.

based method, the accuracy of the UV absorbance method is higher than the accuracy of DXA measurements in HD patients; (iii) the statistically significant difference between LBM_{blood} and LBM_{DXA} is with high probability caused by the higher tissue hydration of the HD patients.

It has been shown previously that UV absorbance originating mostly from uric acid is used to assess creatinine concentration (12). The reason for the systematic difference between LBM_{blood} and LBM_a is that LBM_a calculation utilized dialysate samples collected 10 min after the start of the dialysis session instead of blood samples collected at the start of dialysis. However, the difference between LBM_a and LBM_{blood} was not statistically significant ($p = .06$). Also, LBM_a presented higher accuracy and precision (Fig. 2) than LBM_{DXA} (Fig. 3) when compared to LBM_{blood}. Thus, the results of the present study indicate that the possibility of assessing lean body mass in hemodialysis patients based on creatinine concentration by the SW UV model exists.

A comparison of LBM_{blood} and LBM_{DXA} revealed a significant difference ($p<.05$) between the estimates (Fig. 1). The mean difference between LBM_{blood} and LBM_{DXA} was -8.6 ± 9.6 kg (Fig. 3). It has been demonstrated that the peritoneal fluid in continuous ambulatory peritoneal dialysis (CAPD) patients is measured as lean mass by DXA (8). Moreover, previous studies have shown that LBM is also significantly overestimated by DXA compared to results based on creatinine kinetics in CAPD patients when the abdomen was drained of peritoneal fluid before DXA (9) or the weight of the peritoneal fluid was subtracted from total body weight and LBM by DXA (8). Furthermore, a significant decrease in lean mass was noted in hemodialysis patients as a consequence of dialysis therapy (10). Thus, the difference between LBM_{blood} and LBM_{DXA} could be explained by an effect of the altered fluid balance in HD patients on DXA measurements as the scans were performed on an interdialytic day.

The effect of tissue hydration on DXA measurements was explored further by *in vitro* experiments. It can be seen from the results that the PMP beaker was also identified as tissue by DXA (Fig. 4). The results indicated that excess fluid in tissue influences the DXA estimate of lean mass while the estimate of fat mass remains relatively stable (Fig. 4A). It has been shown previously that if the accumulating fluid has the same ratio of low to high energy attenuation (R) as the lean fraction, it will not cause fat estimation errors irrespective of the amount of the fluid (15). Moreover, as the R value of NaCl 0.9% solution that was used as excess fluid is higher than that of lean tissue (15), overestimation of lean mass change and underestimation of fat mass could be expected. This was also observed in the *in vitro* experiments (Fig. 4B). Hence, the results of the *in vitro* experiments support the hypothesis that the statistically significant difference between LBM_{blood} and LBM_{DXA} can be caused by the higher hydration of the tissues in HD patients. The results also support the suggestion that in order to minimize this type of errors, LBM measurements by DXA should be performed directly after dialysis (10).

In summary, although the LBM estimates based on the SW UV model are slightly lower compared to the estimates based on blood samples, LBM_a presented greater accuracy

and precision compared to LBM determined by DXA measurements. Based on the results of previous studies (8-10, 15) and the *in vitro* experiments, it can be assumed that the statistically significant difference between LBM_{blood} and LBM_{DXA} is with high probability caused by the higher hydration of the tissues in HD patients. Thus, the results suggest that it may be feasible to assess LBM in HD patients based on creatinine concentration estimated by the SW UV model. The study was carried out on a relatively small number of dialysis sessions. To validate the results using a larger database will be an issue of further studies. The merits of the described method are that it does not need blood samples or the patient to undergo additional measurement procedures (e.g. DXA). Moreover, the LBM estimates based on the UV absorbance method are not influenced by the hydration status of the HD patient, which can pose a problem in the case of DXA measurements where excess body water is included in LBM. Also, the UV technique enables monitoring of LBM trends in an individual patient, which is suggested to be more important than the validity of the absolute value (5).

Acknowledgment

The authors wish to thank Andrus Paats and Eirik Lepp at North Estonia Medical Center, Tallinn, Estonia, for assistance with the *in vitro* experiments with DXA.

Disclosures

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Conflict of interest: None to declare.

References

1. Laville M, Fouque D. Nutritional aspects in hemodialysis. Kidney Int Suppl. 2000;58:S133-S139.
2. Locatelli F, Fouque D, Heimburger O, et al. Nutritional status in dialysis patients: a European consensus. Nephrol Dial Transplant. 2002;17(4):563-572.
3. Fouque D, Vennegoor M, ter Wee P, et al. EBPG guideline on nutrition. Nephrol Dial Transplant. 2007;22(Suppl 2):ii45-ii87.
4. Desmeules S, Lévesque R, Jaussent I, Leray-Moragues H, Chalabi L, Canaud B. Creatinine index and lean body mass are excellent predictors of long-term survival in haemodiafiltration patients. Nephrol Dial Transplant. 2004;19(5):1182-1189.
5. Keshaviah PR, Nolph KD, Moore HL, et al. Lean body mass estimation by creatinine kinetics. J Am Soc Nephrol. 1994;4(7):1475-1485.
6. Ellis KJ. Human body composition: *in vivo* methods. Physiol Rev. 2000;80(2):649-680.
7. Andreoli A, Scalzo G, Masala S, Tarantino U, Guglielmi G. Body composition assessment by dual-energy X-ray absorptiometry (DXA). Radiol Med. 2009;114(2):286-300.
8. Bhatla B, Moore H, Emerson P, et al. Lean body mass estimation by creatinine kinetics, bioimpedance, and dual energy x-ray absorptiometry in patients on continuous ambulatory peritoneal dialysis. ASAIO J. 1995;41(3):M442-M446.
9. Nielsen PK, Ladefoged J, Olgaard K. Lean body mass by Dual Energy X-ray Absorptiometry (DEXA) and by urine and dialysate creatinine recovery in CAPD and pre-dialysis patients compared to normal subjects. Adv Perit Dial. 1994;10:99-103.
10. Horber FF, Thomi F, Casez JP, Fonteille J, Jaeger P. Impact of hydration status on body composition as measured by dual energy X-ray absorptiometry in normal volunteers and patients on haemodialysis. Br J Radiol. 1992;65(778):895-900.
11. Fridolin I, Magnusson M, Lindberg L-G. On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: technique description. Int J Artif Organs. 2002;25(8):748-761.
12. Tomson R, Fridolin I, Uhlin F, Holmar J, Lauri K, Luman M. Optical measurement of creatinine in spent dialysate. Clin Nephrol. 2013;79(2):107-117.
13. Holmar J, Fridolin I, Uhlin F, Lauri K, Luman M. Optical method for cardiovascular risk marker uric acid removal assessment during dialysis. Scientific World Journal 2012;2012:506486.
14. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;327(8476):307-310
15. Pietrobelli A, Wang Z, Formica C, Heymsfield SB. Dual-energy X-ray absorptiometry: fat estimation errors due to variation in soft tissue hydration. Am J Physiol. 1998;274(5 Pt 1):E808-E816.

