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Dialysis Dose and Nutrition Assessment by an Optical Method

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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 any academic degree.

Merike Luman

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CONTENTS

INTRODUCTION

For approximately 40 years, dialytic therapy has provided successful “long-term” life sustaining replacement for absent renal function. Defining an appropriate dose of solute removal during maintenance dialysis therapy has long been a crucial interest, especially as mathematical models or formulations were being developed to assess the adequacy of hemodialysis (HD).

Many putative uremic toxins are products from protein metabolism (Vanholder *et al* 1994). Because it is impossible to measure the large number of water-soluble uremic toxins in routine practice, a derivative of protein catabolism –urea– has emerged as the most popular marker for quantifying the dose of small solute removal. Urea *Kt/V* is the most widely accepted measurement of the dose of small solute removal (Gotch *et al* 1985).

Many studies have demonstrated a relationship between delivered dialysis dose as measured in *Kt/V* and morbidity and mortality in chronic hemodialysis patients (Acchiardo *et al* 1983, Blumenkrantz *et al* 1982, Canaud *et al* 1997, Daugirdas *et al* 1995). The clinical application of mathematical terms (such as *Kt/V*) to describe the removal of urea during hemodialysis is called urea kinetic modeling (UKM). Based on blood samples formal UKM calculates the urea distribution volume (*V*) and the urea generation rate (*G*) by mathematical iteration (Gotch and Keen 2005). The delivered *Kt/V* is usually calculated by means of the single-pool variable volume urea kinetic model (VVSP UKM) and is suggested for dialysis adequacy estimation (NKF K/DOQI, Fouque *et al* 2007).

Formal urea kinetic modeling also allows to calculate the normalized protein nitrogen appearance (*nPNA*), which correlates closely with dietary protein intake among patients who are not markedly catabolic or anabolic (Garred *et al* 1995). Protein-energy malnutrition is frequently present in patients undergoing hemodialysis therapy and several studies have suggested that malnutrition is an important risk factor for morbidity and mortality in HD patients (Flanigan *et al* 1995). Maintaining an adequate dose of hemodialysis may improve nutrition and patient survival. In order to optimize the diet of patients with renal diseases, *PNA* provides a more reliable estimate of dietary protein intake and enables the dialysis care team to perform long-term analysis of the patients’ nutritional status and to more soundly guide dietary counselling about protein intake (Leypoldt 2005).

UKM requires an accurate measurement of urea clearance and the collection of blood samples at the start and at the end of dialysis session, which depends on accuracy and timing of drawing the samples and laboratory errors (Canaud *et al* 1997).

In addition to collecting blood samples the complexity of calculations in formal urea kinetic modeling requires the use of computers, and software is still a restriction. Even if the cost is not very high, this remains a consideration for smaller hemodialysis units. Secondly, extra work and time required from the staff to accurately collect and process all patient data for these calculations may be significant in all hemodialysis centres, especially larger ones (Leypoldt 2005).

The total dialysate collection (TDC) technique, or direct dialysis quantification (DDQ), uses the total removed urea nitrogen (*TRU*) through collecting the entire dialysate, exiting the dialyser over a dialysis treatment (Garred 1995). The modified DDQ, mDDQ, using *TRU* and blood samples, is successfully applied for validation of dialysis adequacy (Depner *et al* 1996), enabling similarly to UKM to iteratively calculate the *G* and *V* values. Total dialysate collection technique needs the collection of all spent dialysate in a special tank calibrated against a weighting-machine, which is not possible to use in everyday practice.

The possibility of estimating *Kt/V*, *TRU* and *PNA* by ultraviolet (UV) light-absorbance measurements on the spent dialysate has been demonstrated (Uhlin *et al* 2003 and 2005). Moreover, an optical dialysis adequacy monitor (DIAMON) prototype based on on-line UV-absorbance measurements has been developed (Fridolin *et al* 2007).

The main aim of this work was to assess dialysis adequacy and nutrition by on-line UV method and compare the results with the clinical condition of the patients.

The aim of the first part of the study (Publication I) was to compare the adequacy of dialysis treatment and the patient's nutritional status using the parameters *eKt/V* and *nPNA* estimated using pre- and post-dialysis blood samples, total dialysate collection, and the optical dialysis adequacy monitor. A comparative picture about every patient obtained using variable volume single pool urea kinetic modeling (UKM VVSP) was given to estimate agreement of clinical condition of the patients assessed by the spent dialysate and UKM.

In Publication II a comparison is made to estimate the patient's nutritional parameter *nPNA* individually by the on-line DIAMON prototype, by formal urea kinetic modeling (VVSP UKM), and by modified direct dialysis quantification (mDDQ). *nPNA* was normalized by *V*/0.58 and by the measured dry body weight, *efBW*.

Uric acid (UA) is a water-soluble compound (molecular weight of 168.1 Da) that is the final metabolite of purine in humans. Elevated serum UA contributes to endothelial dysfunction and increased oxidative stress within the glomerulus and the tubulo-interstitium, with associated increased remodeling fibrosis of the kidney (Hayden *et al* 2004). A high level of serum UA, hyperuricemia, has been suggested to be an independent risk factor for cardiovascular and renal disease especially in patients with heart failure, hypertension and/or diabetes (Feig *et al* 2008, Viazzi *et al* 2006, Høieggen *et al* 2004).

The high correlation between UV-absorbance and UA due to the dominant absorbance for UA, compared to other compounds in spent dialysate at the wavelength range 270-310 nm (Uhlin *et al* 2003) raises a question weather UA removal could be estimated by UV-absorbance during dialysis.

The aim of the study in Publication III was to estimate the dialysis dose by means of total removed uric acid (TR_{UA}) by the on-line UV-absorbance measurements in the spent dialysate in two different dialysis centers in two countries, Estonia and Sweden.

In the future, UV-absorbance measurement of uric acid as a risk factor for cardiovascular disease could give extra value for diagnosis, prevention and treatment in cardiovascular and renal medicine.

This thesis is a summary of the author's work at the Department of Biomedical Engineering of the Technomedicum of Tallinn University of Technology. The thesis consists of publications and an overview. The thesis presents the results of the study of the dialysis dose assessment by a novel optical method. The overview consists of a review of the current status of the research problem and the main results presented in the author's publications.

The present thesis is based on the following papers that are referred to in the text by their Roman numerals I-III.

I **Merike Luman**, Jana Jerotskaja, Kai Lauri, Ivo Fridolin. (2009). "Dialysis dose and nutrition assessment by optical on-line dialysis adequacy monitor", *Clinical Nephrology*, vol 72 (4), pp.303-311.

II Fridolin, Ivo; Lauri, Kai; Jerotskaja, Jana; **Luman, Merike**. (2008). "Nutrition estimation of dialysis patients by on-line monitoring and kinetic modelling", *Estonian Journal of Engineering, Special issue on Biomedical Engineering*, June 14(2): 177-188.

III Jerotskaja J, Uhlin F, Fridolin I, Lauri K, **Luman M**, Fernström A (2010) "Optical on-line monitoring of uric acid removal during dialysis", *Blood Purification*, 29:69-74 (on-line DOI: 10.1159/000264269).

Author's own contribution

In all the publications the author participated in the planning of the experiments, in supervising and performing all dialysis treatments in the Department of Dialysis and Nephrology, North-Estonian Medical Centre, collecting the data of the patients and dialysis sessions, collecting the samples, contributing to the writing of the papers, discussing the presentation and clinical value of the results, except the calculation of the dose and nutrition by the iterative VVSP UKM and mDDQ algorithms, and regression analysis for uric acid estimation.

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I would like to express my sincere appreciation and gratitude to all who have contributed to the creation of this thesis.

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ABBREVIATIONS

AGE products – advanced glycation end products
APD – automated peritoneal dialysis
BUN – blood urea nitrogen
CAPD – continuous ambulatory peritoneal dialysis
CKD – chronic kidney disease
CVD – chronic cardiovascular disease
Da – dalton
DDQ – direct dialysis quantification
mDDQ – modified direct dialysis quantification
DIAMON – dialysis adequacy monitor
EBPG – European Best Practice Guidelines
ERA-EDTA –European Renal Association – European Dialysis and Transplantation Associaton
ESAO – European Society of Artificial Organs
ESRD – end-stage renal disease
EUTox – European Uremic Toxin Work Group
GFR – glomerular filtration rate
HD – hemodialysis
HDF – hemodiafiltration
HF – hemofiltration
HPLC – high performance liquid chromatography
K – clearance
Kt/V – dialysis dose efficacy parameter
eKt/V – equilibrated dialysis dose Kt/V
spKt/V – single-pool Kt/V
MW – molecular weight
NKF K/DOQI – National Kidney Foundation Kidney Disease Outcomes Quality Initiative
OCM – on-line clearance monitor from Fresenius Medical Care
PCR – protein catabolic rate
nPCRw – protein catabolic rate normalized to the bodyweight
PD – peritoneal dialysis
PNA – protein nitrogen appearance
nPNA – normalized protein nitrogen appearance
RRT – renal replacement therapy
TDC – total dialysate collection
TRU – total removed urea
Trua – total removed uric acid
UA – uric acid
UKM – urea kinetic modeling
URR – urea reduction ratio

UV – ultraviolet
VVSP UKM – variable volume single-pool urea kinetic modeling
WHO – World Health Organization

1. THE KIDNEY, KIDNEY FAILURE AND RENAL REPLACEMENT THERAPY

1.1 Background

The idea of removing solutes from body fluids by dialysis dates back to the beginning of the last century. The first experimental hemodialysis in dogs was performed by Abel *et al* 1913 at the Johns Hopkins Medical School in Baltomore. The first human dialysis was performed by Georg Haas from Giessen, Germany. Willem Kolff at the Groningen University Hospital in Netherlands introduced the first dialyser suitable for use in humans in 1943 (Yeun and Depner 2005). However, problems of vascular access and technical problems limited the use of dialysis for patients with acute renal failure who only needed renal replacement therapy for a short time. Technical and clinical research and improvements have made it possible to use hemodialysis treatment in humans for years, not only for a short period of time, but as dialysis treatment still is not as efficient as healthy kidneys the patients on the dialysis treatment still suffer from uremic syndrome.

The main functions of the kidney can be named as excretory and metabolic. The excretory function is the excretion of water-soluble metabolic waste products e.g. urea, creatinine and uric acid. The kidney is responsible for the regulation of body water volume and osmolarity, acid-base and electrolyte balance. The kidneys also have hormonal functions, such as conversion of Vitamin D which influences the composition of the skeleton, the regulation of the production of red blood cells as well as the regulation of blood pressure. About 25% of cardiac output at rest passes the kidneys, which allows a great glomerular filtration rate (*GFR*). The primary filtrated volume can reach 180L per day before reabsorbtion of water and selection of dissolved substances from the tubular fluid back to the blood stream. The end-product of this process is the 1-2L urine (Vander 1994).

The kidneys have a large overcapacity. Not before renal function has fallen to about 20 -15% of its capacity is it necessary to observe dietary restrictions, and not before it has fallen to about 15 - 5% must the need for dialysis or transplantation be considered (Lote 2000).

1.2 Kidney failure

Renal (or kidney) failure is characterized by the progressive decline in the capacity of the kidneys to eliminate toxic solutes (Vanholder *et al* 1993). Kidney failure can be distinguished as acute or chronic failure. Acute kidney failure is an acute damage of the kidney after serious trauma, surgery, intoxications etc and could be reversible. Chronic kidney failure usually shows a progressive decrease of glomerular filtration rate and is usually irreversible and related to increased blood level of azotemic substances. Chronic renal failure develops in stages during

different time periods, up to decades. Only in the last stage – the end stage renal disease (ESRD) – is renal replacement therapy e.g. hemodialysis needed.

The most common primary kidney diseases to cause chronic kidney failure are glomerulonephritis, urinary tract infections and inherited diseases, such as polycystic kidney disease. The main secondary renal diseases to cause kidney failure are diabetes mellitus and arteriosclerotic kidney disease e.g. due do hypertension (leading to nephosclerosis), systemic vasculitis, amyloidosis and myeloma. There has been a dramatic global increase in the occurrence and prevalence of diabetes over the last 2 decades. According to data from WHO the number of patients with diabetes will increase from the 154 million in the year 2000 to 370 million in the year 2030 (Wild *et al* 2004). Hypertension is the second most common attributed etiology of end stage renal disease.

Chronic kidney disease increases cardiovascular risk and cardiovascular disease is the predominat cause of death among patients with chronic kidney disease. The uremic state is cardiomyopathic because of its hemodynamic milieu and also because of specific uremic toxins. Also most kidney patients are hypertonic and often the reason for kidney failure is diabetes mellitus which increases cardiovascular risk through different factors. The patients with chronic kidney failure have also anemia, uremic bone disease, malnutrition, chronic inflammatory milieu, insomnia and other disturbances which all increase cardiovascular risk (Vanholder 1998).

1.3 Uremic toxins

Uremic syndrome is attributed to the progressive retention of a large number of compounds, which under normal conditions are excreted by healthy kidneys. These compounds are called uremic retention solutes, or uremic toxins, when they interact negatively with biological functions.

The uremic syndrome is a complex “intoxication” of the retention of waste products resulting in multifactorial problems where disturbances in several metabolic functions are reflected in clinical problems. Several organs and organ systems are affected: cardio-vascular system (hypertension, pericarditis and heart failure), peripheral nervous system (polyneuropathy), central nervous system (poor memory, loss of concentration and slower mental ability), hematology (anemia, bleeding tendencies), coagulation, immune status (immunosupression), nausea, vomiting etc. Uremic toxins retain in the blood and tissues where the removal of the toxins is complicated (Vanholder *et al* 1993).

At the beginning of the dialysis the concentration of the uremic toxins such as urea is high and fast removal with dialysis treatment causes low osmolarity of the blood and oedema of the brain which cause headache, nausea and vomiting (Vanholder *et al* 2004).

The European Society of Artificial Organs (ESAO) and The European Uremic Toxin Work Group (EUTox) have done a lot of research and have had great success in identifying uremic toxins and connecting uremic toxins with the clinical status of the renal patients (Vanholder *et al* 2003, 2005).

The concentrations of in total 90 uremic solutes are listed in the uremic toxins database: 68 of 90 are low molecular weight with MW < 500 Da, 22 are with MW > 500 Da, 25 are protein bound, 2 are protein bound in the range of MW > 500 Da (Vanholder *et al* 2003).

The retained organic compounds may be divided into three groups: (i) small water soluble solutes with a molecule weight MW < 500 Da (e.g. urea, creatinine, uric acid); (ii) middle molecules MW > 500 Da (e.g. β2-microglobulin, cytokines (Interleukin 6), Leptin); and (iii) protein-bound solutes (e.g. homocysteine, P-cresol, AGE products, hippuric acid, indoxyl sulfate) (Vanholder *et al* 2003).

Different uremic toxins effect the patient in several different ways and to various extent. In order to improve the quality of dialysis, monitoring of several uremic toxins is essential.

Urea (MW 60.06 Da) is a small water soluble molecule. Urea is the main nitrogenous end-product of protein metabolism and a well-accepted marker for the severity of renal failure (Rigoir 1997). Most of the direct toxic effects have been proven in vitro. Urea inhibits NaK_2Cl co-transport in human erythrocytes, inhibits in vitro L-arginine transport and endothelial NO synthase activity, stimulates a heat shock response to induce macrophage proliferation, and increases the expression of the oxidative stress-responsive transcription factor, GADD153/CHOP. There is a controversy to its role in the uremic intoxication (Rigoir 1997). In a classical study conducted by Johnson *et al* (1972), urea was being added to the dialysate of a patient cohort in the course of 3 months, so that blood urea never decreased below 200 mg/dl, no impact on the clinical status of the patients was observed. Urea seems to be a surrogate marker and representative for the removal of other solutes with impact on morbidity and survival (Vanholder *et al* 2003).

Uric acid, a final product of purine (MW 168,11 Da) metabolism, is mostly excreted from human body through kidneys in the form of urine. The concentration of uric acid in blood increases when the source of uric acid increases or the kidney malfunctions. High level of serum uric acid, hyperuricaemia, is suggested to be an independent risk factor for cardiovascular and renal disease especially for a patient with heart failure, hypertension or diabetes (Feig *et al* 2008, Viazzi *et al* 2006, Høieggen *et al* 2004). Hyperuricaemia is also a novel risk factor for type 2 diabetes mellitus and has proven to be the cause of renal disease in the rat model (Nakagawa *et al* 2006). Uric acid is removed from plasma during dialysis treatment in a similar manner as urea, but it has not yet been investigated in respect to the outcome of patient's sickness/ailment/treatment as urea has been. Uric acid is mostly associated with gout, but studies have found that uric acid affects biological systems, and could also be the cause of higher mortality in dialysis patients (De Smet *et al* 1997, Perlstein *et al* 2004). According to The European Society of Cardiology guidelines dated 2008 for the diagnosis and treatment of heart failure, elevated uric acid level is associated with poor prognosis in heart failure and uric acid is one of the biomarker in heart failure.

1.4 Treatment of renal failure

Predialysis – low protein diet. If renal function is impaired, but 15 - 20 % is still left, the low protein diet is prescribed. When the protein content of the diet is restricted (but still enough to satisfy the requirements of the body) the amount of waste products formed will naturally also be smaller. It might also be necessary to use a little less salt. As the kidney function falls, dietary measures will no longer be sufficient and renal replacement therapy (RRT) must be started.
For RRT there are three different possibilities available today:

- Hemodialysis (HD);
- Peritoneal dialysis (PD) – continuous ambulatory PD (CAPD) and automated PD (APD);
- Transplantation.

Hemodialysis means cleaning of the blood by an artificial kidney. Artificial kidneys may differ in appearance, but they all work according to the same principle. An important element in treatment by means of the artificial kidney is the dialysis machine, which consist of a blood unit and a fluid unit.

In the artificial kidney – the dialyser – the blood flows between thin membranes with microscopically small pores. On the other side of the membranes there is a flow of fluid which contains no waste products. There is thus a solution on each side of the membrane. The blood is generally withdrawn from a forearm vein through a needle, a so-called cannula, and is pumped through the dialyser where it is cleansed before it returns to the patient's vein through another cannula. To accommodate good flows through a dialyser, the vessels must be quite large and a minor operation is necessary to create better flows for this purpose. During the operation an arteria and vein on the forearm are connected together, which expands the veins and makes it possible to insert dialysis cannulas into the fistula and get good blood flow through the dialyser. The problems with fistulas can be infections and clotting.

Hemodialysis must be done at least 3 times per week and one dialysis session is usually 4-5 hours long. Most often patients have the treatment in hospital or other dialysis centers. Modern dialysis machines are entirely automatic and this together with a number of safety devices enables the patient to sleep in safety during dialysis at night. The technique is so reliable that some patients requiring dialysis could have a machine at home and manage their own treatment. Patients undergoing hemodialysis have to keep to a diet that avoids foods containing the mineral potassium (fruit, vegetables, chocolate etc). Due to over-storage of water in the body, the permitted daily amount of drinking water will be restricted.

Peritoneal dialysis uses the patient's peritoneum, a thin membrane situated inside the abdomen. This membrane surrounds the organs inside the abdomen and, like all body tissue, has its own blood circulation. The special feature of the peritoneum, apart from the fact that it is extremely well supplied with blood, is that it has small pores through which toxic substances can exit. The patient's abdomen is filled with a liquid precisely adapted to the needs of the patient, via catheter. This liquid, called the dialysis solution, "rinses" the peritoneum and absorbs the

toxic substances by the diffusion. With the aid of osmosis, the water is extracted from the body as well. The dialysate that has absorbed the toxic substances is removed after 4-5 hours and replaced with fresh, clean dialysis solution by changing the bag. There is usually 2-2.5 liters of dialysis fluid per exchange and 4 to 5 changes per day must be done. Peritoneal dialysis functions continuously 24 hours a day and is often used for children, adults and diabetic patients. Before the start of peritoneal dialysis a small operation has to be done, a catheter (a thin silicone tube) must be implanted into the abdominal cavity and the dialysis fluid is then exchanged via this catheter by the patient.

There are two types of peritoneal dialysis:

- CAPD – continuous ambulatory peritoneal dialysis, exchange of dialysis fluid is done by patient, 4 to 5 exchanges per day.
- APD – automated peritoneal dialysis when fluid is exchanged by the machine with a special program inserted for every patient. This is usually done at night, so the patient is free at day time and can go to school or work.

Peritoneal dialysis could be carried out at home, at work, wherever the patient is. The patient is trained to do peritoneal dialysis at the nephrology centre, an important point stressed constantly during the patient's period of training is the extremely high degree of cleanliness needed as infection can easily occur if bacteria get inside abdominal cavity.

A successful **kidney transplant** is, without doubt, the best solution when one's own kidney fails. But not all patients are suited to an operation of this kind or to the subsequent long-term medical treatment that must certainly follow. Nor is a kidney ideally suitable to meet the recipient's body's needs immediately. It is thus necessary to bridge the waiting period before a transplant operation. This waiting period can vary in length from months to years and for that time dialysis is needed anyway.

