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Optical Method for Uric Acid Removal Assessment During Dialysis

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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.

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Optiline meetod kusihappe eemaldamise määramiseks dialüüsiravi käigus

JANA HOLMAR

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KIRJASTUS

CONTENTS

INTRODUCTION

From the start of delivering dialysis treatment, its performance has been improved on an ongoing basis. Advances in dialysis machines, filters, water systems etc., have been spectacular, and great efforts have been made to find proper dialysis quality measures. Still, patients' survival has not been improved accordingly. Naturally, renal failure is often only one of many health problems of a patient, and a hope that dialysis treatment only could do miracles, is overbold. However, more individual dialysis, finding new markers and connecting them with a patient's status and other medical conditions could help to achieve better procedure quality and survival for dialysis patients.

Uric acid (UA) (MW=168.1) is a final product of the metabolism of purine and a very important biological molecule present in body fluids.

Hyperuricemia is a symptom when the UA level is above 6.8 mg/dl, at this level urate, ionized form of UA, exceeds its solubility in most biological fluids [1].

It is suggested that increased purine and fructose rich diets that cause hyperuricemia can play an essential role in epidemic spread of obesity and diabetes [2]. Several studies have shown a strong relationship between obesity and development of chronic- and end-stage renal diseases [3].

Number of studies speculate that high level of UA may play an essential role in developing hypertension, cardiovascular events and renal damage [4-6]. Moreover, a number of studies [7-10] (but not all [11]) point out that high level serum UA is an independent risk factor of mortality in end-stage renal disease (ESRD) patients. UA is considered to be one of the small water souble uremic toxins [12]. Therefore, it is an essential solute to monitor in hemodialysis patients.

An earlier research by our group [13] determined that nearly 90% of the measured cumulative ultraviolet (UV)-absorbance originates from the ten main peaks of a dialysis session, one of which is UA. An additional study where the HPLC analysis was used revealed that the main solute producing the UV-absorbance around 280 nm is UA [14, 15]. These findings confirm that it is possible to use an optical method for quantifying removal of UA during the dialysis.

The general aim of the thesis was to determine the concentration and removal of UA optically and on-line. In addition, a possibility to use UA and urea removal assessment to achieve better survival among dialysis patients was studied.

The first part of the study (Publication I and II) estimates the wavelength dependence of UV-absorbance and uremic retention solutes in the spent dialysate. In publication II the total removed uric acid (TR_UA) was monitored by the on-line UV-absorbance measurements of the spent dialysate. Data from two dialysis centers in two countries, Estonia and Sweden, were used.

Publication III introduces algorithms for the estimation of UA concentration. These algorithms are based on data collected over 10 years in Sweden and Estonia, using original or derivative UV-absorbance information from certain wavelengths.

The aim of the study in Publication IV was to analyze connections between serum UA levels, clinical picture and survival; relate the level and removal of two small

molecules, UA and urea, to patients' survival, and offer some indication of levels of these two for medical personnel. Also, models using parameters of two small molecules removal for patient outcome prediction, are presented.
In the future, UV-absorbance monitoring of the spent dialysate and determining the levels and removal of UA as a risk factor for several diseases, e.g. cardiovascular disease (CVD), could give valuable information for medical personnel and could be beneficial for dialysis patients.

This thesis summarizes the author's work at the Department of Biomedical Engineering of the Technomedicum of Tallinn University of Technology. The thesis consists of an overview of the current state of the research problem and the main results reported in the author's publications.

The current thesis is based on the following publications referred to in the text by their Roman numerals I-IV.

I **Jerotskaja (Holmar) J,** Lauri K, Tanner R, Luman M, Fridolin I (2007) "Optical dialysis adequacy sensor: wavelength dependence of the ultraviolet absorbance in the spent dialysate to the removed solutes", *In: Proceedings of 29th Annual International Conference of the IEEE EMBS, Lyon, France August 23-26*, 2960-63 (DOI 10.1109/IEMBS.2007.4352950).