Publication III

Tomson R, Uhlin F, Fridolin I (2014)"Urea rebound assessment based on UV absorbance in spent dialysate", *ASAIO Journal*, 60 (4): 459-465 (DOI: 10.1097/MAT.0000000000000091)

Urea Rebound Assessment Based on UV Absorbance in Spent Dialysate

Ruth Tomson,* Fredrik Uhlin,*† and Ivo Fridolin*

The aim of the study was to examine the possibility of postdialysis urea rebound assessment using UV-absorbance measurements in spent dialysate. Twenty-six patients on chronic three-times-a-week hemodialysis (HD) were studied in two separate studies. Double-beam spectrophotometer was used for the determination of UV absorbance in the collected spent dialysate samples. Also, on-line UV absorbance was monitored. The equilibrium concentration (C_{eq}) of urea at the end of the rebound phase was calculated based on urea concentration in blood and dialysate and UV absorbance in spent dialysate. Based on C_{eq}, urea rebound was expressed relative to urea concentration at the end of HD (R_1) and relative to the decrease in urea concentration during HD (R_2). Estimates based on UV-absorbance values in spent dialysate (R_{1_a}, R_{2_a}) slightly over assess postdialysis rebound compared with results based on the blood sample drawn 30 min after HD (R_{1_30post}, R_{2_30post}), but R_{1_a} and R_{2_a} presented greater consistency and accuracy compared with the estimates based on the intradialytic blood sample (R_{1_b}, R_{2_b}). In summary, the results show that it is possible to assess postdialysis urea rebound in blood based on UV-absorbance measurements in spent dialysate. *ASAIO Journal* 2014; 60:459–465.

Key Words: hemodialysis monitoring, rebound, urea, UV absorption

Urea, a low-molecular-weight metabolic end-product of the catabolism of proteins, is considered to be the most suitable marker for uremic toxins in the range of low-molecular-weight solutes.[1] Urea Kt/V is viewed as a sensitive measure of the overall dialysis dose that characterizes dialysis adequacy.[2] Traditionally, Kt/V is derived from formal urea kinetic modeling which is based on blood samples at the start and end of dialysis.[1]

If the immediate postdialysis urea concentrations are used for the calculation of dialysis dose, it can be significantly overestimated because of the increase in blood urea concentration—urea rebound—which occurs after completion of the hemodialysis (HD) session. Early urea rebound (≤3 min after dialysis) has two components—access recirculation and cardiopulmonary recirculation.[2,3] The late phase of urea rebound (>3 min after dialysis) is caused by the entry of urea into blood from relatively undialysed tissues and is complete within 30–60 min after cessation of HD, when the concentration gradients are dissipated and urea concentrations equilibrated across body water spaces.[2,4] Thus, the most accurate way for the calculation of Kt/V would be to wait 30–60 min after the completion of HD before drawing the postdialysis blood sample. However, this approach is impractical for patients and dialysis facilities.

Two alternative mathematical two-compartment models have been proposed to describe the removal of solutes during dialysis. First, the serial two-compartment model where in case of urea in blood plasma, red blood cell water, and interstitial water are viewed as the extracellular fluid compartment, from which dialysis occurs, and intracellular water represents the intracellular compartment. Transport between the compartments is assumed to be mainly diffusive and governed by intercompartmental clearance, which is thought of as a function of the permeability surface product of the cell membrane.[4] According to this model, the urea disequilibrium during HD is caused by the decrease in urea concentration in the intracellular fluid compartment lagging behind that in the extracellular during HD so that after the end of HD urea from the intracellular fluid compartment continues to diffuse into the extracellular fluid compartment until concentrations are equal.[5] The alternative approach is the regional blood flow model that divides organs based on perfusion into a low-blood-flow group and a high-blood-flow group. The arrangement of compartments is parallel and transport between compartments mainly convective.[4] This model explains the urea disequilibrium during HD with a delay in bringing urea from peripheral organs to the dialyzer when the solute is located in organs with relatively low blood flow.[6]

To avoid the delay of waiting for an equilibrated postdialysis blood sample, algorithms for anticipating postdialysis rebound of urea have been developed based on the serial two-compartment model[5] and the regional blood

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flow model.[7] The Smye algorithm[5] estimates the postdialysis equilibrated urea concentration in blood based on conventional pre- and postdialysis blood samples and an additional intradialytic blood sample. The algorithm is based on the presumption that after an initial period, during which an intercompartmental urea concentration gradient is established, the mean or equilibrated concentration of urea in body water and the extracellular urea concentration decrease with equal log linear slopes. The drawback of the Smye algorithm is that it suffers from the effects of small urea concentration measurement errors.[8] The Smye algorithm has also been modified for the use together with a continuous urea sensor[9] showing good agreement between the estimated equilibrated urea concentration and urea concentration 25–40 min after termination of dialysis.