1.5 Dialysis technologies

The principle of hemodialysis.

The major components of a hemodialysis system include the blood circuit and the dialysate circuit. The central part of both circuits is the dialyser where waste products, excess electrolytes, and water are removed from the patient's blood. Dialysis fluid and blood are pumped through the dialyser in a countercurrent direction, and separated by the semipermeable membrane. The blood-flow compartment is monitored to control the pressures, flow, and accidental entry of air into the blood circuit. In the dialysis-fluid compartment, the composition of the dialysis fluid, flow, pressure, and accidental entry of the blood into dialysate due to rupture of the dialyser membrane need to be monitored (Fig1).

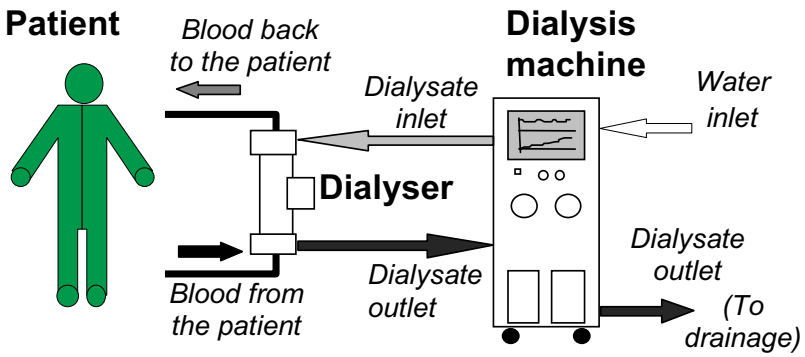

Figure 1: Principle of hemodialysis.

Solute transport across the membrane can occur by diffusion or ultrafiltration – based convection. In hemodialysis, diffusion is the main transport mechanism. Ultrafiltration is applied in hemodialysis to remove excess water from the patient.
There are two types of dialyser membrane: cellulose-based and synthetic (polysulphone, polycarbonate, polyamide, and polyacrylonitrile). The membranes differ in their solute permeability, hydraulic permeability, and interaction with blood. The new synthetic dialysers have greater convective permeability allowing faster removal of larger solutes from the blood and improve the quality of the dialysis treatment

Another possibility to improve quality of the dialysis is to use different methods of filtration.

Hemofiltration (HF) provides solute clearance by convection through high permeability (High Flux) membrane. Hemofiltration is more effective to the uremic toxins with middle and large size as convection increases removal of the large sized molecules. The filtration rate is as high as 30 - 40 liters per treatment session. Thus, the rate of convective solute removal can be modified either by changes in the rate of solvent (plasma water) flow or by changes in the mean effective pore size of the membrane. The blood concentration of a particular solute also influences its convective removal rate.

Hemodiafiltration (HDF) is a renal replacement treatment method where the hemodialysis is combined with hemofiltration and provides solute removal by diffusion and convection. Hemodiafiltration provides better removal of larger molecules and protein bound uremic toxins. Ultrafiltration rate up to 100 ml/min and substitution fluid with volume 20 - 25 liters per dialysis are usually applied. Replacement fluid must be ultra-pure, with minimal endotoxins contamination, since fluid is administered directly into the patient and is produced online within the dialysis machine from dialysate concentrates and water using two or three ultrafilters.

Hemodiafiltration provides better cardiovascular stability, blood pressure control and solute removal than hemodialysis, and is used today more and more often.

1.6 Dialysis quality and adequacy

The prescription of dialysis requires the knowledge of the normal function of the kidney, of the patient metabolism and physiology, and of the dialysis technology. Dialysis should reduce the concentration of the uremic toxins to an acceptably low level. Adequate renal replacement treatment maximizes well-being, minimizes morbidity, and helps a patient retain social independence.

Despite being a routinely used treatment in clinical praxis, dialysis has still remained a complex therapy with side effects, requiring a major time commitment by the patient and it is associated with a complex medical and dietary regimen. Moreover, patients undergoing hemodialysis suffer from several complications such as hypotension and dizziness due to hypovolemia etc.

Because dialysis is a time consuming and expensive treatment, there is great interest in shortening the duration of the procedure from the patient's perspective and reducing the costs of treatment and hospitalisation from the economical viewpoint.

Many studies have demonstrated a relationship between delivered dialysis dose and the morbidity and mortality of chronic hemodialysis patients.

Methods and remedies that allow monitoring of hemodialysis patients might enable early recognition of inadequate dialysis or cardiovascular instability in which action needs to be taken.

There are several guidelines worked out to standardize dialysis treatment in clinical practice and guarantee the best quality. The most used guidelines are the "National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF K/DOQI) Guidelines" and the "European Best Practice Guidelines - EBPG".

Dialysis adequacy parameters are traditionally calculated from urea concentrations in the blood, usually sampled at the start and at the end of dialysis. Two parameters are generally used to assess dialysis adequacy: urea reduction ratio (*URR*), and *Kt/V*, describing how effectively a dialysis treatment removes waste products from the body.

The *URR* is calculated simply from the fractional pre- and post-dialysis urea concentration, which equals C_t/C_0 where C_t is the post-dialysis urea and C_0 the pre-dialysis urea as:

$$URR(\%) = 100 * \left(1 - \frac{C_t}{C_0} \right) \tag{1}$$

URR may vary considerably from treatment to treatment and for this reason, on average the *URR* should be $\geq$ 70%.

Another way of measuring dialysis adequacy is the *Kt/V*. In this measurement, *K* stands for dialyser clearance, expressed in mL/min, and *t* stands for time. The top part of the fraction represents the volume of fluid completely cleared of urea during a single treatment. In the bottom part of the fraction, *V* is the volume of water that a

patient's body contains, defined also as the distribution volume of urea in the body in mL. Assuming that urea is distributed in a single pool volume in the body, that urea generation rate and ultrafiltration are negligible during the session and that the ratio *K/V* remains constant over the dialysis, the following equation holds

$$Kt/V = -ln\left(\frac{C_t}{C_0}\right) \tag{2}$$

The single pool volume *Kt/V* for blood, *spKt/Vb*, can be calculated according to the second-generation Daugirdas formula (Daugirdas 1995)

$$spKt/Vb = -\ln\left(\frac{C_t}{C_0} - 0.008\frac{T}{60}\right) + \left(4 - 3.5\frac{C_t}{C_0}\right)\frac{UF}{W} \tag{3}$$

where *UF* is the total ultrafiltration in kg and *W* is the patient's dry body weight in kg.

The equilibrated *Kt/V*, *eKt/V*, according to the rate adjustment method (Daugirdas 1995), is predicted from the rate of dialysis (*K/V*) and the single pool *Kt/V* as:

$$eKt/V = spKt/V - \frac{0.6}{(T/60)}spKt/V + 0.03 \tag{4}$$

The rate adjustment method predicts that the urea rebound is related to the rate of dialysis or dialysis efficiency (Daugirdas *et al* 1997).

Under European Best Practice Guidelines (EBPG), the prescribed target *eKt/V* should be at least 1.2 for anuric patients treated by dialysis three times per week (Tattersall *et al* 2007). To prevent the delivered dose falling below the recommended minimum dose, the prescribed dose of HD should be $spKt/V \geq 1.3$ (NKF-DOQI 2006). Based on the available evidence the minimum prescribed HD dose per session for a thrice-weekly schedule should be: urea $eKt/V \geq 1.2$ ($spKt/V \sim 1.4$) (Tattersall *et al* 2007).

However, following *Kt/V* is not enough to ensure the best survival and quality of life for the patients on dialysis treatment. Protein–energy malnutrition is frequently present in patients undergoing hemodialysis therapy. Several studies have suggested that malnutrition is an important risk factor for morbidity and mortality in HD patients. In order to optimize the diet of patients with renal disease, dietary protein intake has to be controlled. Protein nitrogen appearance (*PNA*), formerly protein catabolic rate (*PCR*), correlates closely with dietary protein intake and is highly recommended to follow by "EBPG Guideline on

Nutrition" (Fouque *et al* 2007). Hemodialysis patients have increased protein losses and catabolism from the chronic inflammatory state of uremia and dialysis procedure itself, and toxic middle molecules that are normally excreted in the urine accumulate in uremia and suppress appetite.

To ensure the best survival, quality of the treatment and the quality of life for the dialysis patients, all parameters should be followed together and in complex.

Seldom (often in connection to scientific research) the sampling is more frequent and even total dialysate collection, *TRU*, *PNA* may be included into the analysis, but in everyday practice the international guidelines are implemented unsatisfactorily in the dialysis centres. According to a study based on the data from 14 European countries (255 HD centres) (Couchoud *et al* 2009, Jager *et al*. 2009):

- 6% dialysis centres never perform adequacy measurements
- 9% dialysis centres do only every 6 months or less frequently
- 4 dialysis centres never used *eKt/V*
- Total % of *eKt/V* usage – 13% of dialysis centres.

To guarantee that the dose of dialysis can be ascertained as needed and corrections can be made immediately, dialysis quality assessment should be as simple as possible, without extra work and time or blood sampling. The incidence of end-stage renal disease patients on renal replacement therapy continues to increase world-wide. However, staff in dialysis centers is not increasing with the same rate. Extra work and time needed for collect and accuarately process all data for dialysis dose calculations will be significant for staff. Despite wide availability of computers, this kind of special software is still a restriction for many centers, especially for smaller ones.

2. DIALYSIS QUALITY ASSESSMENT TECHNOLOGIES

Dialysis adequacy estimation is currently performed by laboratory analysis (blood and dialysate samples) or/and by real-time monitoring (mostly on the spent dialysate side).

2.1 Laboratory analysis

Usually, the blood samples are analysed at the laboratory yielding the raw data in the form of blood urea concentration or blood urea nitrogen (BUN) for calculation of dialysis quality parameters. Dialysis quality as *Kt/V* or *URR* is recommended to be measured monthly in stable patients to assure the adequacy of HD if the session-to-session variation in *Kt/V* is small. However, averaged values of two to three measurements are insisted to reliably assess the dose of HD in non-compliant or unstable patients, when delivery of the prescribed dose presents frequent problems, or measurements yield variable results (ERA-EDTA 2002).

In practice, this analysis is carried out after every month or after every three months. Considering that the dialysis frequency is three times per week there is no data about the most of the treatments. Doubts about the uniform dialysis quality over all treatments arise from the usual clinical practice when the control or so called “sampling time dialysis” is performed far more carefully than the “ordinary” treatments.

Additional source of errors are sampling, storage and analysis by itself. A survey on 15 000 HD patients of 202 HD centres in the USA participating in a Collaborative Study of the NKF showed a 5.0% error in pre-HD blood drawing and an 8.4–41.6% error in the post-HD counterpart (Beto *et al* 1998).

Laboratory analyses are rather complicated and utilise disposables or chemicals, thus non-fitted for on-line, continous dialysis monitoring. It is suggested that on-line, real-time dialysis monitoring during every dialysis procedure is the ideal way to ensure the dialysis quality and implementation of the international guidelines.

A need for continous, real-time dialysis monitoring is promoted by the results of a large international study on dialysis quality – HEMO Study – which concluded that dialysis quality improvement can not be achieved solely by increasing *Kt/V*, several other factors are important as well (Locatelli 2003, Green *et al* 2003). One possible alternative may be introducing the everyday (or nocturnal) dialysis (Locatelli *et al* 2005). This contributes, in turn, continous real-time dialysis monitoring.

Additionally, continuous real-time dialysis monitoring offers novel possibilities for personalized dialysis treatment. A study in UK examined the feasibility of using continuous online assessment of dialysis quality to allow dialysis sessions to be altered on an individual basis (Chesterton *et al* 2006). The use of individualized variable dialysis treatment time using online monitoring of dialysis quality appears both practicable and effective at ensuring consistently delivered adequate dialysis.

2.2 On-line monitoring of the dialysis quality

On-line monitoring of the dialysis dose has been suggested as a valuable tool to ensure adequate dialysis prescription (Locatelli *et al* 2005). Different monitors are available for automatical dialysis quality estimation based on the following principles:

- electrochemical method;
- conductivity or ionic dialysance method;
- optical method (UV-method).

Electrochemical method includes the ammonium ion sensors to measure the amount of ammonium ion (NH_4^+) determined directly by an ion-specific electrode (Canaud *et al* 1998). Hydrolysis of urea produces NH_4^+, thus creating an electrical potential difference between two electrodes that is then amplified and recorded. The sensor is implemented in the Biostat 1000 Urea Monitor (Baxter Healthcare, Dirfield, Illinois, USA) and in Biotrack (Biocare Corp., Taiwan). Very close to the mentioned principle are also the electrochemical sensors which sense the increase in solution conductivity associated with urea hydrolysis after conversion into ammonium and bicarbonate ions with the help of the enzyme urease. The ions formed result in a conductivity increase which is proportional to the original urea concentration (Calzavara *et al* 1998). This principle is utilized in the dialysate monitor DQM 200 (Gambro Lundia AB, Sweden) (Sternby 1998).

Method can estimate dialysis quality parameters *URR, Kt/V, TRU, PCR* and *nPCRw*.

The drawbacks of the electrochemical method are utilization of disposables or chemicals and rather complicated and intermittent measurement procedure which hampers wider clinical usage.

Conductivity or ionic dialysance method assumes that sodium clearance is an accurate estimate for urea clearance (Mercadal *et al* 1998). The sodium clearance is assessed by temporarily increasing dialysate conductivity (sodium concentration) in the dialysate inlet and measuring the change in conductivity at the dialysate outlet (Polaschegg 1993, Petitclerc *et al* 1993). *KT/V* can be calculated when patient urea distribution volume (*V*) and blood Hematocrit (Hct) are entered.

The method is utilised in: (i) Diascan module (Hospal-Gambro, Mirandola Italy) in Gambro's dialysis machines, and (ii) OCM, (Fresenius Medical Care, Germany) in Fresenius's dialysis machines. The method can estimate the dialysis quality parameter *Kt/V*.

The drawbacks of the conductivity or ionic dialysance method are dependence of the *Kt/V* measurements on the patient urea distribution volume (*V*), and the validity of the assumption that sodium clearance is an accurate estimate for urea clearance. The method is capable to estimate only a single parameter – *Kt/V*.

2.3 Optical UV-method

Recently the spectrophotometrical sensors for on-line monitoring of total ultra-violet (UV) absorbance (Fridolin *et al* 2002, Vasilevski *et al* 1999) of urea (Jensen *et al* 2004*,* Olesberg *et al* 2004) in the spent dialysate have been presented, aiming to follow continuously a single hemodialysis session. A good correlation between UV-absorbance and a small removed waste solute such as urea enables the determination of *Kt/V* for urea (Uhlin *et al* 2003), and *nPNA* (Uhlin *et al* 2005). The UV-method does not need blood samples, disposables or chemicals, is fast, and allows direct and continuous measurement of *Kt/V* without any additional calculations of *V*. Furthermore, a new prototype device – dialysis adequacy monitor (DIAMON, AS Ldiamon, Estonia) has been designed for continuous on-line estimation of delivered dialysis dose from optical dialysate measurements (Fridolin *et al* 2007). The measurements by this prototype generated the results presented in Publications I and II.

The DIAMON prototype (AS Ldiamon, Estonia), applied for the measurement in Publications I and II, was connected to the fluid outlet of the dialysis machine with all spent dialysate passing through an optical cuvette during the on-line experiments. The optical cuvette was a quartz tube permeable to the UV-radiation with a diameter of 10 mm. The intensity of light (280 ± 5 nm) transmitted through the spent dialysate was measured. The utilised wavelength was shown to be both technically and methodologically suitable for dialysis dose estimation having a good correlation to the dialysis quality marker solute urea (Fridolin and Lindberg 2003). The sampling frequency was set to 20 samples per minute. The obtained intensity values were processed to obtain the UV-absorbance presented on the computer screen by a PC using Ldiamon's software (AS Ldiamon, Estonia, for Windows). The UV-absorbance was calculated as following:

$$A = log \frac{I_{\mathrm{r}}}{I_{\mathrm{r+s}}} \tag{5}$$

where I_{r} is the intensity of light transmitted through the reference solution (pure dialysate) and $I_{\mathrm{r+s}}$ is the summated intensity of transmitted light through the reference solution containing the solutions under study (pure dialysate + waste products from the blood).

3. EXPERIMENTAL STUDIES: DESIGN OF THE METHOD, RESULTS AND DISCUSSION

3.1 Selection of patients and analysis of methods

Ten uremic patients, three females and seven males, mean age 62.6 ± 18.6 years, on chronic thrice-weekly hemodialysis treatment were included in the study at the Department of Dialysis and Nephrology, North-Estonian Medical Centre, Estonia (Publication I and II). Each patient was monitored during three consecutive dialysis sessions during one week. All patients were dialysed with polysulfone membrane dialysers (Fresenius Medical Care, Germany): 5 patients with a low flux dialyser with an effective membrane area of 1.8m^2 and 2.2 m^2 and 5 patients with a high flux dialyser with effective membrane area of 1.8m^2. The dialysers used were same as they were for these patients before and after study by medical indication. The dialysate flow rate was constant at 500 mL/min. The prescribed blood flow was 350 or 300 mL/min during the two treatments within the week according to the patient records and clinical need and 245 mL/min for the one treatment, and was kept constant throughout the dialysis session. The duration of dialysis sessions was from 190 to 240 min. Four patients had dialysis access in the form of an arterio-venous fistula, 3 patients in the form of an artificial graft, and 3 patients had a temporary catheter of the jugular or femoral vein. All patients were dialysed using a two-needle system. The dialysis machine used in the study was a Fresenius 4008H (Fresenius Medical Care, Germany).

In order to analyse general validity of the optical on-line monitoring of uric acid removal a group of patients both from Estonia and Sweden was included into the study in Publication III. For this purpose, 6 uremic patients, all males, mean age 64.3 ± 18.5 years, were added at the Department of Nephrology, University Hospital of Linköping, Sweden (Publication III). All these patients were on chronic thrice-weekly hemodialysis and were monitored during three to six dialysis treatments each with the duration from 240 to 300 minutes. The dialysate flow was fixed at 500 mL/min and the blood flow varied between 200 and 350 mL/min. Several dialyzers were used, with an effective membrane area of 1.4 to 2.2 m^2 and two dialysis monitors, Fresenius 4008H (Fresenius Medical Care, Germany) and AK 200 (Gambro Lundia AB, Sweden).

Methods

The adequacy of dialysis treatment and the patient's nutritional status was investigated using the parameters from pre-and post-dialysis blood samples, the optical dialysis adequacy monitor and total dialysate collection.

Blood samples were drawn before the start of the dialysis treatment and at the end of dialysis using the slow flow sampling technique by K/DOQI Guidelines (K/DOQI, 2001) (Publication I, II and III).

Dialysis Adequacy Monitor (DIAMON) prototype incorporated a light source (280 ± 5 nm UV LED), a detector (GaNi UV-photodiode), an electronic circuit board, and an optical cuvette. The monitor was connected to the fluid outlet of the dialysis machine with all spent dialysate passing through during the on-line experiments. The transmitted light intensity of the spent dialysate was measured. The sampling frequency was set to 20 samples per minute. The obtained intensity values were processed to obtain UV-absorbance and presented on the computer screen by a PC using Ldiamon software (AS Ldiamon, Estonia, for Windows). The experimental set-up is shown in Fig 2. (Publication I, II and III). Instead of the DIAMON prototype, the spectrophotometers UVIKON – 943 Kontron, Italy – in Linköping and HR2000 – Ocean Optics, Inc., USA – in Tallinn were used for the determination of UV-absorbance on-line for the study described in Publication III. The spectrophotometer was connected to the fluid outlet of the dialysis machine with all spent dialysate passing through an optical flow cuvette with a depth of 10 mm. The geometry of the cuvette was rectangular in Tallinn and circular in Linköping.

The obtained UV-absorbance values were processed and presented on the computer screen by a PC incorporated in the spectrophotometer using Kontron's software (UVIKON 943, Kontron, Italy, version 7.0 for Windows) in Linköping and Ocean Optics' software (OOIBase32, Ocean Optics, Inc., USA, version 2.0.2.2 for Windows) in Tallinn.

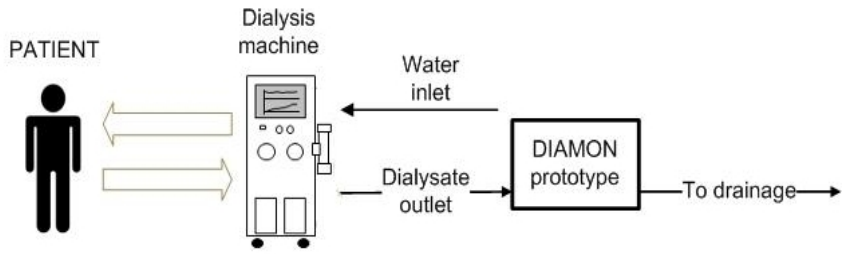

Figure 2. The clinical experimental set-up

Total dialysate collection (TDC) started when the blood had filled the dialyser and ended when the blood was returned to the patient at the end of the dialysis. All spent dialysate was collected in a tank calibrated against a weighing-machine. After careful stirring and recording of the weight of the collected spent dialysate, the TDC sample ($Urea_{TDC}$) was collected.