II **Jerotskaja (Holmar) 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).

III **Holmar J**, Fridolin I, Uhlin F, Lauri K, Luman M (2012) "Optical Method for Cardiovascular Risk Marker Uric Acid Removal Assessment during Dialysis," *The Scientific World Journal*, vol. 2012, Article ID 506486, 8 pages, 2012. (DOI:10.1100/2012/506486).

IV **Holmar J**, Uhlin F, Fridolin I, Luman M, Fernström A (2013) "Can multicomponent on-line monitoring of small molecule uremic markers be beneficial for dialysis patients?", *Manuscript submitted*

Approbation

- Optical method and device for measuring concentrations of substances in biological fluids; Priority number: US60/992156 ; Priority date: 04.12.2007
- Optical method and device for quantitative concentration measurements of compounds in the biological fluids; Priority number: P201000049; Priority date: 27.05.2010

- Device and method for middle and protein bound uremic toxins measurements in the biological fluids; Priority number: P201000056; Priority date: 28.06.2010
- Method for middle and protein bound uremic toxins measurements in the biological fluids; Priority number: P201000085; Priority date: 10.12.2010
- Method and device for monitoring removal of hardly diffusible uremic retention solutes during dialysis by UV-absorbance; Priority number: P201100002; Priority date: 14.01.2011
- 29th Annual International Conference of the IEEE EMBS Cité Internationale, Lyon, France August 23-26, 2007.
- 14th Nordic-BalticConference on Biomedical Engineering and Medical Physics, Riga, Latvia, June 16-20, 2008.
- World Congress of Nephrology, Milano, Italy, May 22-26, 2009.
- 31st Annual International Conference of the IEEE EMBS, Minneapolis, USA, September 2-6, 2009.
- 11th International Congress of the Medical Physics and Biomedical Engineering, Munich, Germany, September 7-12, 2009.
- 12th Mediterranean Conference on Medical and Biological Engineering and Computing, Chalkidiki, Greece, May 27-30, 2010.
- 10th Baltic Nephrology Conference, Jurmala, Latvia, October 14-16, 2010.
- 32nd Annual International Conference of the IEEE EMBS, Buenos Aires, Argentina, August 31 - September 4, 2010.
- 15th Nordic–Baltic Conference on Biomedical Engineering and Medical Physics, Aalborg, Denmark, June 14-17, 2011.
- 4th Meeting of Uremic Toxins and Cardiovascular Disease, Groningen, The Netherlands, May 20-22, 2011.
- 5th European Conference of the IFMBE, Budapest, Hungary, September 14 – 18, 2011.
- World Congress on Medical Physics and Biomedical Engineering, Beijing, China, May 26-31, 2012.
- 50th ERA-EDTA Congress, Istanbul, Turkey, May 18-21, 2013.
- 35th Annual International Conference of the IEEE EMBS, Osaka, Japan, July 3-7, 2013.

Author's own contribution

In all the publications the author processed the spectra, created algorithms for UA concentration and removal assessment, completed statistical analysis for the estimation of UA clinical importance and finding possibilities for combining two small molecules for the patient's survival prognosis and contributed to the writing of the papers. In some clinical experiments the author participated in the planning of the experiments, collecting the samples and measuring the UV-absorbance of the samples.

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ABBREVIATIONS

ABI − ankle-brachial index
ACE − angiotensin converting enzyme
AII − angiotensin II
ATP − adenosine triphosphate
BMI − body mass index
BP − blood pressure
CAD − coronary artery disease
CKD – chronic kidney (renal) disease
CRP − C-reactive protein
CVD – cardiovascular disease
TDC – total dialysate collection
ECV − extracellular volume
ESC − European Society of Cardiology
ESRD − end stage renal disease
GFR – glomerular filtration rate
HD − hemodialysis
HDF − hemodiafiltration
HFCS − high fructose corn syrup
HPLC − high performance liquid chromatography
K − clearance
Kt/V − dialysis dose efficacy parameter
LVMI − left ventricular mass index
MLR − multiple linear regression
MW − molecular weight
PAD − peripheral arterial disease
PD − peritoneal dialysis
PNA − protein nitrogen appearance
nPNA − normalized protein nitrogen appearance
RAS − renin-angiotensin system
RR − reduction ratio
RT − renal transplantation
TR − total removed
TR_UA − total removed uric acid
UA − uric acid
URR − urea reduction ratio
UV − ultraviolet