There is a need for an instrument capable of directly and easily assessing postdialysis urea rebound without the need to have the patient wait 30–60 min after the treatment and repeated blood samples. An optical method using UV-absorbance has been proposed for the monitoring of dialysis adequacy.[10,11] A good linear relation has been found between UV absorbance and dialysate urea concentration in the wavelength range 210–330 nm, with the highest correlation at 280–320 nm.[12] It has been shown that because of the good correlation between UV absorbance and urea concentration in dialysate, the latter can be estimated from UV-absorbance measurements even if the UV technique does not measure urea itself.[13] Moreover, urea concentration in spent dialysate is a fixed fraction of arterial urea concentration as long as dialysate flow rate, dialyser clearance, and recirculation rate remain unchanged.[9]

This study was undertaken to explore whether postdialysis urea rebound in blood can be assessed by UV-absorbance measurements in spent dialysate and to estimate the method's accuracy compared with the blood-based methods.

Methods

Subjects

A total of 26 patients on chronic three-times-a-week HD were studied in two separate studies at the Department of Nephrology, University Hospital, Linköping (**Table 1**) (study 1) and at the Department of Dialysis and Nephrology, North Estonia Medical Center, Estonia (**Table 1**) (study 2). The data of the studies, including the number of dialysis sessions, number of participating patients, and the age of the patients, are presented in **Table 1**. Treatment durations ranged from 210 to 270 min. The dialysate flow rate was fixed at 500 ml/min. The type of dialysis machines used were Fresenius 4008H and Fresenius 5008 (Fresenius Medical Care, Germany).

Table 2. Discrete Sampling of Blood and Spent Dialysate

	Sampling Time (min)	
Study	Blood	Dialysate
1	0, 60, 120, 180, 240, 255, 270; 30 post-HD	5, 60, 120, 180, 240, 255, 270
2	0, 180/210/240 (end of dialysis); 30 post-HD	

The Regional Ethics Committee, Linköping, Sweden, and the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia, approved the study protocol, and informed consent was obtained from all patients.

Sampling and Laboratory Analysis

Samples of blood and spent dialysate were taken at discrete times for analysis (**Table 2**). The numbers for "Sampling time" correspond to minutes after the start of HD. Blood and dialysate samples were taken at 255 and 270 min when the duration of the session was long enough.

Blood and dialysate samples were sent to the laboratory for analysis within 2–4 h. Laboratory's standard sampling procedures were followed. No additional preparation of the blood and dialysate samples was carried out. The concentration of urea was determined at the Clinical Chemistry Laboratory at the Linköping University Hospital (study 1) and at the Clinical Chemistry Laboratory at the North Estonia Medical Centre (study 2) using a standardized method. The ADVIA 1800 automated analyzer (Siemens, Deerfield, IL) was used in Linköping and the Hitachi 912 technology in Tallinn. The accuracy of the method for the determination of urea in blood and dialysate was ±5%.

A double-beam spectrophotometer (in Linköping UVIKON 943, Kontron, Milano, Italy; in Tallinn Shimatzu UV-2401 PC, Shimadzu, Kyoto, Japan) was used for the determination of UV-absorbance in the collected spent dialysate samples. Spectrophotometric analysis over a wavelength range of 190–380 nm was performed using an optical cuvette with an optical path length of 1 cm. Pure dialysate was tested for UV absorbance showing an absorbance value of approximately zero. Pure dialysate was collected before the start of each dialysis session when the dialysis machine was prepared and the conductivity was stable. The collected sample was used as reference solution during the UV scanning of spent dialysate and placed

Table 1. Data of the Studies

Study	No. of Sessions	No. of Patients	Age (yr)	Dialyzer	Area (m^2)	n	Blood flow (ml/min)	Modality (n)
1	30	10	23–78	Polyflux17L	1.7	18	200–350	HD
				TCA150G	1.5	3		
				Nephral300	1.3	9		
2	22	16	40–80	F8	1.8	8	200–350	HD (16)
				FX10	1.8	2		HDF (6)
				FX80	1.8	12		

HD, hemodialysis; HDF, hemodiafiltration.

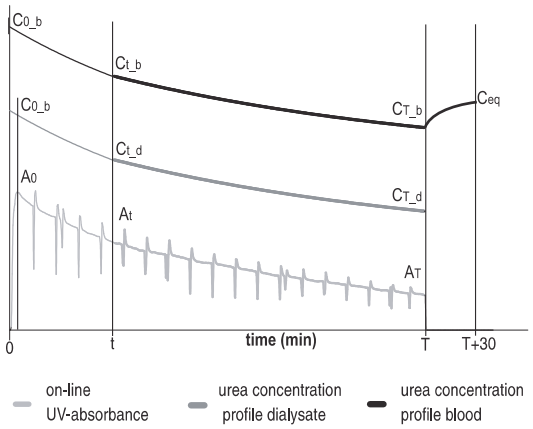

Figure 1. Schematic representation of blood, dialysate, and UV-absorbance parameters used for the calculation of C_{eq}.

in the reference holder of the spectrophotometer. This means that the measured absorbance expresses the absorbance of additional solutes that the dialysate contains after passing the dialyser. Some of the measured values (absorbance or concentration) were excluded from data before analysis. The exclusion criteria were incorrect or illogical values of the measured concentration or absorption, for example, sampling coexisting with self-tests of the dialysis machine.

UV-Absorbance Monitoring

The instrumentation setup for the determination of UV absorbance on-line has been described earlier.[14] The spectrophotometers UVIKON 943 (Kontron) in Linköping and HR2000 (Ocean Optics Inc., Dunedin, FL) in Tallinn were used. The wavelength used in this part of the study was 297 nm. The sampling frequency was set at two samples per minute.