Five dialysate samples were also taken from the drain tube: 10, 60, 120 and 180 minutes after the start of the dialysis session, and immediately after the end of the treatment. The samples were sent to the laboratory and analysis of urea concentration was performed within 1 to 4 hours.

The concentration of urea was determined at the Clinical Chemistry Laboratory at North Estonia Medical Centre (Publication I, II, III) and at the Clinical

Chemistry Laboratory at University Hospital of Linköping (Publication III) using a standardized method. The accuracy of the method for determining urea in blood and dialysate was ± 5% (Publication I, II, III). The concentrations of uric acid were determined at the Clinical Chemistry Laboratory at both Hospitals using enzymatic colorimetric test method. The accuracy for UA in blood and dialysate was ± 3 % in Linköping and ± 2 % in Tallinn (Publication III).

3.2 Dialysis dose assessment by optical on-line monitoring

Despite ongoing technical improvements in both dialysis and overall patient care, the annual mortality rate of patients with end-stage renal disease treated with thrice-weekly hemodialysis remains high (10–22%) (Rainer *et al*. 2004, Ganesh *et al*. 2003). Many studies have demonstrated a relationship between delivered dialysis doses as measured in *Kt/V* and morbidity and mortality in chronic hemodialysis patients (Keshaviah *et al.* 2002, Port *et al.* 2002, Salvatore *et al*. 2005).

Due to great variations between patients and dialysis sessions (McIntyre *et al*. 2003), evaluation of every hemodialysis session by an on-line monitoring system makes it possible to provide an adequate dialysis dose to the HD patients (Lambie *et al.* 2004). To achieve and maintain the pre-set treatment dose, an on-line monitoring system has been suggested as a more accurate method compared to monthly blood sampling (K/DOQI 2001, Hernandez-Herrera *et al.* 2001, Locatelli *et al.* 2005).

The possibility of estimating the dialysis dose as *Kt/V* by ultraviolet (UV) light-absorbance measurements on the spent dialysate has been demonstrated earlier (Uhlin *et al*. 2003 and 2005). The UV-method measures all UV-absorbing compounds in the spent dialysate. A good correlation between the UV-absorbance and urea enables to estimate urea based dialysis adequacy parameters by the UV-technique even if the urea is not directly measured (Uhlin *et al.* 2003 and 2005). Moreover, a new optical dialysis adequacy monitor (DIAMON) prototype based on on-line UV-absorbance measurements was tested instead of the unwieldy complicated spectrophotometer utilized in earlier studies (Fridolin *et al*. 2007), (Publication I).

One of the aims of the study was to compare the adequacy of dialysis treatment by pre- and post-dialysis blood samples, the DIAMON prototype and Total Dialysate Collection (TDC).

For our study (Publication I) dialysis dose from blood, *eKt/V*(blood), was calculated according to the Daugirdas second-generation formula (Daugirdas 1995) using the pre- and post-dialysis urea concentrations (C_0 and C_t) utilised as a comparative well-established method for dialysis quality evaluation. To determine the dialysis dose from UV-absorbance measured by the DIAMON prototype, *eKt/V*(DIAMON), instead of the pre- and post-dialysis blood urea concentrations the maximum UV-absorbance value in the beginning and the minimum UV-

absorbance value at the end of dialysis (A_0 and A_t) were utilized. The single pool volume *Kt/V*, *spKt/Va* was calculated as:

$$spKt/Va = -ln\left(\frac{A_t}{A_0} - 0.008\frac{T}{60}\right) + \left(4 - 3.5\frac{A_t}{A_0}\right)\frac{UF}{BW}. \quad (6)$$

where *T* is the dialysis session length in minutes, *UF* is the total ultrafiltration in kg and *BW* is the patient's dry body weight in kg. *spKt/Va* was used to obtain the equilibrated *Kt/V, eKt/V*(DIAMON).

According to Publication I the mean values for dialysis quality indicator *eKt/V* by blood, as a reference method, and *eKt/V* by DIAMON prototype, were not statistically different ($p < 0.05$) and estimated dialysis dose by DIAMON prototype utilising UV-method confirms the results obtained from the blood urea. Similar results, confirming good agreement between dialysis dose by blood and UV-absorbance, has also been reported earlier (Uhlin *et al* 2003 and 2005).

As we can see in Publication I, changes in values of *eKt/V* by DIAMON in a particular dialysis session are according to the changes in blood flow, except for some patients when the reason was a clinically unstable treatment due to unsteady blood pressure and access problems during which the adequate *eKt/V* determination was difficult. This was well observed from the continuously recorded UV-absorbance indicating deviations during the sessions as presented in Publication I. The DIAMON prototype estimated a higher dialysis dose for one patient (#8) than expected from the blood measurements that seems to be related to the relatively low body weight (< 55 kg) and thus lower urea distribution volume compared to other patients in this study. This difference can be eliminated by taking account this kind of clinical data – for example by utilizing more advanced algorithms for *eKt/V* calculation from UV-absorbance (Fridolin *et al* 2007).

The patient centric personalised HD treatment monitoring by the on-line optical method could be a step towards promoting more individualized dialysis treatment.

Many studies highlight the value of estimating urea removal as an index of dialysis adequacy (Bloembergen *et al* 1996, Shinzato *et al* 1997). The Dialysis Outcomes and Practice Patterns Study (DOPPS) grouped facilities similarly by changes in adherence to the K/DOQI dialysis dose guidelines using the percentage of patients with Urea Removal Rate (*URR*) <65% as an indication for improvement in this practice over time. The results showed again that those facilities with greatest improvement in adherence to *URR* guidelines had the greatest improvement in their patients' mortality risk and those with no change over time had essentially no improvements in outcomes (Port *et al* 2004).

The improvement in patient survival could be due to greater attention paid to all the details involved in delivering a more optimal dialysis prescription. On-line monitoring of dialysis adequacy and immediate identification of deviations in dialysis session is one of those important tools to follow.

In summary, Publication I demonstrated that a new, simple and miniaturized on-line monitoring device, DIAMON, instead of the unwieldy complicated spectrophotometer can estimate the delivered dose, and is reflecting the clinical condition of the patients and is very similar to that obtained by formal urea kinetic modeling.

3.3 Nutrition estimation of dialysis patients by optical on-line monitoring

Another parameter characterising dialysis adequacy and quality is the normalised protein nitrogen appearance *nPNA*. The *nPNA* is one tool to assess malnutrition, which is a strong predictor of death among hemodialysis patients (Fouque *et al* 2007).

A good correlation between UV-absorbance and a small removed waste solute such as urea enables the determination of *nPNA* (Uhlin *et al* 2005). Because protein requirements are determined primarily by fat-free, oedema-free body mass, *PNA* is usually normalized (*nPNA*) to some function of body weight (e.g., actual, adjusted, or standardized [by Second National Health and Nutrition Examination Survey – NHANES II] body weight) (NKF-DOQI 2006). A way to normalize *nPNA* is to use the "kinetic body weight" $V/0.58$ where V is calculated using some iterative algorithm or anthropometric formula (Suri *et al* 2004). The most common anthropometric formula is called the Watson formula (NKF-DOQI 2006).

The aim of this part of our study was to compare the patient's nutritional status by pre- and post-dialysis blood samples, the DIAMON prototype and Total Dialysate Collection (TDC).

The value of *nPNA* in g/kg/day (Publication I, II) was estimated according to the equation by VVSP UKM as

$$nPNA = 9.35 \frac{G}{V / 0.58} + 0.17 \qquad (7)$$

Formal urea kinetic modeling (UKM), based on the blood samples, calculates the urea distribution volume (V) and the urea generation rate (G) by mathematical iteration (Gotch *et al* 2005).

The total dialysate collection technique (TDC), or direct dialysis quantification (DDQ), uses *TRU* through collecting the entire dialysate exiting the dialyser over a dialysis treatment (Garred 1995). The modified DDQ (mDDQ), utilising the *TRU* and the blood samples, is successfully applied for validation of dialysis adequacy (Depner *et al.* 1996) enabling similarly to UKM to iteratively calculate the G and V values (Publication II).

$$V = \frac{\text{TRU} - G \times (T + 0.5) - \text{UF} \times \frac{C_{\text{pre}}}{0.93}}{(C_{\text{pre}} - C_{\text{r}})/0.93} \tag{8}$$

$$G = \frac{V \times \left(\frac{C_{\text{pre}} - C_{\text{r}}}{0.93} \right) + \left(W \times \frac{C_{\text{pre}}}{0.93} \right)}{\theta - 30} \tag{9}$$

where C_{pre} and C_{r} are the blood concentration of urea at the start of dialysis and the rebound urea concentration in mmol/L, W is the total interdialytic weight gain in kg, T and θ are the dialysis session length and the interdialytic time interval in min, respectively.

The *PNA* calculation in Publication I and II, from TDC and UV-absorbance, were based on earlier exploited methodology (Garred *et al.* 1995), assuming that the amount of urea could be approximated from measuring urea concentration from only one of the three treatments, and *nPNA* can be calculated as:

$$nPNA = Factor1,2or3 \left(\frac{TRU1,2or3}{V / 0.58} \right) + 0.17 \tag{10}$$

where *TRU* (or *TRU*a for the DIAMON prototype) 1, 2 or 3 is the amount of urea nitrogen in mg removed from the patient from the first (1), midweek (2) or last dialysis of the week (3). Factor 1, 2 or 3 is the fractional factor for, respectively, the first (1), midweek (2) and last (3) treatment in the week; factor 1 = 2.45; 2 = 2.89; 3 = 3.10. The V value was obtained from the Watson formula. Obligatory loss of dietary protein in stool and via skin shedding is represented by the constant term 0.17 (g protein/kg body weight/day).

TRU was estimated by the DIAMON prototype using the on-line UV-absorbance measurements according to the total dialysate collection method (TDC) under assumptions that the dialysate flow Q_{d} in L/min is constant, the total ultrafiltration *UF* in kg is known, and 1kg = 1L of the dialysate (Uhlin *et al.* 2005), as:

$$\begin{aligned} &\text{TRU}(mmol) = U_{\text{TDC}} * \left(Q_{\text{d}} * T + \text{UF} \right) = \\ &= \left(S * A_{\text{mean}} + I \right) * \left(Q_{\text{d}} * T + \text{UF} \right) \end{aligned} \tag{11}$$

where U_{TDC} in mmol/L is the urea concentration of the collected spent dialysate during the particular hemodialysis session, A_{mean} is the mean of all UV-absorbance values from the start to the end of the dialysis, T is the dialysis session length in minutes. The dialysate urea values from the last treatment of the week and the

corresponding on-line UV-absorbance values were used for a regression line between the UV-absorbance and dialysate urea from which the parameters slope *S* and intercept *I* were obtained. *G* (mg/min) was estimated using totally removed urea and interdialytic time interval θ, assuming that the urea generated is equal to the amount of urea removed.

The individual *nPNA* for each patient for three consecutive dialysis treatments during a seven-day period from UV-absorbance measured on-line by the DIAMON prototype is presented in Publication II, and as an average of a seven day period in Publication I.

Publication II shows the individual *nPNA* monitoring of each patient during a seven-day period by the DIAMON prototype and how *nPNA* can vary depending on the treatment day and the patient. Similar *nPNA* behaviour was obtained also for mDDQ (Publication II) and VVSP UKM (Publication I and II). The lower outcome for two patients in relation to others, who had several dialysis related difficulties (#9 was a new dialysis patient with hypoalbuminemia, and #10 noncompliant with the treatment), is clearly seen from the *nPNA* recordings which show the sensibility of the method (Publication I and II). Urea generation rate (*G*) is not constant over the interdialytic period (Raj *et al* 1997), and day-to-day variations in daily protein intake may result in *nPNA* fluctuating significantly. Considering the abovementioned, an individual *nPNA* for a seven-day period, calculated as an average of *nPNA* values over a seven-day period for each patient, could be a reasonable alternative for decision making instead of *nPNA* from individual sessions.

According to Publication I and II calculated dialysis adequacy and dietary protein intake from spent dialysate and urea kinetic modeling were comparable and are in correlation with the clinical condition of the patients. Simultaneous analysis of *Kt/V*, *URR* and *nPNA* permits us to get a picture of the adequacy of dialysis treatment and dietary protein intake in order to evaluate nutritional status of the patients, and gives the clinicians good opportunity to make correct decisions about dialysis effectiveness and in dietary counselling.

The present study shows that the new on-line monitoring device, DIAMON, is capable of estimating parameters regarding both the delivered dose of dialysis and nutritional status during hemodialysis (Publication I and II). The optical dialysis adequacy monitoring device enables individual *nPNA* estimation for each patient utilising continuous, on-line UV-absorbance measurements and the results are comparable to the *nPNA* values obtained by the kinetic modeling as shown in Publication I and II.

Each dialysis session can result in the removal of amino acids (about 10 to 12g), some peptides, and smaller amounts of protein (< 1 to 3g) (Chazot *et al* 1997). Moreover, uremic complications can promote protein catabolism to increase dietary protein requirements (Kopple 2001). Unfortunately, dietary counseling is not always successful to maintain an adequate protein and calorie intake (Caglar *et al* 2002). From this viewpoint it would be a great advantage to have a continuous on-line monitor for dialysis adequacy and nutrition without extra work from staff

and blood samples. Early detection of malnutrition along with utilized interventions could also save costs in health care.

3.4 Optical on-line monitoring of uric acid removal during dialysis

In fact, there is still research ongoing to find out the best and optimal method to estimate the dialysis adequacy and quality (Charra *et al* 2001). An optical method that makes it possible to acquire both the traditional parameters of dialysis quality (*Kt/V, URR*) as well as untraditional would be an excellent tool for the clinicians to validate the novel hypotesis. An alternative may be the quality parameters of dialysis that are based on other substances than urea (Gotch *et al* 2000).

It is generally accepted that urea, a traditional marker for dialysis quality, is not toxic per se, and can not be responsible for multifactorial and cumulative causes of uraemic toxicity (Vanholder *et al* 2003). An optical method, offering possibility to measure the elimination of other toxic or non-toxic substances that are retained in the uremic patients and with potential clinical significance (e.g. new potential biomarkers polypeptides (Weissinger *et al* 2004)), would enable to validate new hypotheses in this field. Moreover, a parameter based on total UV-absorbance and characterising overall retention of accumulated UV-absorbing solutes, may be an interesting alternative.

Several studies of the general population have suggested an association between uric acid level and cardiovascular outcomes (Hayden *et al* 2004, Feig *et al* 2008, Viazzi *et al* 2006, Høieggen *et al* 2004).

Uric acid is a water-soluble compound (molecular weight of 168.1 Da) that is the final metabolite of purine in humans. Elevated serum UA contributes to endothelial dysfunction and increased oxidative stress within the glomerulus and the tubulo-interstitium, with associated increased remodeling fibrosis of the kidney (Hayden *et al* 2004). A high level of serum UA, hyperuricemia, has been suggested to be an independent risk factor for cardiovascular and renal disease especially in patients with heart failure, hypertension and/or diabetes (Feig *et al* 2008, Viazzi *et al* 2006, Høieggen *et al* 2004).

Hyperuricemia is highly prevalent in patients with chronic kidney disease (CKD). Thus, uric acid may have a role as a uremia related cardiovascular risk factor in patients with CKD. It is unclear whether uric acid level is a marker for increased cardiovascular disease (CVD) and all-cause mortality in this patient population and whether the relationship between uric acid level and mortality is independent of traditional CVD risk factors. Also the pathogenic role of hyperuricemia in dialysis patients is not completely established (Navaneethan *et al* 2009).

In previous studies a good correlation between ultra-violet (UV) absorbance in the spent dialysate and the concentration of several solutes both in the spent dialysate and in the blood of dialysis patients has been presented, indicating that the technique can be used to estimate the removal of retained substances (Fridolin

et al 2002), and the fact that UA is a UV-absorbing solute (Jerotskaja *et al* 2007) makes this study even more interesting.

The aim of this part of the study (Publication III) was to estimate the total removed uric acid (TR_{UA}) by the on-line UV-absorbance measurements in the spent dialysate in two different dialysis centers in two countries, Estonia and Sweden.

The results in Publication III show the possibility to estimate Total Removed Uric Acid (TR_{UA}) from on-line UV-absorbance measurements during hemodialysis. The study also highlights the importance of standardization of issues such as geometry of the flow cuvette and dialyzer characteristics when general models are to be built. The work is ongoing to find a universal model to predict uric acid concentration in the spent dialysate. Many possibilities are available (e.g. regression models taking into account the dialyzer characteristics, multiwavelength approach by least square, inverse least square, partial least square methods) to create a general model with a satisfactory accuracy.

Similarly to the urea based *TRU* calculations which enables to estimate *nPNA*, related to the patient nutritional status, *TRua* could establish a new parameter related to CVD risk factors.

In the future, on-line UV-absorbance measurement during dialysis may be a monitoring tool for dialysis dose and also help the dialysis team to reach a less detrimental level of solutes such as uric acid that could have a direct impact on the patient morbidity and mortality.

CONCLUSIONS

The results of the study add knowledge to on-line optical monitoring of dialysis treatment.

1. A new, simple and miniaturized on-line monitoring device, DIAMON, instead of the unwieldy complicated spectrophotometer utilized in earlier studies, can estimate the delivered dose of dialysis and nutritional status during hemodialysis.
2. Identifying deviations in dialysis treatment enables the dialysis team to act immediately during the treatment to improve the session adequacy.
3. The UV optical on-line monitor enables continuous and on-line *eKt/V* and *nPNA* estimation for each patient as indicators of dialysis adequacy and dietary protein intake in order to evaluate quality of renal replacement therapy and nutritional status of the patients from spent dialysate without blood samples, disposables or chemicals or extra work for the staff.
4. Simultaneous analysis of *Kt/V*, *URR* and *nPNA* permits the dialysis team to get a picture of the adequacy of dialysis treatment and nutritional status of the patients, and gives the dialysis team the possibility to make a choice between increasing dialysis effectiveness, dietary counselling, or both.
5. The dialysis dose delivered to the patients estimated by the UV optical dialysis adequacy monitor is reflecting the clinical condition of the patients and is very similar to that obtained by formal urea kinetic modeling, and by modified direct dialysis quantification.
6. The UV optical on-line monitor is available to measure uric acid in the spent dialysate and in the future, on-line UV-absorbance measurement during dialysis may be a monitoring tool for dialysis dose and also help the dialysis team to reach a less detrimental level of solutes such as uric acid that could have a direct impact on the patient morbidity and mortality.

In the future, a multi-centre study to evaluate optical dialysis adequacy monitoring in a larger scale and for different dialysis modalities (HD vs HDF) from the perspective of conventional dialysis adequacy parameters and removal of uremic retention solutes would be valuable. Different uremic toxins affect the patient in many various ways and to a different extent, and improving the quality of dialysis monitoring of several uremic toxins is essential. Also, the exact identification of the remained prevalent peaks on the HPLC UV-absorption profiles of uremic fluids should be performed.

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II Fridolin, Ivo; Lauri, Kai; Jerotskaja, Jana; **Luman, Merike**. (2008). "Nutrition estimation of dialysis patients by on-line monitoring and kinetic modelling", *Estonian Journal of Engineering, Special issue on Biomedical Engineering*, June 14(2): 177-188.

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KOKKUVÕTE

Dialüüsravi doosi ja patsientide toitumuse hindamine optilise meetodiga

Käesolev töö uurib dialüüsravi doosi ja patsientide toitumuse hindamise võimalust reaalajas ilma vereproove võtmata optilise dialüüsi adekvaatsuse monitoriga DIAMON ja võrdleb tulemusi kahe dialüüsi kvaliteeti hindava kineetilise mudeliga – kogu dialüsaadi kogumisel põhineva meetodiga (TDC) ning dialüüsieelsetel ja -järgsetel vereanalüüsidel baseeruva uurea kineetilise mudeliga UKM. Tulemuste põhjal võrreldakse parameetrite vastavust patsientide kliinilise seisundiga. Uurimistööd on laiendatud kusiaine määramise metoodikaga dialüsaadis UV-meetodiga reaalajas.

Esimeses osas antakse ülevaade neerude osast organismis, neerupuudulikkusest ja tänapäeval kindlaks tehtud ureemiliste toksiinide osast kroonilise neerupuudulikkuse kliinilises pildis. Samuti antakse ülevaade neeruasendusravi meetoditest ja põhimõtetest ning dialüüsravi adekvaatsuse hindamise võimalustest ja olulisusest igapäevases kliinilises praktikas.