1 THE KIDNEY, KIDNEY FAILURE AND DIALYSIS

1.1 Kidneys

The urinary system includes two kidneys and ureters, urinary bladder and urethra. The paired kidneys are bean-shaped reddish organs located right above the waist. A typical adult kidney is 10-12 cm long and weighs around 135-150g. The kidneys do the majority of work of the urinary system and functions of the kidneys include the following:

- regulation of volume, ionic composition, pH, osmolarity, pressure and glucose level of blood;
- production of hormones;
- excretion of waste materials, excessive liquid and foreign substances.

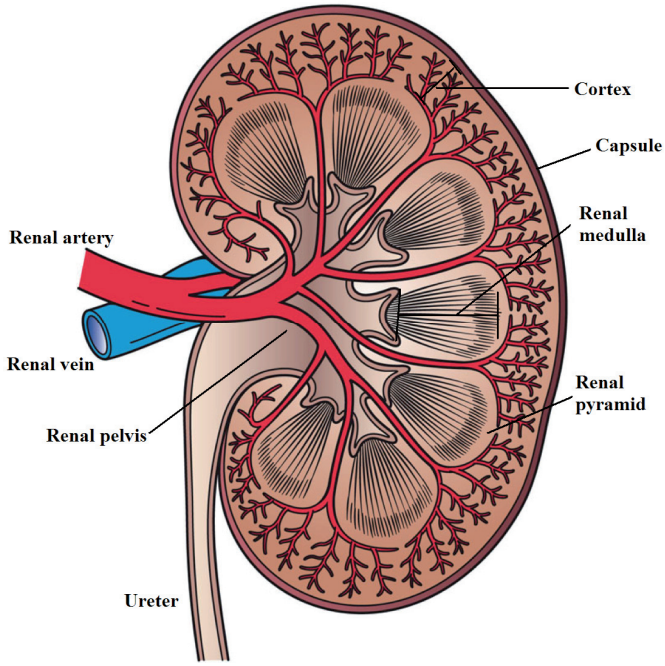

Figure 1. Cross section of the kidney [CC: By Holly Fischer, © Regents of the University of Michigan http://creativecommons.org/licenses/by/3.0/]

Each kidney consists of around 1 million nephrons, which are the functional units of the kidneys. In the course of producing urine, a three-step process is performed by nephrons and collecting ducts: a) glomerular filtration, b) tubular reabsorption and c) tubular secretion. Approximately 150-180 liters of filtrate flow through the nephron system each day [16].

1.2 Kidney failure

Renal (kidney) failure is a loss of renal function inducing a fall of glomerular filtration rate (GFR) below 80 ml/min and to accumulation of creatinine, urea and other nitrogenous end-products. Renal failure can occur rapidly (acute) or slowly and progressively (chronic) [17].
Chronic kidney disease (CKD) is a severe health problem that influences millions of people all over the world. Diabetes mellitus (diabetes) is the prime cause of CKD, and since the occurrence of diabetes is rapidly increasing, prevalence of CKD concurrent to diabetes will continue to rise as well [18].
Three treatment modalities for patients with end-stage chronic kidney failure are available: hemodialysis (HD), peritoneal dialysis (PD) and renal transplantation (RT). The most common way to treat chronic kidney failure is dialysis; majority of renal failure patients spend some time on dialysis while waiting for a donor kidney [19].