The baseline was measured a few minutes before the start of each dialysis treatment on the flowing pure dialysate (reference solution) when the temperature and conductivity had been stabilized and the sodium and bicarbonate levels had been preset according to the patient records.

The obtained UV-absorbance values were processed and presented on computer screen by a PC incorporated into the spectrophotometer using Kontron software (UVIKON 943, version 7.0 for Windows; Kontron Instruments) in Linköping and Ocean Optics' software (OOI-Base32, version 2.0.2.2 for Windows) in Tallinn. Data were then transformed to an Excel file at the end of the treatment.

Data Analysis

The equilibrium concentration (C_{eq}) of urea at the end of rebound phase was calculated according to the Smye algorithm[5] as:

$$C_{eq} = C_0 e^{-\lambda T} \quad (1)$$

$$\lambda = \frac{1}{T-t} \ln\left(\frac{C_t}{C_T}\right) \quad (2)$$

where C_0 is the concentration of urea before HD, C_t is the concentration of urea at time t after the onset of HD, C_T is the concentration of urea at the end of HD, and T is the duration of HD in minutes (**Figure 1**). C_{eq} was calculated based on values of C_0, C_t, and C_T obtained by different methods (**Table 3**) using one intradialytic sample and all available samples from 60 min to the end of HD (marked by index "exp"). In case of C_{eq_a}, urea concentrations in the Smye algorithm were substituted by UV-absorbance values (A_0, A_t, A_T, respectively) in the respective dialysate samples (**Figure 1**), to explore the possibility of estimating urea rebound based on these measurements. The obtained substitute values for C_{eq} were subsequently used to calculate urea rebound. The most suitable wavelength (296 nm) (**Table 3**) for the calculation of $C_{eq\_a\_60}$ and $C_{eq\_a\_exp}$ was determined by forward stepwise regression with urea concentration in blood as the dependent variable and UV-absorbance values in the corresponding dialysate samples at the wavelength 210–330 nm as the independent variables.

The material was divided into a calibration set and a validation set, which consisted of the data of study 1 (**Table 1**) and

Table 3. Urea Concentrations Used for the Calculation of C_{eq}

C_{eq}	Based on	Sample Used for C_0	Calculation of λ
$C_{eq\_b\_60}$	Urea concentration in blood	0 min	According to Equation 2 60-min sample used as C_t
$C_{eq\_b\_exp}$		0 min	Obtained by line fitting based on 60, 120, 180, 240, 255, and 270 min values
$C_{eq\_d\_60}$	Urea concentration in dialysate	5 min	According to Equation 2 60-min sample used as C_t
$C_{eq\_d\_exp}$		5 min	Obtained by line fitting based on 60, 120, 180, 240, 255, and 270 min values
$C_{eq\_a\_60}$	UV absorbance in spent dialysate samples	5 min	According to Equation 2 60-min sample used as C_t
$C_{eq\_a\_exp}$			Obtained by line fitting based on 60, 120, 180, 240, 255, and 270 min values
$C_{eq\_a\_on\text{-}line}$	On-line UV absorbance in spent dialysate	Average value of 2–6 min from the beginning of HD	Obtained by line fitting based on on-line UV-signal from 60 min to end of HD session

HD, hemodialysis.

Table 4. Urea Concentrations Used for the Calculation of R_1 and R_2

R_1	R_2	Based on	Sample Used for C_0	C_{eq}
R_{1_30post}	R_{2_30post}	Urea concentration in blood	0 min	Urea concentration in the 30-min post-HD blood sample (C_{eq_30post})
$R_{1\_b\_60}$	$R_{2\_b\_60}$	Urea concentration in blood	0 min	$C_{eq\_b\_60}$
$R_{1\_b\_exp}$	$R_{2\_b\_exp}$			$C_{eq\_b\_exp}$
$R_{1\_d\_60}$	$R_{2\_d\_60}$	Urea concentration in dialysate	5 min	$C_{eq\_d\_60}$
$R_{1\_d\_exp}$	$R_{2\_d\_exp}$			$C_{eq\_d\_exp}$
$R_{1\_a\_60}$	$R_{2\_a\_60}$	UV absorbance in spent dialysate samples	5 min	$C_{eq\_a\_60}$
$R_{1\_a\_exp}$	$R_{2\_a\_exp}$			$C_{eq\_a\_exp}$
$R_{1\_a\_on\text{-}line}$	$R_{2\_a\_on\text{-}line}$	On-line UV absorbance in spent dialysate	Average value of 2–6 min from the beginning of HD	$C_{eq\_a\_on\text{-}line}$

HD, hemodialysis.

the data of study 2 (**Table 1**), respectively. For the validation set, only $C_{eq\_a\_on\text{-}line}$ was calculated.

Urea rebound (R) was expressed relative to C_T:

$$R_1 = \frac{C_{eq} - C_T}{C_T} 100\% \quad (3)$$

and relative to the decrease in urea concentration during HD

$$R_2 = \frac{C_{eq} - C_T}{C_0 - C_T} 100\% \quad (4)$$

R_2 expresses better the meaning of rebound as the ratio to the pre–post concentration drop, but the percentage value of R_1 better approximates the percentage difference between the single-pool Kt/V (Kt/V_{sp}) and the equilibrated Kt/V (Kt/V_{eq}).[3] R_1 and R_2 were calculated based on values of C_0, C_T, and C_{eq} obtained by different methods (**Table 4**). In case of R_{1_a} and R_{2_a}, urea concentrations were substituted by UV-absorbance values at the wavelength used for the calculation of C_{eq_a} in the respective dialysate samples.