Teises osas on toodud ülevaade dialüüsravi kvaliteedi mõõtmise võimalustest ja tehnoloogiatest tänapäeval, alustades vere analüüsidel baseeruva ühekordse mõõtmise võimalusega kuni reaalajas dialüüsravi kvaliteedi mõõtmise meetoditeni. Täpsemalt on kirjeldatud ka antud uuringus kasutatud optilist UV-meetodil baseeruvat dialüüsravi kvaliteedi ja patsientide toitumuse jälgimise meetodit.

Kolmandas osas annab autor ülevaate tehtud eksperimentaalsetest uurimistöödest. Uuriti kümmet kroonilise dialüüsravi patsienti Põhja-Eesti Regionaalhaigla dialüüsi ja nefroloogia osakonnas. Hinnati kolme järjestikust dialüüsi ühe nädala jooksul ja võrreldi kvaliteedi ning toitumuse näitajaid vereanalüüside alusel, kogu dialüsaadi kogumise alusel ja monitoriga DIAMON mõõdetuna reaalajas. Kusiaine määramise uuringusse kaasati lisaks 6 hemodialüüsravi patsienti Rootsi Linköpingi Ülikooli Haiglast.

Töö tulemusena leiti järgmist:

1. Uue, lihtsa ja miniatuurse reaalajas monitoriga DIAMON on võimalik mõõta dialüüsi doosi ja patsiendi toitumust dialüüsravi patsientidel varem esitletud komplitseeritud spektrofotomeetri asemel.
2. Kõrvalekallete avastamine dialüüsravi ajal võimaldab kohest ravi korrigeerimist adekvaatsuse parandamiseks.
3. Optiline reaalajas monitor võimaldab pidevat *eKt/V* ja *nPNA* kui dialüüsi adekvaatsuse ja patsiendi toitumuse näitajate määramist igal patsiendil dialüsaadi põhjal ilma vereanalüüside võtmiseta ning lisavahendite ja -tööta neeruasendusravi kvaliteedi ja patsiendi toitumuse hindamiseks.
4. Samaegne *Kt/V* ja *nPNA* hindamine annab pildi dialüüsi adekvaatsusest ja patsiendi toitumusest ning aitab meditsiinipersonalil otsustada, kas on vaja tõsta dialüüsi efektiivsust või nõustada patsienti dieedi alal või on mõlemad vajalikud.
5. Optilise dialüüsi adekvaatsuse monitoriga mõõdetud dialüüsidoos vastab patsiendi kliinilisele seisundile ja on väga lähedane dialüüsidoosile, mis on

saadud uurea kineetilisest modelleerimisest, ja kogu dialüsaadi kogumise alusel saadud tulemustele.

6. Optilise reaalajas monitoriga on võimalik mõõta kusihapet dialüsaadis ja tulevikus võib reaalajas UV absorbtsiooni mõõtmine dialüüsiravi jooksul muutuda üheks dialüüsidoosi mõõtmise parameetriks ning võimaldada vähendada selliste kahjulike ja võimalik, et otseselt patsiendi surevusega seotud ainete nagu kusihape taset.

Võtmesõnad: dialüüs, uurea, dialüüsi doos, toitumus, reaalajas monitooring, optiline meetod, kineetiline modelleerimine, kusiaine.

ABSTRACT

Dialysis dose and nutrition assessment by an optical method

The thesis focuses on the estimation of dialysis dose and nutrition of the patients by on-line optical dialysis adequacy monitor (DIAMON) prototype without blood sampling. Results were compared with the results from the total dialysate collection (TDC) and with results from urea kinetic modeling (UKM) using pre- and post-dialysis blood samples. The study was extended to on-line monitoring of uric acid in the spent dialysate by UV-absorbance.

Section 1 presents an overview of the functions of the kidneys, kidney failure and the importance of uremic toxins in the clinical picture of renal failure. Also, the methods of renal replacement therapy (RRT) and technologies of dialysis quality assessment with association to importance in everyday clinical practice are represented.

Section 2 gives an extended picture of dialysis quality assessment technologies today based on pre- and post-dailysis blood samples to on-line methods. More exact description of the on-line optical UV method used in the study is included as well.

Section 3 presents an overview of the results of the author's experimental studies. Ten chronic dialysis patients from Dialysis and Nephrolgy Department of North Estonia Medical Centre were included in the study. Three consecutive dialysis sessions during one week were monitored and the indicators of the adequacy of dialysis treatment and the patients' nutritional status were compared by pre- and post-dialysis blood samples, Total Dialysate Collection and the DIAMON prototype. 6 more patients from Linköping University Hospital in Sweden were included to the study on the estimation of uric acid.

The results of the study add knowledge to on-line optical monitoring of dialysis treatment.

1. A new, simple and miniaturized on-line monitoring device, DIAMON, instead of the unwieldy complicated spectrophotometer utilized in earlier studies, can estimate the delivered dose of dialysis and nutritional status during hemodialysis.
2. Identifying deviations in dialysis treatment enables the dialysis team to act immediately during the treatment to improve the session adequacy.
3. The optical on-line monitor enables continuous and on-line *eKt/V* and *nPNA* estimation for each patient as indicators of dialysis adequacy and dietary protein intake in order to evaluate quality of RRT and nutritional status of the patients from the spent dialysate without blood samples, disposables or chemicals or extra work for the staff.
4. Simultaneous analysis of *Kt/V*, *URR* and *nPNA* permits the dialysis team to get a picture of the adequacy of dialysis treatment and nutritional status of the patients, and gives the dialysis team the possibility to make a choice between increasing dialysis effectiveness, dietary counselling, or both.

5. The dialysis dose delivered to the patients estimated by the optical dialysis adequacy monitor is reflecting the clinical condition of the patients and is very similar to that obtained by formal urea kinetic modeling, and by modified direct dialysis quantification.
6. The optical on-line monitor is available to measure uric acid in the spent dialysate and in the future, on-line UV-absorbance measurement during dialysis may be a monitoring tool for dialysis dose and also help the dialysis team to reach a less detrimental level of solutes such as uric acid that could have a direct impact on the patient morbidity and mortality.

Keywords: dialysis, urea, dialysis dose, nutrition, on-line monitoring, optical method, kinetic modeling, uric acid.

APPENDIX 1

PUBLICATIONS

Publication I

Merike Luman, Jana Jerotskaja, Kai Lauri, Ivo Fridolin. (2009). "Dialysis dose and nutrition assessment by optical on-line dialysis adequacy monitor", *Clinical Nephrology*, vol 72 (4), pp.303-311.

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Dialysis dose and nutrition assessment by optical on-line dialysis adequacy monitor

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Key words
hemodialysis efficiency – nutrition – ultraviolet absorption – on-line monitoring

Abstract. Aim: In light of the variability of dialysis sessions, on-line monitoring could improve hemodialysis (HD) adequacy. A new optical Dialysis Adequacy Monitor (DIAMON) prototype enables to estimate dialysis dose and protein nitrogen appearance (PNA) at every dialysis session. The aim of this study was to compare the adequacy of dialysis treatment and the patient's nutritional status by pre-and post-dialysis blood samples, the DIAMON prototype and Total Dialysate Collection (TDC). Material and methods: Ten patients were monitored during three consecutive hemodialysis sessions during one week. Blood samples were drawn before the start of dialysis and at the end of dialysis. The DIAMON prototype was connected to the fluid outlet of the dialysis machine with all spent dialysate passing through during the on-line experiments, and TDC was performed for all dialysis treatments. Equilibrated Kt/V (eKt/V) values were estimated from blood-urea (eKt/Vb) and from DIAMON (eKt/Va), and normalized PNA (nPNA) values from TDC and DIAMON, respectively. The variable volume single pool urea kinetic modeling (VVSP UKM) was also utilized for single-pool Kt/V (spKt/V) and nPNA estimation. Results: The mean ± SD given by eKt/Vb was 1.08 ± 0.22 (n = 30), and eKt/Va 1.05 ± 0.21 (n = 28). The mean ± SD of nPNA was 0.73 ± 0.15 g/kg/day (n = 29) from TDC, and 0.73 ± 0.14 g/kg/day (n = 28) using DIAMON prototype. The mean values of eKt/V from blood samples and nPNA from TDC were not statistically different from the corresponding values estimated by DIAMON ($p < 0.05$). Generally the delivered dialysis dose and dietary protein intake of the patients observed during the study using the DIAMON prototype was very similar to that obtained by TDC and VVSP UKM. Conclusion: The optical dialysis adequacy sensor, DIAMON, provides continuous, on-line measurements of dialysis adequacy and permits longitudinal analysis of the delivered dialysis dose and patient's nutritional status, and can immediately identify, and alert to, any deviations in dialysis treatment.

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Introduction

Many studies have demonstrated a relationship between delivered dialysis doses as measured in Kt/V and morbidity and mortality in chronic hemodialysis patients [Keshaviah et al. 2002, Port et al. 2002, Salvatore et al. 2005]. Under K/DOQI Clinical Practice Guidelines for Hemodialysis Adequacy, 2000, the dialysis care team should deliver a single-pool variable volume Kt/V of at least 1.2 and equilibrated Kt/V (eKt/V) of 1.05 for both adult and pediatric hemodialysis patients, and under European Best Practice Guidelines on Hemodialysis (EBPG), the prescribed target eKt/V should be at least 1.2 for anuric patients treated by dialysis three times per week.

The delivered Kt/V is usually calculated by means of the single-pool, variable volume urea kinetic model (VVSP UKM), which requires an accurate measurement of urea clearance and the collection of blood samples at the start and at the end of dialysis session, which depends on accuracy and timing of drawing the samples and laboratory errors [K/DOQI].

High blood concentration of urea is not necessarily related with a poor outcome if removal is sufficient (e.g., in CAPD patients and/or in patients receiving a high protein diet) [Blumenkrantz et al. 1982], but also protein-energy malnutrition is frequently present in patients undergoing hemodialysis therapy. Several studies have suggested that malnutrition is an important risk factor for morbidity and mortality in HD patients [Lindsay et al. 1994]. Maintaining an adequate dose of hemodialysis may improve nutrition and patient survival. In order to optimize the diet of patients with renal diseases, dietary protein

intake has to be controlled. Protein nitrogen appearance (PNA), formerly protein catabolic rate (PCR) [EBPG], is easily obtainable from UKM, and in patients who are not markedly catabolic or anabolic, the normalized PNA (nPNA) correlates closely with dietary protein intake [Flanigan et al. 1995, Keane and Collins 1994]. A common way to normalize PNA is to use V/0.58 or the dry body weight [Canaud et al. 1997].

The NCDS showed that a high nPNA (presumably reflective of better dietary protein intake) was associated with lower morbidity or lesser likelihood of treatment failure [Gotch and Sargent 1985]. An nPNA below 1g/kg/day has been shown to be associated with increased morbidity and mortality [Acchiardo et al. 1983].

As the delivered dialysis dose may be lower than prescribed and can vary between dialysis sessions due to fistula function, patient's tolerance of the treatment, workload of the nurses etc., it is extremely important to know how much of the prescribed dose is actually delivered.

Due to great variations between patients and dialysis sessions [McIntyre et al. 2003], evaluation of every hemodialysis session by an on-line monitoring system makes it possible to provide an adequate dialysis dose to the HD patients [Lambie et al. 2004]. To achieve and maintain the pre-set treatment dose, an on-line monitoring system has been suggested as a more accurate method compared to monthly blood sampling [Hernandez-Herrera et al. 2001, K/DOQI, Locatelli et al. 2005].

The possibility of estimating Kt/V, TRU and PCR by ultraviolet (UV) light-absorbance measurements on the spent dialysate has been demonstrated. The UV-method measures all UV-absorbing compounds in the spent dialysate. A good correlation between the UV-absorbance and urea enables to estimate urea based dialysis adequacy parameters by the UV-technique even if the urea is not directly measured [Uhlin et al. 2003, 2005]. Moreover, an optical dialysis adequacy monitor (DIAMON) prototype based on on-line UV-absorbance measurements has been developed [Fridolin et al. 2007].

The aim of this study was to compare the adequacy of dialysis treatment and the patient's nutritional status using the parameters eKt/V and nPNA estimated using pre-and post-dialysis blood samples, the optical dialysis adequacy monitor and total dialysate collection.

Subjects and Methods

Subjects

Ten patients were included in the study: 3 females and 7 males, mean age 62.6 ± 18.6 years on chronic thrice-weekly hemodialysis treatment. The Ethics Committee approved the study protocol and an informed consent was obtained from every patient.

Each patient was monitored during three consecutive dialysis sessions during one week. All patients were dialyzed with polysulfone membrane dialyzers (Fresenius Medical Care, Bad Homburg, Germany): 1) 4 patients on 12 treatments with a low flux F8 HPS dialyzer with an effective membrane area of 1.8 m^2; 2) 1 patient, i.e. three treatments, was dialyzed with a low flux membrane F10 HPS dialyzer with a membrane area of 2.2 m^2 and 3) 5 patients on 15 sessions with a high flux FX 80 dialyzer with effective membrane area of 1.8 m^2. The dialysate flow rate was constant at 500 ml/ min. The prescribed blood flow was 350 or 300 ml/min during the two treatments within the week according to the patient records and 245 ml/min for the one treatment, and was kept constant throughout the dialysis session. Duration of dialysis sessions was from 190 – 240 min. Four patients had dialysis access in the form of an arterio-venous fistula, 3 patients in the form of an artificial graft, and 3 patients had a temporary catheter of the jugular or femoral vein. All patients were dialyzed using a two-needle system. Additional information about the studied patients is shown in Table 1. The dialysis machine used in the study was a Fresenius 4008H (Fresenius Medical Care, Bad Homburg, Germany).

Dialysis Adequacy Monitor (DIAMON) prototype

DIAMON has incorporated a light source (280 ± 5 nm UV LED), a detector (GaNi UV-photodiode), an electronic circuit board,

Table 1. Data of the studied patients.

Patient	Kidney disease	Age	Gender	Dialyzer type and membrane area	BMI	Serum albumin (g/l)	Dialysis access
1	Goodpasteure's syndrome	69	F	F8HPS-1.8 m^2	28.6	41.1	graft
2	Renal tuberculosis	63	M	F10HPS-2.2 m^2	28.1	37.8	a/v fistula
3	Diabetic nephropathy	70	M	FX-80-1.8 m^2	25.5	42.4	graft
4	Tubulointerstitial nephritis	65	M	F8HPS-1.8 m^2	23.9	42.7	a/v fistula
5	Myeloma	52	F	F8HPS-1.8 m^2	24.5	43.4	graft
6	Hypertension	80	M	F8HPS-1.8 m^2	19.6	41	a/v fistula
7	Diabetic nephropathy	73	M	FX80-1.8 m^2	38.3	42	a/v fistula
8	Glomerulonephritis	46	F	FX80-1.8 m^2	23.9	41	Temporary catheter
9	Diabetic nephropathy	44	M	FX80-1.8 m^2	25.0	29	Temporary catheter
10	Glomerulonephritis	65	M	FX80-1.8 m^2	24.9	27	Temporary catheter

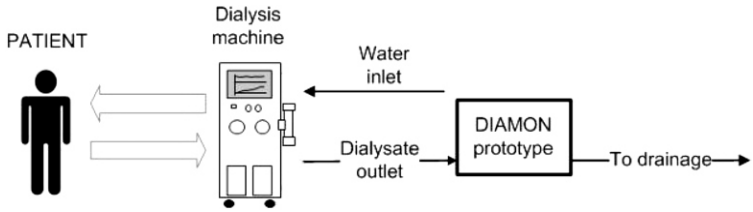

Figure 1. The clinical experimental set-up.

and an optical cuvette. The monitor was connected to the fluid outlet of the dialysis machine with all spent dialysate passing through during the on-line experiments. The transmitted light intensity of the spent dialysate was measured. The sampling frequency was set to 20 samples per minute. The obtained intensity values were processed to obtain UV-absorbance and presented on the computer screen by a PC using Ldiamon software (AS Ldiamon, Estonia, for Windows). The experimental set-up is shown in Figure 1.

Sampling and laboratory analysis

Blood samples were drawn before the start of the dialysis treatment and at the end of dialysis using the slow flow sampling technique by K/DOQI Guidelines.

Total dialysate collection (TDC) started when the blood had filled the dialyzer and ended when the blood was returned to the patient at the end of the dialysis. All spent dialysate was collected in a tank calibrated against a weighing-machine. After careful stirring and recording of the weight of the collected spent dialysate, the TDC sample (UreaTDC) was collected.

Five dialysate samples were also taken from the drain tube: 10, 60, 120 and 180 minutes after the start of the dialysis session, and immediately before the end of the treatment. The samples were sent to the laboratory and analysis of urea concentration was performed within 1 to 4 hours.

The concentration of urea was determined at the Clinical Chemistry Laboratory at North Estonia Medical Centre using a standardized method. The accuracy of the method for determination of urea in blood and dialysate was ± 5%.

Estimation of dialysis dose and nutrition

Dialysis dose from blood, eKt/V (blood), was calculated according to the Daugirdas [1995] second-generation formula using the pre-and post- dialysis urea concentrations (C_0 and C_t). For determination of the dialysis dose from UV-absorbance measured by the DIAMON prototype, eKt/V(DIAMON), instead of the pre-and post-dialysis blood urea concentrations the maximum UV-absorbance value in the beginning and the minimum UV-absorbance value at the end of dialysis (A_0

and A_t) were utilized. The single pool volume Kt/V, spKt/Va was calculated as:

$$spKt/Va = \ln\left(\frac{A_t}{A_0} - 0.008\frac{T}{60}\right) + \left(4 - 3.5\frac{A_t}{A_0}\right)\frac{UF}{BW} \quad (1)$$

Where T is the dialysis session length in minutes, UF is the total ultra filtration in kg and BW is the patient's dry body weight in kg. spKt/Va was used to obtain the equilibrated Kt/V, eKt/V(DIAMON).

Totally removed urea, TRU, from TDC was calculated as urea concentration $Urea_{TDC}$ (mmol/l)* collected dialysate weight (kg), assuming that 1 kg = 1 l of the dialysate. Assuming that the dialysate flow, Qd(t), is constant and the total ultra filtration (UF) is known, TRU may be calculated from the on-line UV-absorbance (TRUa) as [Uhlin et al. 2005]

$$TRUa = (Slope * Mean\ A + Intecept) * (Qd * T + UF)/1000 \quad (2)$$

Where Mean A is the mean of all on-line UV-absorbance values from the start to the end of the dialysis and Qd is the rate of the dialysate flow in ml/min. The value 1000 is used to convert TRUa into mmol. The parameters Slope and Intercept are obtained from the regression line between the UV-absorbance and dialysate urea [Uhlin et al. 2005] where the dialysate urea values from the last treatment of the week and the corresponding on-line UV-absorbance values were used.

The PNA calculation, from TDC and UV-absorbance, were based on earlier exploited methodology [Garred et al. 1995], assuming that the amount of urea could be approximated from measuring urea concentration from only one of the three treatments, and nPNA can be calculated as:

$$nPNA = Factor\,1,2,or\,3\left(\frac{TRU\,1,2,or\,3}{V/0.58}\right) + 0.17 \quad (3)$$

where TRU (or TRUa for the DIAMON prototype) 1, 2 or 3 is the amount of urea nitrogen in mg removed from the patient from the first (1), midweek (2) or last dialysis of the week (3) and Factor 1, 2 or 3 is the fractional factor for, respectively, the first (1), midweek (2) and last (3) treatment in the week; factor 1 = 2.45; 2 = 2.89; 3 = 3.10. The V value was obtained from the Watson formula. Obligatory loss of dietary protein in stool and via skin shedding is represented by the constant term 0.17 (g protein/kg body weight/day).

Variable volume single pool urea kinetic modeling (VVSP UKM) was also utilized for spKt/V and nPNA estimation. nPNA was estimated according to the equation

$$nPNA = 9.35\frac{G}{V/0.58} + 0.17 \quad (4)$$

The G and V values were obtained from the iterative VVSP UKM calculation. The dialysers blood urea clearance in vitro, Kb, was calculated as in Daugirdas and Depner [1994]. Effective blood urea clearance in vivo was estimated as being 20% lower from the Kb value [Daugirdas and Depner 1994].

Assuming that the urea generation rate (G) is equal to the amount of urea removed [Garred et al. 1995], and considering that the urea generation rate (G) is not constant over the interdialytic period [Raj et al. 1997]; day-to-day variations in daily protein intake may result in PNA fluctuating significantly. Therefore an individual nPNA for a seven-day period was calculated as an average of nPNA values over a seven-day period for each patient. A residual renal function was not taken into account.

Statistical analysis

The results are presented as mean ± SD. The DIAMON prototype data was not obtained for two sessions because of a technical computer failure during the data collection. eKt/V from blood samples and nPNA from TDC were not calculated for one session due to laboratory errors. Paired t-test (two-tailed) was used to compare means for different methods, and $p < 0.05$ was considered significant. The different methods were compared using Bland and Altman plot.