Systematic error was calculated as:

$$BIAS = \frac{\sum_{i=1}^{N} e_i}{N} \quad (5)$$

where e_i is the i-th residual and N is the number of observations.[15]

Standard error of performance, corrected for BIAS was calculated as[15]:

$$SE = \sqrt{\frac{\sum_{i=1}^{N} (e_i - BIAS)^2}{N-1}} \quad (6)$$

Student's t-test for dependent samples was used to compare means for all estimated parameters, and p value 0.05 or less was considered significant. Individual differences in R_{1_b}, R_{1_d}, R_{1_a} compared with R_{1_30post} and R_{2_b}, R_{2_d}, R_{2_a} compared with R_{2_30post} were also examined using Bland and Altman analysis.[16]

For the analysis Statistica 6.0 (Statsoft, Inc. [Tulsa, OK] for Windows) and Excel (Microsoft Corp., Seattle, WA; version 2003 for Windows) were used.

Results

Average values (mmol/L) of C_{eq} calculated for the calibration set based on urea concentrations obtained by different methods are presented in **Figure 2A**. $C_{eq\_b\_60}$ and $C_{eq\_b\_exp}$ were significantly higher compared with C_{eq_30post} ($p < 0.05$), whereas $C_{eq\_d\_60}$ and $C_{eq\_d\_exp}$ were significantly lower compared with C_{eq_30post} ($p < 0.05$). The systematic errors and standard errors of C_{eq_b} and C_{eq_d} compared with C_{eq_30post} are presented in **Figure 2B**. The smallest systematic error and standard error were obtained in case of $C_{eq\_b\_exp}$ compared with C_{eq_30post}.

Average values (%) of R_1 for the calibration set are presented in **Figure 3A**. $R_{1\_b\_60}$ and $R_{1\_b\_exp}$ significantly overestimated urea rebound expressed relative to C_T compared with R_{1_30post}

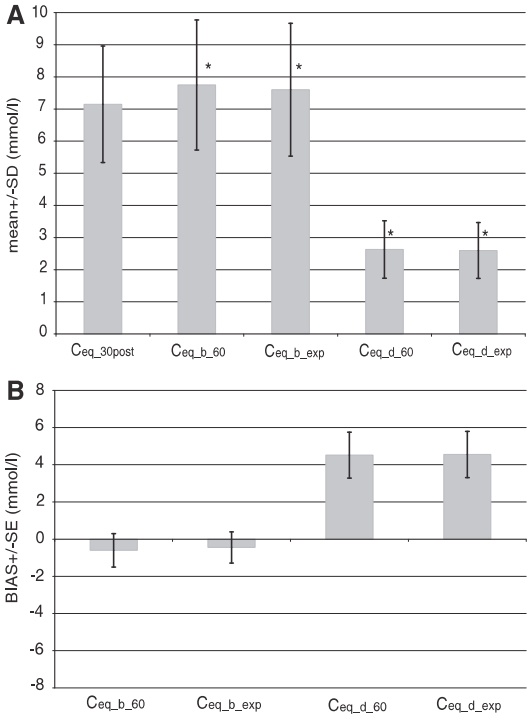

Figure 2. A: Average values and standard deviations (mean ± SD) of C_{eq_30post}, $C_{eq\_b\_60}$, $C_{eq\_b\_exp}$, $C_{eq\_d\_60}$, and $C_{eq\_d\_exp}$ (N = 29) for the calibration set. **B**: The systematic errors and standard errors of $C_{eq\_b\_60}$, $C_{eq\_b\_exp}$, $C_{eq\_d\_60}$, and $C_{eq\_d\_exo}$ compared with C_{eq_30post}. *Represents statistical significance.

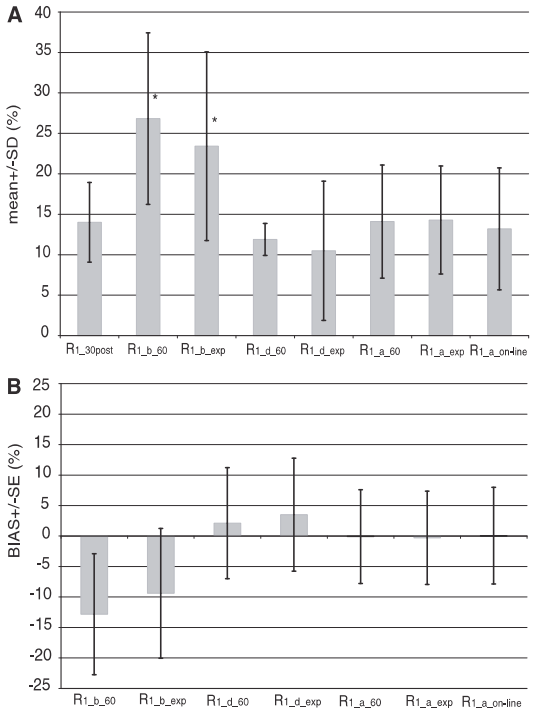

Figure 3. A: Average values and standard deviations (mean ± SD) of R_{1_30post}, $R_{1\_b\_60}$, $R_{1\_b\_exp}$, $R_{1\_d\_60}$, $R_{1\_d\_exp}$, $R_{1\_a\_60}$, $R_{1\_a\_exp}$, and $R_{1\_a\_on\text{-}line}$ (N = 25) for the calibration set. **B**: The systematic errors and standard errors of $R_{1\_b\_60}$, $R_{1\_b\_exp}$, $R_{1\_d\_60}$, $R_{1\_d\_exp}$, $R_{1\_a\_60}$, $R_{1\_a\_exp}$, and $R_{1\_a\_on\text{-}line}$ compared with R_{1_30post}. *Represents statistical significance.

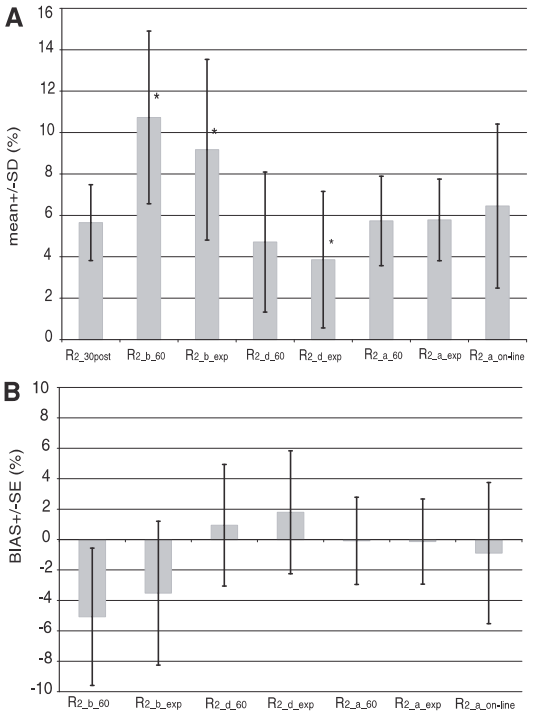

Figure 4. A: Average values and standard deviations (mean ± SD) of R_{2_30post}, $R_{2\_b\_60}$, $R_{2\_b\_exp}$, $R_{2\_d\_60}$, $R_{2\_d\_exp}$, $R_{2\_a\_60}$, $R_{2\_a\_exp}$, and $R_{2\_a\_on\text{-}line}$ (N = 25) for the calibration set. **B**: The systematic errors and standard errors of $R_{2\_b\_60}$, $R_{2\_b\_exp}$, $R_{2\_d\_60}$, $R_{2\_d\_exp}$, $R_{2\_a\_60}$, $R_{2\_a\_exp}$, and $R_{2\_a\_on\text{-}line}$ compared with R_{2_30post}. *Represents statistical significance.

($p < 0.05$). The systematic and standard errors of R_{1_b}, R_{1_d}, and R_{1_a} compared with R_{1_30post} are presented in **Figure 3B**. The smallest systematic error was obtained in case of $R_{1\_a\_on\text{-}line}$ compared with R_{1_30post}. The smallest standard error was obtained in case of $R_{1\_a\_exp}$ compared with R_{1_30post}.

Average values (%) of R_2 for the calibration set are presented in **Figure 4A**. $R_{2\_b\_60}$ and $R_{2\_b\_exp}$ significantly overestimated urea rebound expressed relative to the decrease in urea concentration during HD compared with R_{2_30post} ($p < 0.05$). $R_{2\_d\_exp}$ significantly underestimated urea rebound compared with R_{2_30post} ($p < 0.05$). The systematic and standard errors of R_{2_b}, R_{2_d}, and $R_{2\_a\_on\text{-}line}$ compared with R_{2_30post} are presented in **Figure 4B**. The smallest systematic error was obtained in case of $R_{2\_a\_60}$ compared with R_{2_30post}. The smallest standard error was obtained in case of $R_{2\_a\_exp}$ compared with R_{2_30post}.

Figure 5 shows the Bland–Altman plot of the differences between 1) R_{1_30post} and $R_{1\_a\_on\text{-}line}$, and 2) R_{2_30post} and $R_{2\_a\_on\text{-}line}$ for the validation set. The mean difference between R_{1_30post} and $R_{1\_a\_on\text{-}line}$ was 0.9 ± 9.0% and the mean difference between R_{2_30post} and $R_{2\_a\_on\text{-}line}$ was 0.3 ± 4.7%.

Discussion

The current study investigated the possibility of postdialysis urea rebound assessment using UV-absorbance measurements in spent dialysate. Urea rebound is relevant because it affects the postdialysis blood urea concentration and thus also the dialysis dose.[3] Because of the double-pool nature of urea intradialytic kinetics, the urea concentration in blood immediately after dialysis is lower than the mean concentration in the body, and consequently, Kt/V can be significantly overestimated if this value is used. As the percentage value of rebound relative to the decrease in urea concentration during HD approximates the percentage difference between single-pool Kt/V and equilibrated Kt/V,[3] this information could be used to estimate the true dialysis dose.

The results indicated that: 1) it is possible to assess postdialysis urea rebound in blood based on UV-absorbance measurements in spent dialysate; and 2) calculation of percent urea rebound based on UV-absorbance values in spent dialysate is preferable to the use of C_{eq} derived by applying the Smye algorithm from urea concentration values in blood samples.

$C_{eq\_b\_60}$, calculated according to the Smye algorithm,[5] was significantly higher compared with C_{eq_30post} ($p < 0.05$). As a consequence, $R_{1\_b\_60}$ and $R_{2\_b\_60}$ also significantly overestimated postdialysis urea rebound compared with R_{1_30post} and R_{2_30post}, respectively. It has been shown that the Smye approximation slightly overestimates the actual equilibrated concentration.[9] However, the results demonstrate the sensitivity of the Smye algorithm to small urea concentration measurement errors in the laboratory that has been pointed out earlier.[8] This

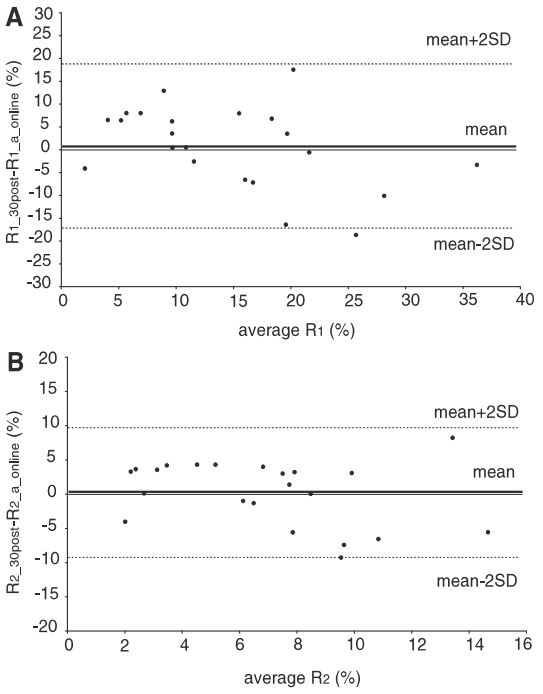

Figure 5. Bland–Altman plot of the differences between (**A**) R_{1_30post} and $R_{1\_a\_on\text{-}line}$, and (**B**) R_{2_30post} and $R_{2\_a\_on\text{-}line}$ for the validation set (N = 22).