Results

The mean ± SD given by eKt/V (blood) was 1.05 ± 0.19 (n = 29), and eKt/V (DIAMON) 1.05 ± 0.21 (n = 28). The mean values for eKt/V(blood) and eKt/V(DIAMON) were not statistically different ($p < 0.05$). Values of

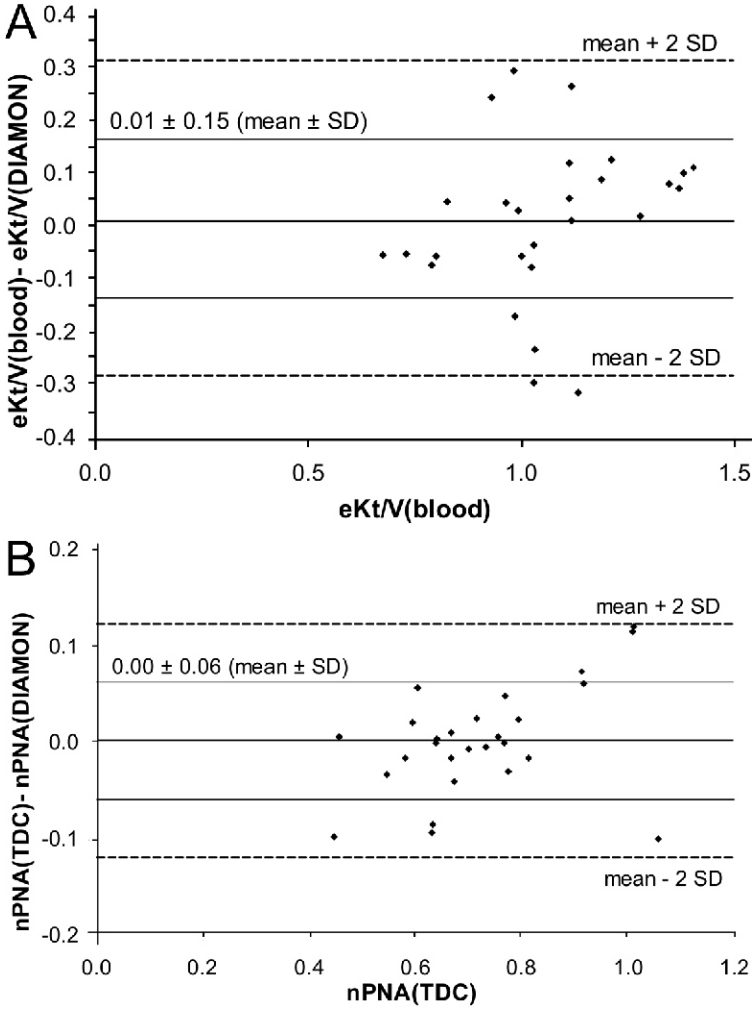

Figure 2. Comparison of the estimated parameters eKt/V and nPNA for all 10 patients as the differences between: (A) eKt/V (blood) and eKt/V (DIAMON) (number of HD sessions n = 27) plotted against eKt/V (blood), and (B) nPNA (TDC) and nPNA (DIAMON) (n = 27) plotted against nPNA (TDC).

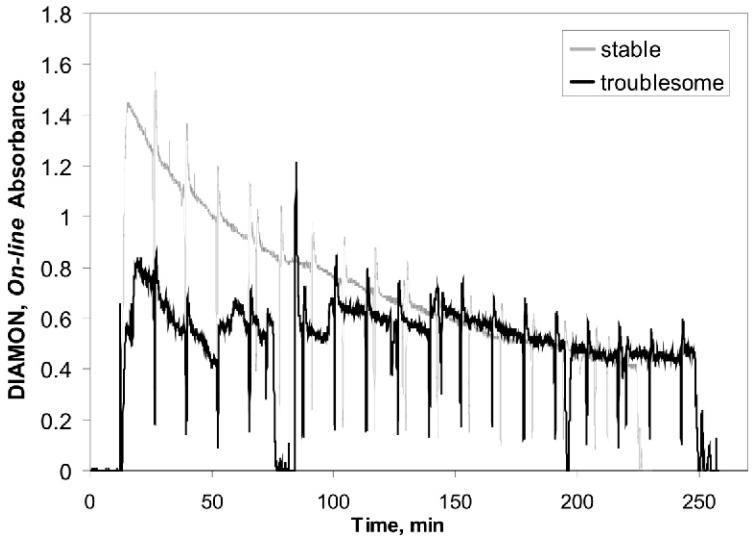

Figure 3. Continuously recorded UV-absorbance during a single troublesome dialysis treatment indicating unstable clearance due to malfunctioning central venous catheter for the patient 10. A stable treatment for the patient 5 is given for comparison.

nPNA (mean ± SD) were 0.73 ± 0.15 (n = 29) from TDC, and 0.73 ± 0.14 (n = 28) using DIAMON prototype. The mean values of nPNA from TDC and DIAMON were not statistically different ($p < 0.05$).

Figure 2 presents Bland and Altman plot for eKt/V and nPNA in all 30 treatments. Figure 2A presents the differences as mean ± SD having the value of 0.01 ± 0.15 (n = 27) for eKt/V(DIAMON) compared to eKt/V(blood) plotted against eKt/V(blood). Figure 2B shows the differences between nPNA (TDC) and nPNA (DIAMON) plotted against nPNA (TDC) having the value of 0.00 ± 0.06 (n = 27). The deviating values larger than ± 1 SD appeared primarily due to troublesome treatments (Figure 3).

Figure 4 shows eKt/Va in 28 treatments separately grouped by the patients followed during one week, 3 dialysis sessions each, measured by the DIAMON prototype. The data from two dialysis sessions (Patient 2, session no. 3, and Patient 3, session no. 1) were not collected due to a technical computer failure. The reduced values can be observed when the blood flow was decreased by approximately 25%.

Figure 5 shows the nPNA individually for each patient as an average of a seven-day period from TDC, and measured by the DIAMON prototype. General picture of the nPNA approaches to that of eKt/V for a single patient, i.e. for higher eKt/V higher nPNA is obtained.

Figure 6 presents the results from UKM VVSP for all patients as TAC versus nPNA. A line that determines minimum dialysis dose (spKt/V = 1.2) is also given. This figure gives a comparative picture about the status of dialysis adequacy and points up differences for the studied patients.

Discussion

The present study investigated the adequacy of dialysis treatment and the patient's nutritional status using the parameters eKt/V and nPNA estimated using pre-and post-dialysis blood samples, the optical dialysis adequacy monitor and total dialysate collection.

The results indicated that: (i) a new, simple and miniaturized on-line monitoring device, DIAMON, instead of the unwieldy com-

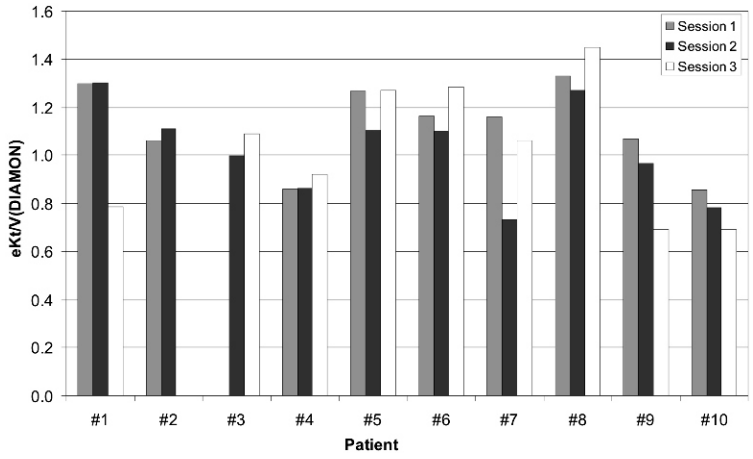

Figure 4. eKt/V (DIAMON) for all treatments separately grouped by the patients followed during one week, 3 dialysis sessions each.

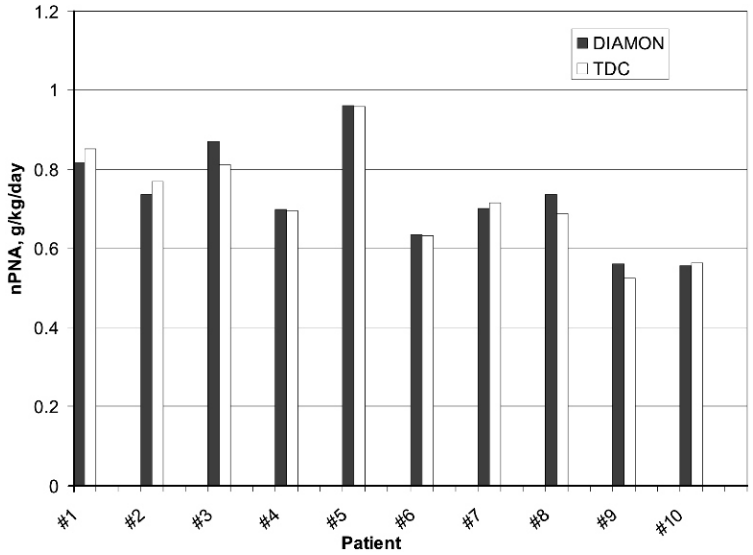

Figure 5. Individual nPNA for each patient as an average of a seven-day period from TDC, and using UV-absorbance measured by the DIAMON prototype.

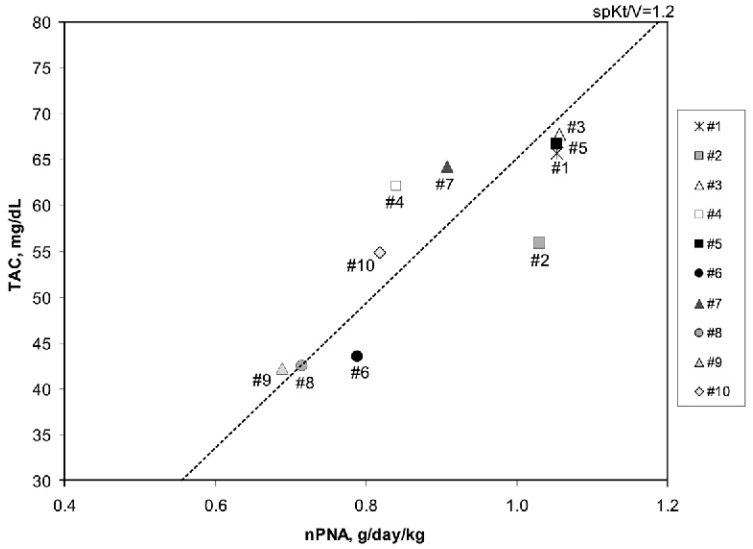

Figure 6. Results from UKM VVSP presented as TAC = f (nPNA) for all patients. A dashed line (spKt/V = 1.2) is also shown.

plicated spectrophotometer utilized in earlier studies [Uhlin et al. 2003, 2005] can estimate the delivered dose of dialysis and nutritional status during hemodialysis; (ii) the dialysis dose delivered to the patients estimated by the optical dialysis adequacy monitor is reflecting the clinical status of the patients and is very similar to that obtained by UKM.

In our study, Patients 1 – 7 have been on dialysis more than 6 months and had a stable clinical condition. Patients 8 and 9 started dialysis in an acute situation via central venous catheter. Patient 10 discontinued treatment himself, but it was started once again due to an emergency.

The prescribed blood flow was decreased for the second dialysis session for all patients except for Patient 1, in the case of whom the decrease was applied for the third dialysis session; this explains the decreased eKt/V for those sessions. Figure 2A depicts the differences between the dialysis dose as eKt/V calculated from the blood urea concentrations and from the UV-absorbance measured by the DIAMON prototype for all patients as Bland and Altman plot. The estimated dialysis dose by DIAMON prototype utilizing UV-method confirms the results obtained from the blood urea calculated as eKt/V. This confirms previously reported studies where dialysis dose measured by the UV-method gives results similar to as calculated from the blood urea [Uhlin et al. 2003, 2005]. The deviating values larger than ± 1 SD were mostly related to troublesome treatments (Figure 3). To decrease the effects of the treatment instability two measurement points were utilized for eKt/V calculations instead of slope in this paper. An algorithm capable to calculate slope based eKt/V from UV-absorbance on-line measurements for troublesome treatments is under development. Figure 2B shows a good agreement between nPNA (TDC) and nPNA (DIAMON) as for the UV-method from the earlier studies [Uhlin et al. 2005].

Figure 4 shows the delivered dialysis dose as eKt/V(DIAMON) at every HD session for the studied patients. With regard to different patients, it should be mentioned that the ordinary prescription for Patients 2 and 4 was 4 dialysis sessions a week compared to the thrice-weekly schedule during the study. The reasons were a high BMI and high ultra filtration need in the case of Patient 2; while Pa-

tient 4 did not tolerate higher dialysis dose during one session due to serious cardiac insufficiency. This explains the lower delivered dialysis dose during this study for Patient 4. Moreover, this indicates that dialysis dose for one session could be on a borderline level to guarantee adequate dialysis dose and nutritional status for a longer period. Changes in values of Kt/V for a particular dialysis session are according to the changes in blood flow, except for Patients 9 and 10, when Kt/V is seen as decreasing with every session despite higher blood flow in the first and last session. The reason was a clinically unstable third treatment due to unsteady blood pressure and access problems for both mentioned patients during which the adequate eKt/V determination was difficult. This was observed from the continuously recorded UV-absorbance indicating deviations during the sessions as presented in Figure 3 for Patient 10. The DIAMON prototype estimated a higher dialysis dose for Patient 8 than expected from the blood measurements that seems to be related to the relatively low body weight (< 55 kg) and thus lower urea distribution volume compared to other patients in this study. This difference can be eliminated by taking account this kind of clinical data – for example by utilizing more advanced algorithms for eKt/V calculation from UV-absorbance [Fridolin et al. 2007].

Figure 5 shows nPNA (g/day/kg) comparison from the total dialysate collection (TDC) and with DIAMON prototype for every patient. Patients 9 and 10 have the lowest nPNA values due to the reasons mentioned above. Patient 5 had a good dialysis dose, but relatively high nPNA could be due to myeloma and paraproteinemia.

Figure 6 gives a comparative picture about every patient obtained using UKM VVSP and represented as Time Average urea Concentration, TAC, relative to nPNA (for a seven-day study period). A line that determines minimum dialysis dose (spKt/V = 1.2) is also given. Considering decreased blood flow during one treatment, it can be seen that the dialysis dose delivered to the Patients 1, 2, 3, 5, 6, 8 seems to be adequate. The result for the Patient 4 is also expected because the effect of less delivered dialysis during the study compared to the ordinary prescription (3 times vs. 4 times per week) and decreased clearance for one session can clearly be observed. The delivered dialysis dose for Patients 9 and 10 needs to be increased, and attention should be paid also to the somewhat low nPNA values. Patient 9 was a new patient with Type 1 diabetes and hypoalbuminemia and the dialysis treatment was initiated at the inception of uremic symptoms and hypervolemia in an acute situation. Patient 10 had an ineffective dialysis treatment because a malfunction of central venous catheter caused severe disturbances during the dialysis treatments (Figure 3), and due to insufficient nPNA and hypoalbuminemia due to chronic alcoholism and non-compliance with the treatment. Moreover, a low dialysis dose for the studied week for Patient 7 can also be seen, probably caused by interrupted second treatment due to problems with blood clotting. This is clearly seen from the eKt/V results estimated by the DIAMON prototype (Figure 4). This example stresses the importance of continuous and long-term monitoring of the delivered dialysis dose and nutritional status, helping to avoid misinterpretation of the dialysis dose estimate due to accidental and non-systematical errors arising when only a single treatment is observed.

The general picture of the dialysis dose delivered to the patients during the study using the DIAMON prototype is very similar to that obtained by UKM. Both methods reveal that out most of the patients are adequately dialyzed and point up some critical patients. The somewhat low dialysis dose is caused by decreased blood flow for one treatment in the week, the purpose of which was to vary dialysis sessions in order to compare results from the DIAMON prototype, total dialysate collection and UKM in different clinical situations. Calculated dialysis adequacy and dietary protein intake from spent dialysate and urea kinetic modeling were comparable and are in correlation with the clinical status of the patients.

Interestingly, the average value of nPNA estimated by the TDC and DIAMON prototype is somewhat lower than the average value of nPNA calculated from VVSP UKM. Similarly, a tendency for lower nPNA values for TDC and UV-method was reported in other studies [Garred et al. 1995, Uhlin et al. 2005]. This could be related to methodological differences that should be explored fur-

ther. One reason could lie in a residual renal function that was not taken into account. Moreover, a rebound effect is not considered in the VVSP UKM calculations. Because the UKM and TDC results are highly dependent on collected blood and dialysate urea values, attention should be paid on sampling and laboratory errors, also correct estimation of in vivo dialyzer's blood urea clearance is important in VVSP UKM.

The complexity of calculations in formal urea kinetic modeling requires the use of computational devices, and software is still a restriction. Even if the cost is not very high, this remains a consideration for smaller hemodialysis units. Secondly, extra work and time required for the staff to accurately collect and process all patient data for these calculations may be significant in all hemodialysis centers, especially larger ones [Pereira et al. 2005].

Simultaneous analysis of Kt/V, URR and nPNA permits us to get a picture of the adequacy of dialysis treatment and dietary protein intake in order to evaluate nutritional status of the patients, and enables the dialysis team to make a choice between increasing dialysis effectiveness, dietary counseling, or both. It would be a great advantage to have a continuous on-line monitor for dialysis adequacy and nutrition without extra work for the staff and blood samples.

Conclusion

The optical dialysis adequacy monitor enables continuous and on-line measurements of the delivered dialysis dose and the patient's dietary protein intake, and can immediately identify any deviations in dialysis treatment due to changes in the dialyzer performance or the removal of solute from the blood.

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PUBLICATIONS

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Nutrition estimation of dialysis patients by on-line monitoring and kinetic modelling

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Abstract. The aim of this study was to estimate a nutritional parameter, normalized protein nitrogen appearance (nPNA), for haemodialysis (HD) patients by on-line monitoring with the optical dialysis adequacy monitor (DIAMON) prototype, by the modified direct dialysis quantification (mDDQ), and by the volume-variable single-pool urea kinetic modelling (VVSP UKM). Ten HD patients were monitored on-line by the DIAMON prototype during three consecutive haemodialysis sessions during one week. Blood samples were taken and the total dialysate collection (TDC) was performed during all dialyses. The nPNA values were estimated by DIAMON, mDDQ and VVSP UKM; nPNA was normalized by $V/0.58$ and by the measured dry body weight, efBW. Individual nPNA for each patient during a seven-day period was estimated using UV-absorbance measured on-line by the DIAMON prototype. The nPNA values (mean $\pm$SD) in g/kg/day for the total material were: 1) 0.74 ± 0.12 from DIAMON ($N = 28$), 0.90 ± 0.26 from mDDQ ($N = 29$) and 0.90 ± 0.23 from VVSP UKM ($N = 30$) normalized by $V/0.58$, and 2) 0.68 ± 0.10 from DIAMON ($N = 28$), 0.72 ± 0.19 from mDDQ ($N = 29$) and 0.80 ± 0.18 from VVSP UKM ($N = 30$) normalized by efBW. The optical device for monitoring the dialysis adequacy enables individual nPNA estimation for each patient using continuous, on-line UV-absorbance measurements. The results are comparable to the nPNA values obtained by the kinetic modelling. Still a question remains concerning the normalization of PNA.

Key words: dialysis, nutrition, urea, protein nitrogen appearance, ultraviolet absorption, on-line monitoring, kinetic modelling.

1. INTRODUCTION

Dialysis is the most common method for treating advanced and permanent kidney failure. The most popular clinical parameters from urea kinetic modelling (UKM), characterizing dialysis adequacy, are the dialysis dose Kt/V and the

normalized protein nitrogen appearance nPNA. The dialysis dose has been reported to have a great significance for the outcome of the dialysis treatment [1,2]. The nPNA is one tool to assess malnutrition, which is a strong predictor of death among haemodialysis patients [2]. Formal UKM, based on the blood samples, calculates the urea distribution volume (V) and the urea generation rate (G) by mathematical iteration [3]. The VVSP UKM model is suggested for dialysis adequacy estimation [1,2].

The total dialysate collection technique, or direct dialysis quantification (DDQ), uses the total removed urea nitrogen (TRU) through collecting the entire dialysate, exiting the dialyser over a dialysis treatment [4]. The mDDQ, using TRU and the blood samples, is successfully applied for validation of dialysis adequacy [5], enabling similarly to UKM iteratively calculate the G and V values. An alternative approach is to estimate TRU over a 7-day period, which corresponds to the urea generated within the same time period for the anuric patients, and calculate nPNA without blood sampling [4]. An approximation of the mentioned method is nPNA determination from a single measurement of dialysate urea, assuming that the first, midweek, and last dialysis account for nearly constant fractions of the week's urea removal for thrice weekly dialysis cycle [6].