can cause substantial errors in estimating the slope of the urea elimination profile if it is based only on the intradialytic and immediate postdialysis blood sample. The fact that the smallest systematic error and standard error compared with C_{eq_30post} were obtained in case of $C_{eq\_b\_exp}$, although it was significantly higher compared with C_{eq_30post} ($p < 0.05$), shows that the results improve if more than one intradialytic sample is used. It has been shown that the problems connected to the implementation of the Smye algorithm could be overcome with the use of an on-line urea sensor that enables the assessment of the slope based on the entire urea elimination profile after the initial period.[9]

Urea concentration in dialysate is only a fraction of arterial urea concentration, which can explain the outcome that $C_{eq\_d\_60}$ and $C_{eq\_d\_exp}$ were significantly lower compared with C_{eq_30post} ($p < 0.05$) (**Figure 2A**). However, $R_{1\_d\_60}$, $R_{1\_d\_exp}$, and $R_{2\_d\_60}$ were not statistically different from R_{1_30post} ($p = 0.258$, $p = 0.07$, respectively) and R_{2_30post} ($p = 0.253$), respectively. This shows that the dialysate urea follows proportionally blood urea concentrations, and calculation of percent urea rebound based on dialysate urea concentrations is possible.

The optical method using UV absorbance proposed for the monitoring of dialysis adequacy[10,11] offers the possibility to continuously follow the urea elimination profile without the need for disposables of chemicals. To explore the possibility of implementing UV absorbance in spent dialysate for the assessment of postdialysis rebound $C_{eq\_a\_60}$ and $C_{eq\_a\_exp}$ were calculated based on UV absorbance values in spent dialysate at the most suitable wavelength (296 nm) so that $R_{1\_a\_60}$, $R_{2\_a\_60}$, $R_{1\_a\_exp}$, and $R_{2\_a\_exp}$ could be derived. $R_{1\_a\_60}$ and $R_{2\_a\_60}$ did not significantly differ from R_{1_30post} ($p = 0.952$) and R_{2_30post} ($p = 0.890$), respectively. Also, $R_{1\_a\_exp}$ and $R_{2\_a\_exp}$ were not statistically different from R_{1_30post} ($p = 0.853$) and R_{2_30post} ($p = 0.818$), respectively. In addition, to take full advantage of the possibilities offered by the UV-technique, the implementation of on-line measurements to derive the slope of the extracellular urea log concentration curve for the calculation of C_{eq} was investigated. $R_{1\_a\_on\text{-}line}$ and $R_{2\_a\_on\text{-}line}$, calculated based on $C_{eq\_a\_on\text{-}line}$, were not statistically different from R_{1_30post} ($p = 0.977$) and R_{2_30post} ($p = 0.355$), respectively, in case of the calibration set. Also for the validation set, $R_{1\_a\_on\text{-}line}$ and $R_{2\_a\_on\text{-}line}$ were not statistically different from R_{1_30post} ($p = 0.660$) and R_{2_30post} ($p = 0.803$), respectively, confirming the results obtained for the calibration set. Thus, calculation of percent urea rebound based on UV-absorbance values in spent dialysate seems preferable to the traditional blood-based method.

In summary, although the estimates based on UV-absorbance values in spent dialysate (R_{1_a}, R_{2_a}) slightly over assess postdialysis urea rebound compared with results based on the blood sample drawn 30 min after HD (R_{1_30post}, R_{2_30post}), R_{1_a} and R_{2_a} presented greater consistency and accuracy compared with the estimates based on the intradialytic blood sample (R_{1_b}, R_{2_b}) (**Figures 3B** and **4B**). Moreover, a very good estimate of the urea rebound can be achieved using on-line UV absorbance.

The results suggest that the possibility of assessing postdialysis urea rebound in blood based on UV-absorbance measurements in spent dialysate exists. The study was carried out on a relatively small number of dialysis sessions. To validate the results using a larger database will be an issue of further studies. The merits of the described method are that it does not need blood samples or the patient to wait 30–60 min after the completion of HD before drawing the postdialysis blood sample. Moreover, the high sampling frequency of the UV signal reduces the effect of measurement errors that could occur with the analysis of blood samples in the laboratory. Also, on-line monitoring of several uremic solutes would be advantageous because urea is not representative of all small and water-soluble uremic toxins.

References

1. European Best Practice Guidelines of Haemodialysis, ERA-EDTA. II.1 Haemodialysis dose quantification: Small solutes. Nephrol Dial Transplant 17: 16–31, 2002.
2. NKF KDOQI guidelines. Clinical practice guidelines for hemodialysis adequacy, update 2006. Guideline 3. Methods for postdialysis blood sampling [National Kidney Foundation web site]. Available at: http://www.kidney.org/professionals/KDOQI/guideline_upHD_PD_VA/hd_guide3.htm. Accessed November 4, 2013.
3. Alloatti S, Molino A, Manes M, Bosticardo GM: Urea rebound and effectively delivered dialysis dose. *Nephrol Dial Transplant* 13(Suppl 6): 25–30, 1998.
4. Schneditz D, Daugirdas JT: Compartment effects in hemodialysis. Semin Dial 14: 271–277, 2001.
5. Smye SW, Evans JH, Will E, Brocklebank JT: Paediatric haemodialysis: Estimation of treatment efficiency in the presence of urea rebound. Clin Phys Physiol Meas 13: 51–62, 1992.