On-line monitoring of the dialysis dose has been suggested as a valuable tool to ensure adequate dialysis prescription [7]. Recently spectrophotometrical sensors for on-line monitoring of total ultraviolet (UV) absorbance or urea in the spent dialysate have been presented, aiming to follow continuously a single haemodialysis session [8–11]. A good correlation between UV-absorbance and a small removed waste solute such as urea enables the determination of Kt/V for urea [12], and nPNA [13]. Furthermore, a new prototype device, DIAMON, has been designed for continuous on-line estimation of delivered dialysis dose from optical dialysate measurements [14].

Because protein requirements are determined primarily by fat-free, oedema-free body mass, PNA is usually normalized to some function of body weight (e.g., actual, adjusted, or standardized [NHANES II] body weight) [1]. A way to normalize nPNA is to use the "kinetic body weight" $V/0.58$, where V is calculated using some iterative algorithm or anthropometric formula [15]. The most common anthropometric formula is called the Watson formula [1]. The measured dry body weight or oedema free body weight (efBW) [2], obtained post-dialysis in HD patients, has been used [6], which may lead to nPNA underestimation [15,16]. However, efBW may be used effectively when the value is close to the standardized body weight [1].

The aim of this study is to estimate patient nutritional parameter nPNA individually by the on-line DIAMON prototype, by formal urea kinetic modelling, and by modified direct dialysis quantification.

2. SUBJECTS AND METHODS

2.1. Subjects

This study was performed after approval of the protocol by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. A consent was obtained from all participating patients.

Ten patients were investigated, three females and seven males, mean age 62.6 ± 18.6 years, on chronic thrice-weekly haemodialysis treatment. Each patient was monitored during three consecutive dialysis sessions during one week. As dialysis access four patients had arterio-venous fistula, three patients had artificial graft, and three patients had temporary catheter of *v. jugularis* or *femoralis*. All patients were dialysed using a two-needle system. All patients were dialysed with polysulfone membrane dialysers (Fresenius Medical Care, Germany): (1) four patients with 12 treatments by low flux dialyser F8 HPS with an effective membrane area of $1.8\ m^2$ and an ultrafiltration coefficient of 18 mL/h·mmHg; (2) one patient with three treatments was dialysed with low flux membrane dialyser F10 HPS with membrane area $2.2\ m^2$, ultrafiltration coefficient of 21 mL/h·mmHg; (3) five patients on 15 sessions with high flux dialyser FX 80 with effective membrane area of $1.8\ m^2$ and ultrafiltration coefficient of 59 mL/h·mmHg. The dialysate flow rate was fixed at 500 mL/min. The prescribed blood flow was 350 or 300 mL/min during the two treatments within the week according to the patient records and 245 mL/min for one treatment, and was kept constant throughout the dialysis session. The durations of dialysis sessions were from 190 to 240 min. The dialysis machine used in the study was Fresenius 4008H (Fresenius Medical Care, Germany).

2.2. Sampling and laboratory analysis

Blood samples were drawn before the start of the dialysis treatment, at the end of dialysis, and 30 min after dialysis, using the slow flow sampling technique. Total dialysate collection (TDC) started when the blood filled the dialyser and ended when the blood was returned to the patient at the end of the dialysis. All spent dialysate was collected in a tank, calibrated against a weighting-machine. After careful stirring and recording of the weight of the collected spent dialysate, the TDC sample (U_{TDC}) was collected. The concentration of urea was measured at the Clinical Chemistry Laboratory at North-Estonia Medical Centre using a standardized method. The accuracy of the method for determination of urea in blood and dialysate was $\pm 5\%$.

2.3. Normalized protein nitrogen appearance

The value of nPNA in g/kg/day, A_{nPNA}, was estimated as

$$A_{\mathrm{nPNA}} = 9.35 \frac{G}{V/0.58} + 0.17. \qquad (1)$$

Obligatory loss of dietary protein in stool and via skin shedding represent the constant term 0.17 (g protein/kg body weight/day). The G and V values were obtained from the iterative mDDQ and VVSP UKM calculations. Patients' residual clearance was considered to be negligible and was not taken account.

2.4. Modified direct dialysate quantification

The parameters V and G were calculated according to the mDDQ method solving iteratively two equations [5]:

$$V = \frac{U_{\mathrm{TRU}} - G(T+0.5) - F_{\mathrm{UF}} \dfrac{C_{\mathrm{pre}}}{0.93}}{(C_{\mathrm{pre}} - C_{\mathrm{r}})/0.93}, \tag{2}$$

$$G = \frac{V\left(\dfrac{C_{\mathrm{pre}} - C_{\mathrm{r}}}{0.93}\right) + \left(W\dfrac{C_{\mathrm{pre}}}{0.93}\right)}{\theta - 30}, \tag{3}$$

where U_{TRU} is the amount of TRU, C_{pre} and C_{r} are the blood concentration of urea at the start of dialysis and the rebound urea concentration in mmol/L, F_{UF} is the value of the total ultra filtration UF, W is the total interdialytic weight gain in kg, T and θ are the dialysis session length and the interdialytic time interval in min, respectively.

2.5. Variable-volume single-pool urea kinetic modelling

The variable-volume single-pool urea kinetic modelling was used to calculate V and G values from the following equations:

$$V = F_{\mathrm{UF}}\left\{\left[1 - \left(\frac{G - C_{\mathrm{post1}}(K + K_{\mathrm{r}} - F_{\mathrm{UF}}/t)}{G - C_{\mathrm{pre1}}(K + K_{\mathrm{r}} - F_{\mathrm{UF}}/t)}\right)^{\frac{F_{\mathrm{UF}}/t}{K + K_{\mathrm{r}} - \mathrm{UF}/t}}\right]^{-1} - 1\right\}, \tag{4}$$

$$G = \frac{(K_{\mathrm{r}} + W/\theta)\left\{C_{\mathrm{pre2}} - C_{\mathrm{post1}}[(V+W)/V]^{\frac{K_{\mathrm{r}} + W/\theta}{W/\theta}}\right\}}{1 - [(V+W)/V]^{\frac{K_{\mathrm{r}} + W/\theta}{W/\theta}}}, \tag{5}$$

where C_{pre1} and C_{post1} are the blood concentrations of urea at the start and at the end of the first dialysis in mmol/L, C_{pre2} is the blood concentration of urea at the start of the second dialysis in mmol/L, K and K_{r} are the dialysers blood urea clearance and patients' renal residual clearance in mL/min, respectively.

The dialysers blood urea clearance in vitro, K_b, was calculated as

$$K_b = Q_b Q_d \frac{1 - e^{\left(-K_0 A \frac{Q_d - Q_b}{Q_d Q_b}\right)}}{Q_d - Q_b e^{\left(-K_0 A \frac{Q_d - Q_b}{Q_d Q_b}\right)}}, \quad (6)$$

where $K_0 A$ is the dialyser mass transfer area coefficient in mL/min, and Q_b is the blood flow rate in mL/min. Effective blood urea clearance in vivo, K, was estimated as being 20% lower than the K_b value [17].

2.6. Dialysis adequacy monitor prototype

The DIAMON prototype (AS Ldiamon, Estonia) was connected to the fluid outlet of the dialysis machine with all spent dialysate passing through an optical cuvette during on-line experiments (Fig. 1). The optical cuvette was a quartz tube, permeable for the UV-radiation, with a diameter of 10 mm. The transmitted light (280 ± 5 nm) intensity of the spent dialysate was measured. The used wavelength was shown to be both technically and methodologically suitable for dialysis dose estimation having a good correlation with the dialysis quality marker solute urea [18]. The sampling frequency was set to 20 samples/min. The obtained intensity values were processed to obtain UV-absorbance, presented on the computer screen by using Ldiamon's software (AS Ldiamon, Estonia, for Windows). The UV-absorbance A was calculated as

$$A = \log \frac{I_r}{I_{r+s}}, \quad (7)$$

where I_r is the intensity of transmitted light through the reference solution (pure dialysate) and I_{r+s} is the summated intensity of transmitted light through the reference solution containing the solutions under study (pure dialysate + waste products from the blood).

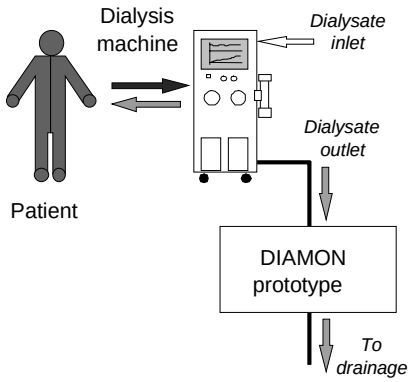

Fig. 1. The clinical experimental set-up.

The calculation of A_{nPNA} for the DIAMON prototype was based on the earlier exploited methodology [6], according to the first, midweek, and last dialyses account for nearly constant fractions (37.9, 32.1, and 30.0%, respectively) of the week's urea removal. This leads to an equation, where the amount of urea is approximated from measuring totally removed urea (TRU) from only one of the three treatments and A_{nPNA} can be calculated as

$$A_{\mathrm{nPNA}} = F_i \left(\frac{U_{\mathrm{TRU},i}}{V/0.58} \right) + 0.17, \tag{8}$$

where $U_{\mathrm{TRU},i}$ and F_i are the amount of urea nitrogen in mg dialysed from the patient, and the fractional factor for the first $(i=1)$, midweek $(i=2)$ or last dialysis in week $(i=3)$, respectively; $F_1 = 2.45$, $F_2 = 2.89$, $F_3 = 3.10$. The value of V was obtained from the Watson formula.

TRU was estimated by the DIAMON prototype using the on-line UV-absorbance measurements according to the total dialysate collection method under assumptions that the dialysate flow Q_{d} in L/min is constant, the total ultra filtration F_{UF} in kg is known and 1 kg = 1 L of the dialysate, as

$$U_{\mathrm{TRU}} = U_{\mathrm{TDC}}(Q_{\mathrm{d}}T + F_{\mathrm{UF}}) = (SA_{\mathrm{mean}} + I)(Q_{\mathrm{d}}T + F_{\mathrm{UF}}), \text{ mmol}, \tag{9}$$

where U_{TDC} in mmol/L is the urea concentration of the collected spent dialysate during the particular haemodialysis session and A_{mean} is the mean of all UV-absorbance values from the start to the end of the dialysis. The dialysate urea values from the last treatment of the week and the corresponding on-line UV-absorbance values were used for a regression line between the UV-absorbance and dialysate urea, from which the parameters slope S and intercept I were obtained. The value of G (mg/min) was estimated using totally removed urea and interdialytic time interval θ, assuming that the urea generated is equal to the amount of urea removed.

2.7. Statistical analysis

The results are presented as mean $\pm$SD. Student's t-test (two-tailed) was used to compare means for different methods and $p = 0.05$ was considered significant.

3. RESULTS

The individual nPNA for each patient for three consecutive dialysis treatments during a seven-day period from UV-absorbance, measured on-line by the DIAMON prototype, is presented in Fig. 2. The results for two sessions are missing due to the technical failure in computer during the data collection. Similar nPNA behaviour was obtained also for mDDQ and VVSP UKM.

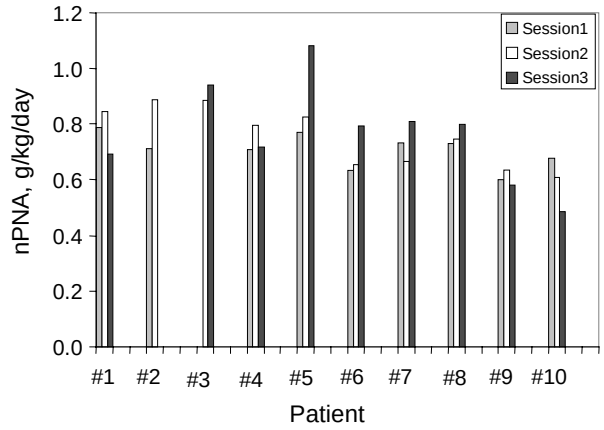

Fig. 2. Individual nPNA in g/kg/day for each patient during a seven-day period from UV-absorbance, measured on-line by the DIAMON prototype.

Figure 3 shows the estimated G values (mean ±SD) in mg/min for the total material from the UV-absorbance measured by the DIAMON prototype, from mDDQ and from VVSP UKM using blood and dialysate urea samples. The mean ±SD values of G in mg/min were: 4.50 ± 1.27 from DIAMON (number of HD sessions $N = 28$), 4.45 ± 1.75 from mDDQ ($N = 29$) and 5.17 ± 1.77 from VVSP UKM ($N = 30$). The mean G values for DIAMON, mDDQ and VVSP UKM were not statistically different ($p < 0.05$).

The estimated mean BW value (mean ±SD) in kg for the total material (Fig. 4), calculated as $V/0.58$, was by the Watson formula 74.84 ± 16.33 ($N = 30$), by mDDQ 58.04 ± 14.31 ($N = 29$), and by VVSP UKM 67.58 ± 13.31 ($N = 30$). Additionally, the measured efBW for the studied patients was

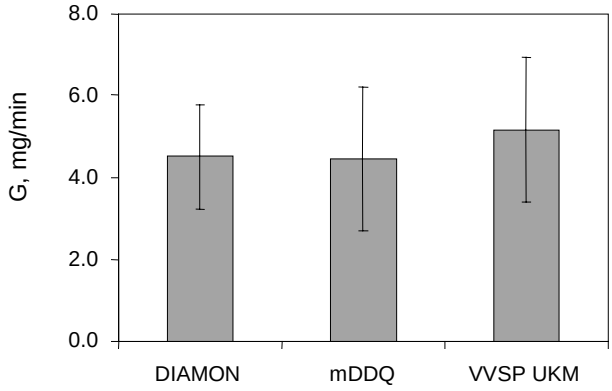

Fig. 3. Estimated urea generation rate G values (mean ±SD) for the total material from the UV-absorbance, measured by the DIAMON prototype (number of HD sessions $N = 28$), from mDDQ ($N = 29$) and VVSP UKM ($N = 30$) calculations using blood and dialysate urea samples.

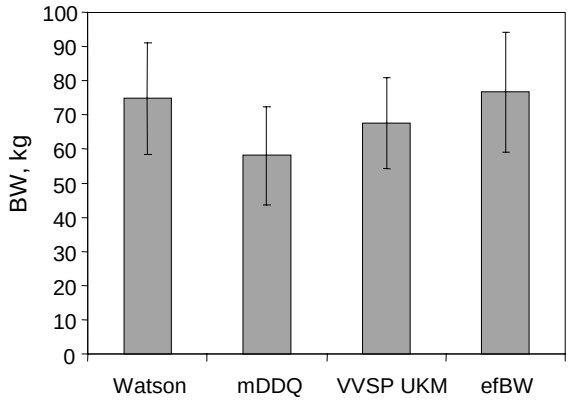

Fig. 4. Estimated BW values (mean ±SD) for the total material calculated by the Watson formula (N = 30), by mDDQ (N = 29) and by VVSP UKM (N = 30) as V/0.58, and the dry body weight, efBW (N = 30).

76.60 ± 17.56 kg $(N = 30)$, and is presented for comparison. efBW was statistically different compared to the kinetic body weights $(p < 0.05)$, and the anthropometric BW was different compared to the BW value by mDDQ $(p < 0.05)$.

Figure 5 depicts the nPNA values (mean ±SD) for the total material from the UV-absorbance, measured by the DIAMON prototype, from mDDQ, and from VVSP UKM calculations, normalized by $V/0.58$ and by the dry body weight, efBW. The mean ±SD values of nPNA (g/kg/day) were: (1) 0.74 ± 0.12 from DIAMON $(N = 28)$, 0.90 ± 0.26 from mDDQ $(N = 29)$, and 0.90 ± 0.23 from VVSP UKM $(N = 30)$, normalized by $V/0.58$; (2) 0.68 ± 0.10 from DIAMON $(N = 28)$, 0.72 ± 0.19 from mDDQ $(N = 29)$, and 0.80 ± 0.18 from VVSP UKM $(N = 30)$ normalized by efBW. The mean nPNA values from mDDQ and from

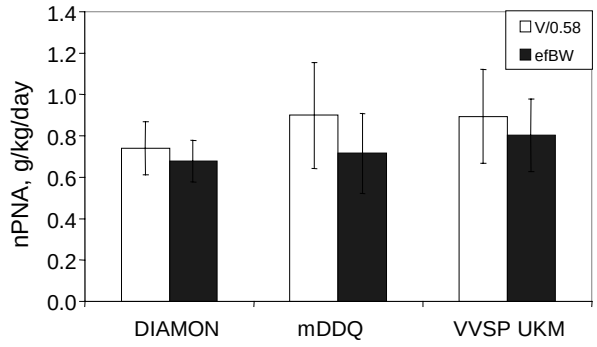

Fig. 5. Estimated nPNA values (mean ±SD) in g/kg/day for the total material from the UV-absorbance, measured by the DIAMON prototype (N = 28), from mDDQ (N = 29) and from VVSP UKM (N = 30) calculations, normalized by V/0.58 and by the dry body weight, efBW.

VVSP UKM, normalized by the kinetic body weights, were higher compared to nPNA values from DIAMON, normalized by the anthropometric BW ($p < 0.05$). Also a higher nPNA value was obtained by UKM VVSP compared to nPNA values from DIAMON, normalized by efBW ($p < 0.05$).

4. DISCUSSION

The optical dialysis adequacy sensor, DIAMON, would facilitate HD dialysis adequacy monitoring, providing continuous, on-line measurements of dialysis dose and individual follow-up of dietary protein intake of HD patients.

The individual nPNA monitoring of each patient during a seven-day period by the DIAMON prototype shows how nPNA can vary depending on the treatment day and the patient (Fig. 2). The lower outcome for two patients relative to others, who had several dialysis related difficulties (#9 was a new dialysis patient with hypoalbuminemia, and #10 noncompliant with the treatment), is clearly seen from the nPNA recordings. Considering that the urea generation rate G is not constant over the interdialytic period [19] and day-to-day variations in daily protein intake may result in significantly fluctuating nPNA; nPNA, calculated as an average of nPNA values over a seven-day period for each patient, could be a reasonable alternative for decision making instead of nPNA from individual sessions.

The estimated mean G values for the total material were relatively close for different methods (Fig. 3). The mean values from DIAMON and mDDQ were slightly lower compared to VVSP UKM being still not statistically different. This can be due to the methodological factors described in relation to nPNA below. The level of mean G values was comparable to the reported values by earlier studies [20].

The estimated mean BW values for the total material, calculated by the kinetic or anthropometric formula, show that the results depend on the methodology (Fig. 4). For comparison, the measured dry body weight efBW is shown. The BW from mDDQ was lower than BW from the anthropometric and VVSP UKM methods, or measured as efBW. Lower V from DDQ than from UKM is observed also by other authors [21].

The malnutrition of patients that can be suspected from these results was still not evident because the mean albumin level was 38.6 g/L. The anthropometric formula by Chertow et al is suggested by guidelines, claiming that the Watson formula underestimates V about 7.5% [1]. This will cause the estimated BW to approach more to efBW and deviate from iteratively calculated values. At the same time, calculation of BW using Chertow's method requires information about the patient's age, sex and diabetic status in addition to measurements of the height and weight. Adjusted oedema-free body weight (aBWef), based on the dry body weight and the standard body weight, has been recommended for nPNA normalization [2]. However, aBWef includes NHANES II (National Health and Nutrition Examination Survey) tables specific to US and may not be suitable for

other countries [15]. With extreme obese or oedematous patients, care must be taken to estimate V and PNA [22] that could be valid for some studied patients but not in general. One solution could be to use the lean body mass to normalize PNA [16].

Figure 5 indicates higher nPNA with mDDQ and VVSP UKM compared to TRU-based equation, used by DIAMON when normalized by the anthropometric and the kinetic body weights. Higher nPNA with VVSP UKM, compared to urea output measurements, is reported also in [16]. Two sources of errors that must be considered when estimating nPNA from UKM are the rebound effect and neglected residual function [22]. The preliminary check revealed that the mean nPNA value from VVSP UKM (about 7% lower when rebound), taken 30 min after dialysis, was used instead of direct postdialysis blood urea. The residual function was not taken into account in this study. However, considering the residual function, a higher nPNA value should be obtained for all methods. Moreover, correct estimation of *in vivo* dialysers blood urea clearance is important in VVSP UKM [4].

Interestingly, the lower mDDQ G value (Fig. 3) is compensated by the lower mDDQ V value (Fig. 4) leading to equal mean nPNA compared to nPNA from VVSP UKM. This is not the case when normalized by efBW (Fig. 5). Considering that DIAMON included only dialysate-based TRU, VVSP UKM solely the blood samples, and mDDQ both plus the rebound effect, certainly some differences will appear. The mDDQ gives higher nPNA compared to DIAMON (using solely TRU-based formulae and normalized by the anthropometric BW) because of the lowest "kinetic body weight" from mDDQ. Similarly, VVSP UKM has the "kinetic body weight" lower than the anthropometric BW but a higher G value, leading to higher nPNA than the TRU-based formulae, applied for the DIAMON prototype. These methodological differences should be explored further.

The overall mean nPNA for the studied patients is lower compared to the recommended value 1 g/kg/day. The reason could lie in the design of the study, since lower blood flow than usually prescribed was applied during one dialysis for all patients. Additionally, two patients (#9 and #10) had several dialysis-related difficulties. Moreover, the patient #4 received thrice-weekly haemodialysis treatment instead of the prescribed four times per week dialysis. These factors together influence the nPNA level, indicating the importance of continuous dialysis adequacy monitoring.

In practice, the clinical judgment and longitudinal assessment of body weight and other nutritional measures should be used to assess the response to dietary therapy and to make further decisions concerning dietary management [1].

5. CONCLUSIONS

The optical dialysis adequacy monitoring device enables individual nPNA estimation for each patient using continuous on-line UV-absorbance measure-

ments. The results are comparable to the nPNA values obtained by the kinetic modelling. Still a question remains concerning the normalization of PNA.

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Dialüüsipatsientide toitumuse hindamine *online*-monitooringu ja kineetilise modelleerimise abil

Ivo Fridolin, Kai Lauri, Jana Jerotskaja ja Merike Luman

Optiline dialüüsi adekvaatsuse monitooring reaalajas pakub võimaluse hinnata dialüüsi doosi ja toitumuse parameetrit vereproove võtmata. Artiklis on käsitletud toitumuse parameetrit nPNA, mida on hinnatud optilise dialüüsi adekvaatsuse monitoriga DIAMON ja kahe dialüüsi kvaliteeti hindava kineetilise mudeliga – mDDQ ning VVSP UKM-iga. Tulemuste põhjal on järeldatud, et DIAMON võimaldab nPNA väärtust individuaalselt jälgida. Tulevikus on vajalik uurida nPNA normaliseerimisega seotud probleeme.

PUBLICATIONS

Publication III

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Original Paper

Blood Purification

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Optical Online Monitoring of Uric Acid Removal during Dialysis

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Key Words
Optical online monitoring · Uric acid removal · Ultraviolet absorbance method

Abstract
This study estimates the total removal of uric acid (TR_{UA}) by online UV absorbance measurements in the spent dialysate in two different dialysis centers in Estonia and Sweden. Sixteen dialysis patients were included. All dialysate was collected that gave the reference for TR_{UA}. Two regression models were investigated: one for each patient (UV1) and one for the entire material (UV2). TR_{UA} from the three methods was in the same order but showed a statistically significant difference when the UV2 model was built on data from both centers together. TR_{UA}, (n = 56) was (mean ± SD, μmol): 5,854 ± 1,377 for reference, 6,117 ± 1,795 for UV1 and 5,762 ± 1,591 for UV2. Six patients were monitored 1 year after the first study session, using the same models as the previous year, still having a nonsignificant difference. The results show the possibility of estimating TR_{UA} by using UV absorbance. The method appeared to be reliable also in long-term patient monitoring.

Introduction

Uric acid (UA) is a water-soluble compound (molecular weight of 168.1) that is the final metabolite of purine in humans. Elevated serum UA contributes to endothelial dysfunction and increased oxidative stress within the glomerulus and the tubulointerstitium, with associated increased remodeling fibrosis of the kidney [1]. A high level of serum UA, hyperuricemia, has been suggested to be an independent risk factor for cardiovascular and renal disease especially in patients with heart failure, hypertension and/or diabetes [2–4] and has been shown to cause renal disease in a rat model [5]. UA is mostly associated with gout but studies have implicated that UA affects biological systems [6] and could also influence risks of higher mortality in dialysis patients [7] but the pathogenic role of hyperuricemia in dialysis patients is not completely established [8].

In previous studies a good correlation between ultraviolet (UV) absorbance in the spent dialysate and the concentration of several solutes both in the spent dialysate and in the blood of dialysis patients has been presented, indicating that the technique can be used to estimate the removal of retained substances [9]. Moreover the possibility to estimate total removed urea [10] by UV absorbance has been presented. The wavelength of 285 nm that was

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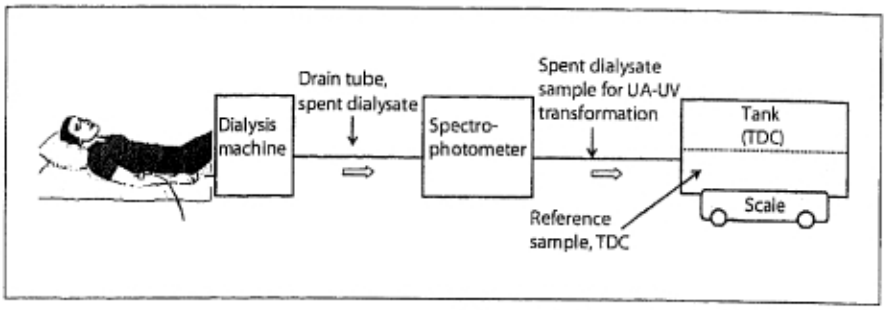

Fig. 1. Schematic clinical setup of the experiments.

utilized for urea removal estimation [10] was even utilized in the present study. The purpose was to find out if it is possible to create a specific model for UA while still using the same wavelength. The fact that UA is a UV-absorbing solute [11] makes this study even more interesting.

The aim of this study was to estimate the total removed uric acid (TR_{UA}) by the online UV absorbance measurements in the spent dialysate in two different dialysis centers in two countries, Estonia and Sweden.

Materials and Methods

Subjects

Ten uremic patients, 3 females and 7 males, mean age 62.6 ± 18.6 years, were included in the study at the Department of Dialysis and Nephrology, North-Estonian Regional Hospital, Estonia, and 6 uremic patients, all males, mean age 64.3 ± 18.5 years, were included at the Department of Nephrology, University Hospital of Linköping, Sweden.

All patients were on chronic thrice-weekly hemodialysis and were monitored during 3–6 dialysis treatments, each with a duration from 240 to 300 min (totally 56 hemodialysis sessions).

The dialysate flow was fixed at 500 ml/min and the blood flow varied between 200 and 350 ml/min. Several dialyzers were used (both low- and high-flux membranes), with an effective membrane area of 1.4–2.2 m^2 and two dialysis monitors, Fresenius 4008H (Fresenius Medical Care, Germany) and AK 200 (Gambro Lundia AB, Sweden).

The study was performed after approval of the protocol by the Regional Ethics Committee, Linköping, Sweden, and by Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. Informed consent was obtained from all participating patients.

Sampling and Laboratory Analysis

Spent dialysate samples were taken at 5 (only in Linköping), 10 (only in Tallinn), 15 (only in Linköping), 30 (only in Linköping), 60, 90 (only in Linköping), 120 and 180 min after the start of the dialysis session and at the end. After finishing the session, one sample was taken from the collection tank. This gave the UA concentration value utilized for TR_{UA} calculation from the total dialysate collection (TDC). The concentrations of UA were determined at the Clinical Chemistry Laboratory at both Hospitals using the enzymatic colorimetric test method. The accuracy for UA in dialysate was ±3% in Linköping and ±2% in Tallinn.

UV Absorbance Monitoring

The spectrophotometers Uvikon 943 (Kontron, Italy) in Linköping and HR2000 (Ocean Optics Inc., USA) in Tallinn were used for the determination of UV absorbance online. The spectrophotometer was connected to the fluid outlet of the dialysis machine (fig. 1) with all spent dialysate passing through an optical flow cuvette with a depth of 10 mm. The geometry of the cuvette was rectangular in Tallinn and circular in Linköping.

The obtained UV absorbance values were processed and presented on the computer screen by a PC incorporated in the spectrophotometer using Kontron's software (Uvikon 943, version 7.0 for Windows) in Linköping and Ocean Optics' software (OOIBase32, version 2.0.2.2 for Windows) in Tallinn. The absorbance A of a solution, obtained by the spectrophotometer using the pure dialysate as the reference solution, was determined as:

$$A = \log \frac{I_r}{I_{r+s}}$$

where I_r is the intensity of transmitted light through the reference solution (pure dialysate) and I_{r+s} is the summated intensity of transmitted light through the reference solution containing the solutions under study (pure dialysate + waste products from the blood). The sampling frequency was set at 2 samples/min.

Transformation of UV Absorbance into Dialysate UA Concentration

The regression line between collected spent dialysate samples and corresponding online UV absorbance values at the used wavelength (285 nm) was assessed to transform UV absorbance into UA concentration. The obtained relationship was used for generating two different models to estimate UA concentration from UV absorbance:

UV1 = individual model, i.e. the regression line (slope and intercept) for the first session of each patient was used to calculate TR_{UA} of the subsequent treatments of the same patient;

UV2 = general model, i.e. the regression line (slope and intercept) for all sessions was used to calculate TR_{UA} for all dialysis sessions (mix of patients and dialyzers).

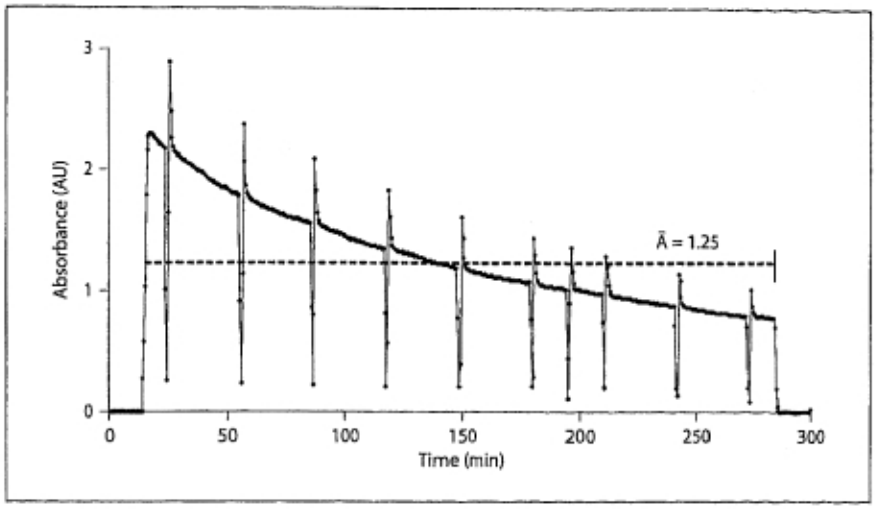

Fig. 2. A typical online monitoring curve during a dialysis treatment lasting 270 min where UV absorbance is plotted against time. AU = Arbitrary units. The spikes correspond to the self-test of the dialysis machine when dialysate automatically has been set in bypass. The mean absorbance value (Ā) is the mean of all UV absorbance values (n = 546) from start to end of dialysis. In this particular session the Ā was 1.25.

Estimation of TR_{UA}

Assuming that dialysate flow rate, $Q_{D(t)}$, is constant, duration of dialysis T (in minutes) and total ultrafiltrated volume UF (in liters) is known, the following equation can be utilized:

$$TR_{UA} = \overline{UA} \cdot (Q_D \cdot T + UF) \quad (2)$$

where $\overline{UA}$ is the mean UA concentration in the spent dialysate of the particular dialysis session. The TR_{UA} from TDC was calculated as UA concentration (in micromoles per liter) in the tank at the end of dialysis multiplied by collected weight (in kilograms), assuming that 1 kg = 1 liter of the dialysate.

In a similar way, TR_{UA} may be calculated from the online UV absorbance as:

$$TR_{UA} = (\alpha \cdot \bar{A} + \beta) \cdot (Q_D \cdot T + UF) \quad (3)$$

where Ā is the mean of all UV absorbance values from the start to the end of the dialysis (fig. 2). The regression line between the UV absorbance and concentration of UA in spent dialysate gives the slope (α) and the intercept (β) inserted into equation 3 when calculating TR_{UA} from UV1 or UV2.

Statistics

TR_{UA} from the two UV models was finally compared with TR_{UA} from the TDC.

Student's t test (two tailed) was used to compare means for different methods and SD values, respectively; $p < 0.05$ was considered significant. Differences between the two UV models (UV1 and UV2) and the TDC were compared using Bland-Altman analysis [12].

Results

Figure 2 shows an online monitoring curve during a dialysis treatment. The mean absorbance value (Ā) is the mean of all UV absorbance values from the start to end of dialysis and inserted in equation 3 when calculating TR_{UA}.

Figure 3 presents the best-fit regression equation of UA (manually taken samples from the drain tube, fig. 1) against UV absorbance in the spent dialysate at the same time point in 4 sessions of the same patient, showing a high correlation of r = 0.99.

Figure 4 shows the regression equation of UA against UV absorbance in all 56 sessions and also with the two centers separated (28 sessions each), and a notable difference is seen. When using UV2, based on all sessions, corresponding to y = 37.14x + 4.41 (the line in the middle), there was a statistical difference ($p < 0.05$) between UV2 and TDC. The two centers were then separated, and a center-specific equation was used: y = 51.97x – 0.67 for Tallinn and y = 38.94x – 3.30 for Linköping; a nonsignificant statistical difference ($p > 0.05$) was then shown.

Table 1 demonstrates TR_{UA} in mean ± SD (in micromoles) of the compared methods to calculate TR_{UA}, i.e. TDC, UV1 and UV2 (center-specific). The best agreement was seen in Tallinn in the case of UV1 and in Linköping in the case of UV2 compared to TDC. There was no statistically significant difference (n = 56) between TDC compared to UV1 ($p > 0.05$) or compared to UV2 ($p > 0.05$).

In the 6 patients in Linköping 1 dialysis session each was performed once again after more than 12 months using UV1 and UV2 from the previous year, as shown in figure 5. Similar means and SD from TDC and UV1 ($p > 0.05$) methods are presented for these 6 patients, and UV2 showed a slightly lower mean and a higher SD ($p > 0.05$).

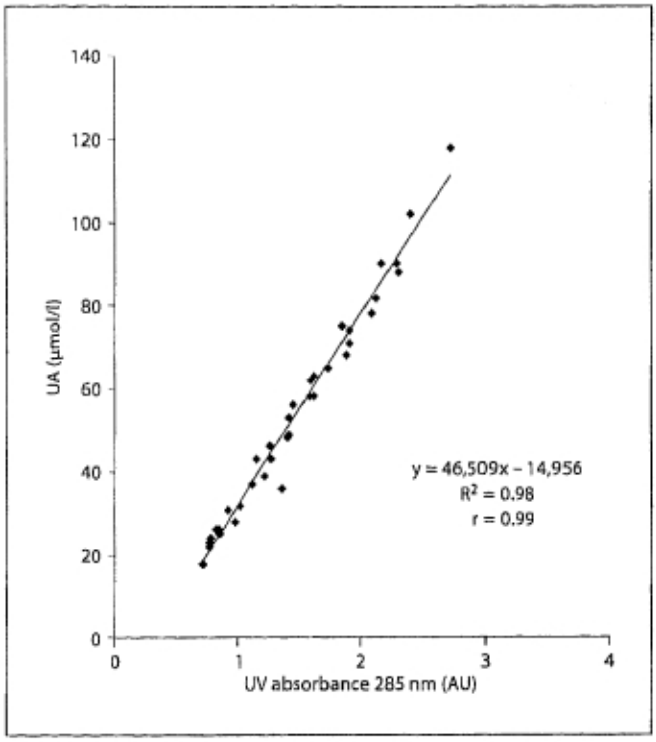

Fig. 3. An example of the regression line between concentration of UA in spent dialysate and UV absorbance in 4 sessions in 1 patient. This relationship was utilized when UV1 was calculated. AU = Arbitrary units.

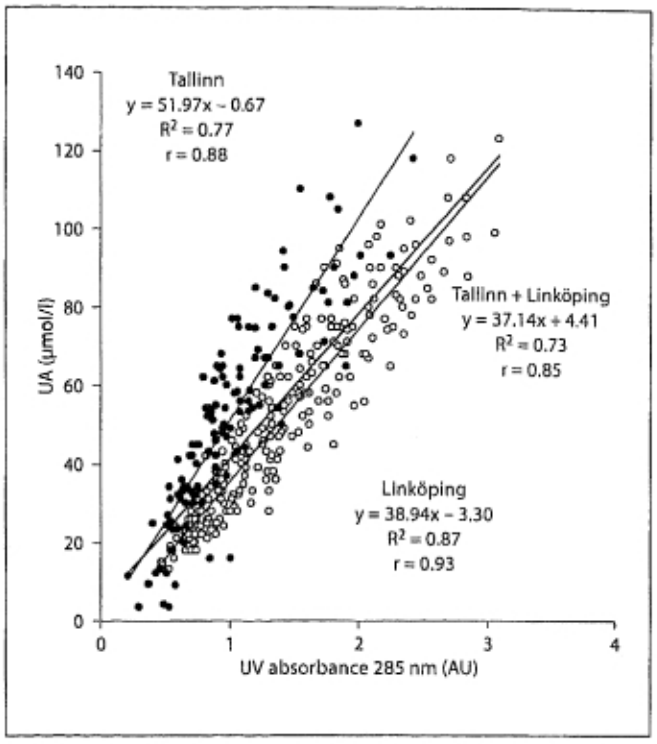

Fig. 4. Regression lines between concentration of UA in spent dialysate and UV absorbance in all sessions (n = 56) and also with the two centers, Linköping (n = 28) and Tallinn (n = 28), with separately calculated TR_{UA} from the general model UV2.

Table 1. The mean ± SD (μmol) when using different methods to calculate TR_{UA}

	Linköping (n = 28)	Tallinn (n = 28)	Linköping + Tallinn (n = 56)
TDC (reference)	5,723 ± 1,032	5,986 ± 1,662	5,854 ± 1,377
UV1 (individual model)	6,162 ± 1,336	6,072 ± 2,184	6,117 ± 1,795
UV2 (general model)	5,725 ± 1,421	5,799 ± 1,770	5,762 ± 1,591

The results were not significantly different ($p > 0.05$) compared to TDC.

Figure 6 presents the difference between the individual values of TDC compared to the UV method plotted against the mean value of TDC and UV method, respectively (TDC vs. UV1 and TDC vs. UV2 in fig. 6a and b). The mean value ± SD of the difference between TDC and UV1 was –262 ± 1,263 (n = 56) and 52 ± 1,167 (n = 56) between TDC and UV2 and showed a similar SD value.

Discussion

The presented results show the possibility to estimate TR_{UA} by using transformation models generated by a UV absorbance technique in two different dialysis centers in two countries, Estonia and Sweden. The mean values of TR_{UA} obtained using the UV1 model were not statistically different from TR_{UA} calculated from TDC (reference method) at the two centers (n = 56). When using the

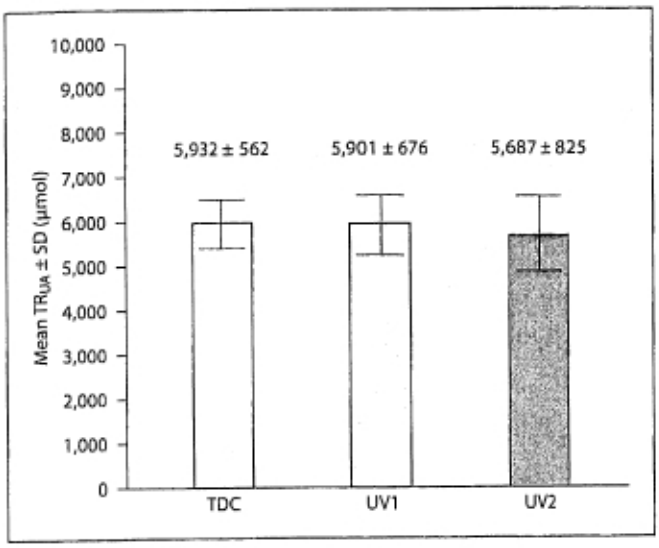

Fig. 5. Mean and SD values of TR_{UA} in micromoles for the 1-year follow-up sessions (n = 6). No significant difference ($p > 0.05$) was seen between TDC and UV1 and between TDC and center-specific (Linköping) UV2.