6. Schneditz D, Platzer D, Daugirdas JT: A diffusion-adjusted regional blood flow model to predict solute kinetics during haemodialysis. *Nephrol Dial Transplant* 24: 2218–2224, 2009.
7. Daugirdas JT, Schneditz D: Overestimation of hemodialysis dose depends on dialysis efficiency by regional blood flow but not by conventional two pool urea kinetic analysis. *ASAIO J* 41: M719–M724, 1995.
8. Gotch FA, Keen ML: Kinetic modeling in hemodialysis. in Nissenson AR, Fine RN (eds), *Clinical Dialysis*. New York, McGraw-Hill, 2005, pp. 153–202.
9. Garred LJ, Canaud B, Bosc JY, Tetta C: Urea rebound and delivered Kt/V determination with a continuous urea sensor. *Nephrol Dial Transplant* 12: 535–542, 1997.
10. Fridolin I, Magnusson M, Lindberg LG: On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: Technique description. *Int J Artif Organs* 25: 748–761, 2002.
11. Castellarnau A, Werner M, Günthner R, Jakob M: Real-time Kt/V determination by ultraviolet absorbance in spent dialysate: Technique validation. *Kidney Int* 78: 920–925, 2010.
12. Fridolin I, Lindberg LG: On-line monitoring of solutes in dialysate using wavelength-dependent absorption of ultraviolet radiation. *Med Biol Eng Comput* 41: 263–270, 2003.
13. Uhlin F, Fridolin I, Lindberg LG, Magnusson M: Estimating total urea removal and protein catabolic rate by monitoring UV absorbance in spent dialysate. *Nephrol Dial Transplant* 20: 2458–2464, 2005.
14. Uhlin F, Fridolin I, Lindberg LG, Magnusson M: Estimation of delivered dialysis dose by on-line monitoring of the ultraviolet absorbance in the spent dialysate. *Am J Kidney Dis* 41: 1026–1036, 2003.
15. Esbensen KH: Multivariate data analysis—In practice. CAMO Process AS, 2009.
16. Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1: 307–310, 1986.

ELULOOKIRJELDUS

1. Isikuandmed

Ees- ja perekonnanimi	Ruth Tomson
Sünniaeg ja –koht	27.12.1981, Oru vald, Eesti
Kodakondsus	Eesti
E-posti aadress	ruth@cb.ttu.ee

2. Hariduskäik

Õppeasutus (nimetus lõpetamise ajal)	Lõpetamise aeg	Haridus (eriala/kraad)
Tallinna Tehnikaülikool	2004	Elektroonika ja biomeditsiinitehnika, bakalaureusekraad
Tallinna Tehnikaülikool	2006	Elektroonika ja biomeditsiinitehnika, magistrikraad

3. Keelteoskus (alg-, kesk- või kõrgtase)

Keel	Tase
Eesti	Emakeel, kõrgtase
Inglise	Kõrgtase
Saksa	Kesktase
Rootsi	Algtase
Vene	Algtase

4. Täiendusõpe

Õppimise aeg	Täiendusõppe läbiviija nimetus
18.-19.02.2016	ERA-EDTA CME Course „Introductory Course on Epidemiology“
08.-10.03.2016	3 TTÜ keelte ja kommunikatsiooni keskuse rahvusvahelist töötuba

5. Teenistuskäik

Töötamise aeg	Tööandja nimetus	Ametikoht
2005-2006	Tallinna Tehnikaülikool	Tehnik
2006-k.a.	Tallinna Tehnikaülikool	Teadur

6. Teadustegevus

Ureemiliste toksiinide uurimine ja nende neeruasendusravi käigus eemaldamise hindamine optiliste meetoditega.

7. Teadustöö põhisuunad

2007-2012	Biosignaalide interpreteerimine meditsiinitehnikas	SF0140027s07
2008-2015	Integreeritud elektroonikasüsteemide ja biomeditsiinitehnika tippkeskus	TAR8077DB
2011-2014	Uudne optiline meetod ureemiliste toksiinide – alatoitumuse ja kroonilise põletiku ning SVH riski potentsiaalsete markerite, monitooringuks	ETF8621

APPENDIX 2 continued

2014-2019	Biooptilised ja bioelektrilised signaalid meditsiinitehnikas	IUT19-2
2016-2023	Eesti Infotehnoloogia Tippkeskus	TAR16013DB

APPENDIX 3

CURRICULUM VITAE

1. Personal data

Name	Ruth Tomson
Date and place of birth	27.12.1981, Oru Parish, Estonia
E-mail	ruth@cb.ttu.ee

2. Education

Educational institution	Graduation year	Education (field of study/degree)
Tallinn University of Technology	2004	Electronics and biomedical engineering, BSc
Tallinn University of Technology	2006	Electronics and biomedical engineering, MSc

3. Language competence/skills (fluent, average, basic skills)

Language	Level
Estonian	mother-tongue, fluent
English	fluent
German	average
Swedish	basic skills
Russian	basic skills

4. Special Courses

Period	Educational or other organizations
18.-19.02.2016	ERA-EDTA CME Course „Introductory Course on Epidemiology“
08.-10.03.2016	3 academic writing workshops at TUT Language Centre

5. Professional Employment

Period	Organisation	Position
2005-2006	Tallinn University of Technology	Technician
2006-...	Tallinn University of Technology	Research scientist

6. Scientific work

Research of uremic toxins and optical estimation of their elimination during dialysis.

7. Main areas of scientific work/Current research topics

2007-2012	Interpretation of Biosignals in Biomedical Engineering	SF0140027s07
2008-2015	Centre for Integrated Electronic Systems and Biomedical Engineering	TAR8077DB

APPENDIX 3 continued

2011-2014	A novel optical technology for monitoring of uremic toxins – potential markers for malnutrition-inflammation syndrome and CVD risk	ETF8621
2014-2019	Biooptical and bioelectrical signals in Biomedical Engineering	IUT19-2
2016-2023	Estonian Centre of Excellence in ICT Research	TAR16013DB

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