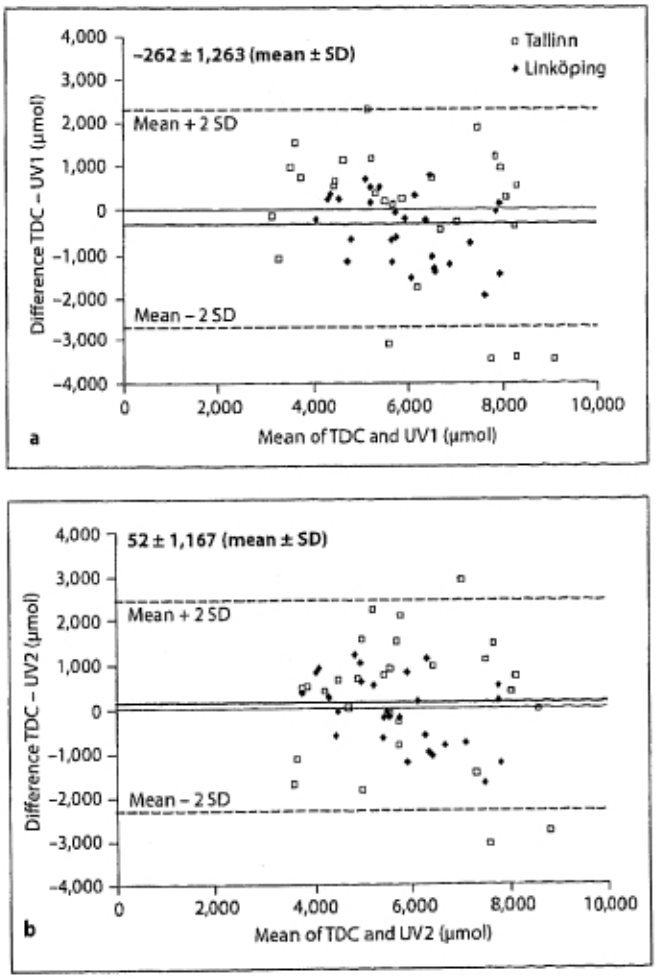

Fig. 6. Bland-Altman plot. **a** The difference between the TDC and UV1 method is plotted against the mean value of TDC and UV1 method (n = 56). **b** The difference between the TDC and UV2 method is plotted against the mean value of the TDC and UV2 method (n = 56).

UV2 model (entire group) to calculate TR_{UA} there was a statistical difference, but no difference when separated regression equations were used at the two centers (fig. 4). Interestingly, the mean values of TR_{UA} exhibited the same good agreement as above (fig. 5) from the main study in Linköping after more than 1 year. This shows that the long-term patient calibration based on the dialysate samples taken during one session, from which a regression line could be assessed for transformation, could be an alternative to calculate TR_{UA} from the online UV absorbance measurements in the spent dialysate.

The need for individual dialysate samples is a tedious and laborious procedure when estimating TR_{UA} with the UV1 model. A general regression model based on the UV absorbance and UA for all subjects (UV2) would be preferable. The obtained significant difference when the UV2 model was built on data from both centers shows that there are several issues to consider: a more accurate general model must be based on standardized optical parameters (wavelength, optical flow cuvette), type of dialyzer (ultrafiltration coefficient, surface area) and probably even some patient-dependent parameters. The reason for the difference within the general model UV2 of the centers can arise because a circular cuvette may result in a different propagation of light compared to a rectangular one. Moreover, elimination of the UV-absorbing compounds/chromophores apart from UA contributing to the total UV absorbance may differ compared to UA and vary depending on the dialyzer characteristics since different dialyzers were used at the centers. However, many other possibilities are available (e.g. regression models taking into account the dialyzer characteristics, multiwavelength approach by least square, inverse least square, partial least square methods) to create a more universal general model with a satisfactory accuracy. Those more advanced algorithms will be the subject for future studies.

The difference between the individual values of TDC and the two models, presented in figure 6a and b, shows that the sessions from Tallinn have a higher distribution from the mean value compared to Linköping. The reason for this could be that in Tallinn 50% of the used mem-

branes were low-flux and 50% were high-flux ones, whereas more homogenous types of dialyzers (92% low-flux and 8% high-flux membranes, respectively) were utilized in Linköping.

The high correlation between UV absorbance and UA in every single patient (fig. 3) could be explained by a dominant absorbance for UA, compared to other compounds in spent dialysate at the wavelength 280 nm [12]. This is due to relatively high millimolar extinction coefficients of UA with 3 distinct maxima around 202, 235 and 292 nm and 2 minima around 220 and 260 nm in the wavelength range from 200 to 380 nm [13]. The absorbance around 292 nm is characteristic of UA and is utilized for UA concentration determination by the enzymatic degradation method [14]. The wavelength 285 nm was chosen in this study because a high correlation with several other solutes, above urea [10], has also been shown and the purpose was to find out if it would be possible to create a specific model for other substances while still using the same wavelength.

A new interest in UA has emerged and several recent studies have shown that elevated serum UA is associated with cardiovascular disease, hypertension, diabetes and renal disease, and that it also plays a role in the metabolic syndrome [2]. Several uremic toxins might be involved in the induction of inflammation and different mechanisms that could cause vascular damage resulting in endothelial dysfunction and finally death, in cardiovascular diseases in the dialysis population [15]. UV absorbance monitoring may allow controlled and optimal removal of a uremic toxin, UA, which is one of the risk factors of cardiovascular disease.

Conclusions

The results show the possibility to estimate TR_{UA} from online UV absorbance measurements during hemodialysis. The study also highlights the importance of the standardization of issues such as the geometry of the flow cuvette and dialyzer characteristics when general models are to be built.

In the future, online UV absorbance measurement during dialysis may be a monitoring tool for dialysis dose and also help the dialysis team to reach a less detrimental level of solutes such as uric acid that could have a direct impact on patient morbidity and mortality.

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APPENDIX 2

ELULOOKIRJELDUS

1. Isikuandmed

Ees- ja perekonnanimi	Merike Luman
Sünniaeg ja -koht	29.06.1957, Elva, Eesti
Kodakondsus	eestlane

2. Kontaktandmed

Aadress	Pargi 22a, 11613 Tallinn, Eesti
Telefon	+372 6706888
E-posti aadress	merike.luman@regionaalhaigla.ee

3. Hariduskäik

Õppeasutus (nimetus lõpetamise ajal)	Lõpetamise aeg	Haridus (eriala/kraad)
Tartu Riiklik Ülikool	1981	Arstiteaduskond/ravi eriala
Tartu Riiklik Ülikool/Tallinna Pelgulinna Haigla	1982	internatuur

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

Keel	Tase
Eesti	Kõrgtase
Inglise	Kõrgtase
Vene	Kõrgtase
Saksa	Algtase
Soome	Algtase

5. Täiendusõpe

Õppimise aeg	Täiendusõppe läbiviija nimetus
1989, 1990	Leningradi Meditsiini Instituudi Nefroloogia Keskus
1993	Hamburg Dialyze Kuratorium
1993	Eskilstuna Haigla
1993	Huddinge Ülikooli Kliinik
1993	Amsterdami Meditsiini Keskus
1995	Harvardi Ülikooli Brigham and Womens Hospital
2000	Rahvusvaheline Nefroloogia Selts
2002	Euroopa Südame Maja
2006	Tallinna Tehnikaülikool
2009	Rahvusvaheline Nefroloogia Selts

6. Teenistuskäik

Töötamise aeg	Tööandja nimetus	Ametikoht
1982 – 1984	Tallinna Pelgulinna Haigla	Sisehaiguste arst
1984 – 2002	Tallinna Pelgulinna Haigla	Dialüüsi osakonna juhataja
2002 – k.a.	SA Põhja-Eesti Regionaalhaigla	Dialüüsi ja nefroloogia osakonna juhataja

7. Teadustegevus

Dialüüsravi doosi ja patsientide toitumuse hindamine reaalajas uue optilise meetodiga, mis baseerub UV absorbtsiooni mõõtmisel.

8. Teadustöö põhisuunad

SF0142084As02, Bioelektriliste signaalide interpreteerimine, 2002-2006

SF0140027s07, Biosignaalide interpreteerimine meditsiinitehnikas, 2007-2012

ETF5871, Uudne optiline multikomponent monitor neerupuudulikkusega patsientide ravi kvaliteedi hindamiseks, 2004-2006

ETF6936, Uudne optiline tehnika dialüüsi kvaliteedi jälgimiseks ja hindamiseks, 2007-2010

TAR8077DB, Integreeritud elektroonikasüsteemide ja biomeditsiinitehnika tippkeskus – CEBE, Estonian centre of excellence in research, 2008-2015

„NephroQUEST“–Euroopa Dialüüsi ja Transplantatsiooni Ühingu Registri poolt algatatud Euroopa Ühisuuring

APPENDIX 3

CURRICULUM VITAE

1. Personal data

Name	Merike Luman
Date and place of birth	29.06.1957, Elva, Estonia

2. Contact information

Address	Pargi 22a, 11613 Tallinn, Estonia
Phone	+372 6706888
E-mail	merike.luman@regionaalhaigla.ee

3. Education

Educational institution	Graduation year	Education (field of study/degree)
Tartu University	1981	Faculty of Medicine/Medical Doctor
Tartu University/Tallinn Pelgulinna Hospital	1982	internship

4. Language competence/skills (fluent; average, basic skills)

Language	Level
Estonian	fluent
English	fluent
Russian	fluent
German	basic skills
Finnish	basic skills

5. Special Courses

Period	Educational or other organisation
1989, 1990	Leningrad Medical Institute, Nephrology Centre
1993	Hamburg Dialyze Kuratorium
1993	Eskilstuna Hospital
1993	Huddinge University Hospital
1993	Amsterdam Medical Centre
1995	Harvard University - Brigham and Womens Hospital
2000	International Society of Nephrology
2002	The European Heart House
2006	Tallinn University of Technology
2009	International Society of Nephrology

6. Professional Employment

Period	Organisation	Position
1982-1984	Tallinn Pelgulinna Hospital	MD of Internal Medicine
1984 - 2002	Tallinn Pelgulinna Hospital	Head of Dialysis Unit
2002 -	North- Estonia Medical Centre	Head of Dialysis and Nephrology Unit

7. Scientific work

Estimation of dialysis quality and adequacy with a new optical technique based on the UV-absorbance measurements

8. Main areas of scientific work/Current research topics

SF0142084As02, Bioelectrical signals interpretation, 2002-2006

SF0140027s07, Interpretation of Biosignals in Biomedical Engineering,2007-2012

ETF5871, Estimation of dialysis quality and adequacy with a new optical technique, 2004-2006

ETF6936, A novel optical multicomponent monitor estimating ESRD patients' treatment quality, 2007-2010

TAR8077DB, Centre for Integrated Electronic Systems and Biomedical Engineering – CEBE, Estonian centre of excellence in research, 2008-2015

"European Nephrology Quality Improvement Network-NephroQUEST"

DISSERTATIONS DEFENDED AT TALLINN UNIVERSITY OF TECHNOLOGY ON *NATURAL AND EXACT SCIENCES*

1. **Olav Kongas**. Nonlinear dynamics in modeling cardiac arrhytmias. 1998.

2. **Kalju Vanatalu**. Optimization of processes of microbial biosynthesis of isotopically labeled biomolecules and their complexes. 1999.

3. **Ahto Buldas**. An algebraic approach to the structure of graphs. 1999.

4. **Monika Drews**. A metabolic study of insect cells in batch and continuous culture: application of chemostat and turbidostat to the production of recombinant proteins. 1999.

5. **Eola Valdre**. Endothelial-specific regulation of vessel formation: role of receptor tyrosine kinases. 2000.

6. **Kalju Lott**. Doping and defect thermodynamic equilibrium in ZnS. 2000.

7. **Reet Koljak**. Novel fatty acid dioxygenases from the corals *Plexaura homomalla* and *Gersemia fruticosa.* 2001.

8. **Anne Paju**. Asymmetric oxidation of prochiral and racemic ketones by using sharpless catalyst. 2001.

9. **Marko Vendelin**. Cardiac mechanoenergetics *in silico*. 2001.

10. **Pearu Peterson**. Multi-soliton interactions and the inverse problem of wave crest. 2001.

11. **Anne Menert**. Microcalorimetry of anaerobic digestion. 2001.

12. **Toomas Tiivel**. The role of the mitochondrial outer membrane in *in vivo* regulation of respiration in normal heart and skeletal muscle cell. 2002.

13. **Olle Hints**. Ordovician scolecodonts of Estonia and neighbouring areas: taxonomy, distribution, palaeoecology, and application. 2002.

14. **Jaak Nõlvak**. Chitinozoan biostratigrapy in the Ordovician of Baltoscandia. 2002.

15. **Liivi Kluge**. On algebraic structure of pre-operad. 2002.

16. **Jaanus Lass**. Biosignal interpretation: Study of cardiac arrhytmias and electromagnetic field effects on human nervous system. 2002.

17. **Janek Peterson**. Synthesis, structural characterization and modification of PAMAM dendrimers. 2002.

18. **Merike Vaher**. Room temperature ionic liquids as background electrolyte additives in capillary electrophoresis. 2002.

19. **Valdek Mikli**. Electron microscopy and image analysis study of powdered hardmetal materials and optoelectronic thin films. 2003.

20. **Mart Viljus**. The microstructure and properties of fine-grained cermets. 2003.

21. **Signe Kask**. Identification and characterization of dairy-related *Lactobacillus.* 2003.

22. **Tiiu-Mai Laht**. Influence of microstructure of the curd on enzymatic and microbiological processes in Swiss-type cheese. 2003.

23. **Anne Kuusksalu**. 2–5A synthetase in the marine sponge *Geodia cydonium*. 2003.

24. **Sergei Bereznev**. Solar cells based on polycristalline copper-indium chalcogenides and conductive polymers. 2003.

25. **Kadri Kriis**. Asymmetric synthesis of C_2-symmetric bimorpholines and their application as chiral ligands in the transfer hydrogenation of aromatic ketones. 2004.

26. **Jekaterina Reut**. Polypyrrole coatings on conducting and insulating substracts. 2004.

27. **Sven Nõmm**. Realization and identification of discrete-time nonlinear systems. 2004.

28. **Olga Kijatkina**. Deposition of copper indium disulphide films by chemical spray pyrolysis. 2004.

29. **Gert Tamberg**. On sampling operators defined by Rogosinski, Hann and Blackman windows. 2004.

30. **Monika Übner**. Interaction of humic substances with metal cations. 2004.

31. **Kaarel Adamberg**. Growth characteristics of non-starter lactic acid bacteria from cheese. 2004.

32. **Imre Vallikivi**. Lipase-catalysed reactions of prostaglandins. 2004.

33. **Merike Peld**. Substituted apatites as sorbents for heavy metals. 2005.

34. **Vitali Syritski**. Study of synthesis and redox switching of polypyrrole and poly(3,4-ethylenedioxythiophene) by using *in-situ* techniques. 2004.

35. **Lee Põllumaa**. Evaluation of ecotoxicological effects related to oil shale industry. 2004.

36. **Riina Aav**. Synthesis of 9,11-secosterols intermediates. 2005.

37. **Andres Braunbrück**. Wave interaction in weakly inhomogeneous materials. 2005.

38. **Robert Kitt**. Generalised scale-invariance in financial time series. 2005.

39. **Juss Pavelson**. Mesoscale physical processes and the related impact on the summer nutrient fields and phytoplankton blooms in the western Gulf of Finland. 2005.

40. **Olari Ilison**. Solitons and solitary waves in media with higher order dispersive and nonlinear effects. 2005.

41. **Maksim Säkki**. Intermittency and long-range structurization of heart rate. 2005.

42. **Enli Kiipli**. Modelling seawater chemistry of the East Baltic Basin in the late Ordovician–Early Silurian. 2005.

43. **Igor Golovtsov**. Modification of conductive properties and processability of polyparaphenylene, polypyrrole and polyaniline. 2005.

44. **Katrin Laos**. Interaction between furcellaran and the globular proteins (bovine serum albumin β-lactoglobulin). 2005.

45. **Arvo Mere**. Structural and electrical properties of spray deposited copper indium disulphide films for solar cells. 2006.

46. **Sille Ehala**. Development and application of various on- and off-line analytical methods for the analysis of bioactive compounds. 2006.

47. **Maria Kulp**. Capillary electrophoretic monitoring of biochemical reaction kinetics. 2006.

48. **Anu Aaspõllu.** Proteinases from *Vipera lebetina* snake venom affecting hemostasis. 2006.

49. **Lyudmila Chekulayeva**. Photosensitized inactivation of tumor cells by porphyrins and chlorins. 2006.

50. **Merle Uudsemaa**. Quantum-chemical modeling of solvated first row transition metal ions. 2006.

51. **Tagli Pitsi**. Nutrition situation of pre-school children in Estonia from 1995 to 2004. 2006.

52. **Angela Ivask**. Luminescent recombinant sensor bacteria for the analysis of bioavailable heavy metals. 2006.

53. **Tiina Lõugas**. Study on physico-chemical properties and some bioactive compounds of sea buckthorn (*Hippophae rhamnoides* L.). 2006.

54. **Kaja Kasemets**. Effect of changing environmental conditions on the fermentative growth of Saccharomyces cerevisae S288C: auxo-accelerostat study. 2006.

55. **Ildar Nisamedtinov**. Application of ^{13}C and fluorescence labeling in metabolic studies of Saccharomyces spp. 2006.

56. **Alar Leibak**. On additive generalisation of Voronoï's theory of perfect forms over algebraic number fields. 2006.

57. **Andri Jagomägi**. Photoluminescence of chalcopyrite tellurides. 2006.

58. **Tõnu Martma**. Application of carbon isotopes to the study of the Ordovician and Silurian of the Baltic. 2006.

59. **Marit Kauk**. Chemical composition of $CuInSe_2$ monograin powders for solar cell application. 2006.

60. **Julia Kois**. Electrochemical deposition of $CuInSe_2$ thin films for photovoltaic applications. 2006.

61. **Ilona Oja Açik**. Sol-gel deposition of titanium dioxide films. 2007.

62. **Tiia Anmann**. Integrated and organized cellular bioenergetic systems in heart and brain. 2007.

63. **Katrin Trummal**. Purification, characterization and specificity studies of metalloproteinases from *Vipera lebetina* snake venom. 2007.

64. **Gennadi Lessin**. Biochemical definition of coastal zone using numerical modeling and measurement data. 2007.

65. **Enno Pais**. Inverse problems to determine non-homogeneous degenerate memory kernels in heat flow. 2007.

66. **Maria Borissova**. Capillary electrophoresis on alkylimidazolium salts. 2007.

67. **Karin Valmsen**. Prostaglandin synthesis in the coral *Plexaura homomalla*: control of prostaglandin stereochemistry at carbon 15 by cyclooxygenases. 2007.

68. **Kristjan Piirimäe**. Long-term changes of nutrient fluxes in the drainage basin of the gulf of Finland – application of the PolFlow model. 2007.

69. **Tatjana Dedova**. Chemical spray pyrolysis deposition of zinc sulfide thin films and zinc oxide nanostructured layers. 2007.

70. **Katrin Tomson**. Production of labelled recombinant proteins in fed-batch systems in *Escherichia coli*. 2007.

71. **Cecilia Sarmiento**. Suppressors of RNA silencing in plants. 2008.

72. **Vilja Mardla**. Inhibition of platelet aggregation with combination of antiplatelet agents. 2008.

73. **Maie Bachmann**. Effect of Modulated microwave radiation on human resting electroencephalographic signal. 2008.

74. **Dan Hüvonen**. Terahertz spectroscopy of low-dimensional spin systems. 2008.

75. **Ly Villo**. Stereoselective chemoenzymatic synthesis of deoxy sugar esters involving *Candida antarctica* lipase B. 2008.

76. **Johan Anton**. Technology of integrated photoelasticity for residual stress measurement in glass articles of axisymmetric shape. 2008.

77. **Olga Volobujeva**. SEM study of selenization of different thin metallic films. 2008.

78. **Artur Jõgi**. Synthesis of 4'-substituted 2,3'-dideoxynucleoside analogues. 2008.

79. **Mario Kadastik**. Doubly charged Higgs boson decays and implications on neutrino physics. 2008.

80. **Fernando Pérez-Caballero**. Carbon aerogels from 5-methylresorcinol-formaldehyde gels. 2008.

81. **Sirje Vaask**. The comparability, reproducibility and validity of Estonian food consumption surveys. 2008.

82. **Anna Menaker**. Electrosynthesized conducting polymers, polypyrrole and poly(3,4-ethylenedioxythiophene), for molecular imprinting. 2009.

83. **Lauri Ilison**. Solitons and solitary waves in hierarchical Korteweg-de Vries type systems. 2009.

84. **Kaia Ernits**. Study of In_2S_3 and ZnS thin films deposited by ultrasonic spray pyrolysis and chemical deposition. 2009.

85. **Veljo Sinivee**. Portable spectrometer for ionizing radiation "Gammamapper". 2009.

86. **Jüri Virkepu**. On Lagrange formalism for Lie theory and operadic harmonic oscillator in low dimensions. 2009.

87. **Marko Piirsoo**. Deciphering molecular basis of Schwann cell development. 2009.

88. **Kati Helmja**. Determination of phenolic compounds and their antioxidative capability in plant extracts. 2010.

89. **Merike Sõmera**. Sobemoviruses: genomic organization, potential for recombination and necessity of P1 in systemic infection. 2010.

90. **Kristjan Laes**. Preparation and impedance spectroscopy of hybrid structures based on $CuIn_3Se_5$ photoabsorber. 2010.

91. **Kristin Lippur**. Asymmetric synthesis of 2,2'-bimorpholine and its 5,5'-substituted derivatives. 2010.