1.3 Hemodialysis

Dialysis is a periodically performed procedure for removing excess water and metabolic toxins from a patient's body, also adjusting electrolytes and metabolic acidocis, i.e. pH. During the dialysis procedure, the patient's blood is in contact with the dialysate solution across a semi–permeable membrane. Dialysis was first described by Thomas Graham in 1854. Hemodialysis is an application of an artificial kidney (extracorporeal hemodialysis machine with hemodialyzer). Usually, hemodialysis is performed in a medical center, three times a week and each procedure lasts for four hours. In the course of the hemodialysis procedure, blood flows from the body to the dialyzer. Waste products are removed by diffusion or ultrafiltration across the dialyzer membrane. Two types of procedures are available: hemodialysis (HD) where molecules are removed by diffusion and hemodiafiltration (HDF), which is combining diffusive and convective transport (ultrafiltration) for solute removal. Filtered blood is returned to the patient and used dialysis fluid is sent to the drain [19, 20].

2 UREMIC SOLUTES, UREA AND URIC ACID

To date, 153 uremic retention solutes, which are removed during dialysis, are described by European Uremic Toxins (EUTox) Work Group. Uremic solutes can be divided into three large groups according their physiochemical characteristics and behavior during dialysis [12, 21-24]:

- small, water-soluble, not protein bound solutes (MW < 300 Da, e.g. Uric Acid (MW = 168), Creatinine (MW = 113) and Urea (MW = 60));
- middle molecules (300 < MW < 12000 Da, e.g. β_2-microglobulin (MW = 11 818) and Cystatin C (MW = 13 300));
- protein bound molecules (e.g. Indoxyl sulphate (MW = 251) and p-cresol (MW = 108)).

In the present thesis, focus is on the small water soluble uremic solutes, specifically on urea and UA, on their impact and removal monitoring possibilities.

2.1 Urea and dialysis dose

Urea (MW=60) is a small water-soluble compound, one of the most commonly used markers for estimating dialysis quality [25]. Urea is quantitatively the main end product of protein catabolism and the primal organic solute which appears in blood in patients with kidney failure [26]. It is possible to estimate dialysis patient nutrition status by calculating PNA (protein nitrogen appearance) and/or nPNA (normalized PNA) using urea nitrogen appearance in serum/urine/dialysate [27].
A classical way to estimate dialysis adequacy is to estimate urea levels in the blood at the start and the end of the dialysis procedure and calculate urea reduction ratio (URR) [28]. Another way of measuring a dialysis dose is the Kt/V, where K stands for dialyzer urea clearance (ml/min), t is dialysis procedure duration and V is the volume of water in a patient's body, defined also as the patient's volume of urea distribution in ml [29].
On the basis of clinical practice guidelines for hemodialysis adequacy, the delivered dose of the dialysis should be measured regularly at least once a month [29]. On the basis of 255 questionnaires from 14 European countries (255 dialysis centers), it turned out [30] that:

- 37% of centers only estimated URR;
- 40% of centers measured urea removal less than monthly;
- 6% dialysis centers never perform adequacy measurements;
- 9% dialysis centers do it only every 6 months or less frequently.

Regardless of wide usage, studies that show direct biologic and toxic effects of urea are rare [31, 32]. Urea seems to act as a surrogate marker reflecting behavior of other uremic toxins with serious impact [23]. However, removal kinetics of urea is different from many small molecular weight uremic solutes removal kinetics (UA among them) [22].
Estimation of dialysis quality on the basis of urea needs blood or urine sampling, laboratory tests and reagents. Therefore, quality estimation can not be performed on-line, and it is impossible to make adjustments in the course of procedure. It has been demonstrated that a dialysis dose could be estimated on-line by optical methods [33] and it could be a beneficial alternative to traditional methods [34].

2.2 Uric acid (UA)

Uric acid (UA) is an end product of the metabolism of purines (MW=168.1) and an important biological molecule present in body fluids. Purines play a particularly important role in human metabolism: they are needed for the transmission and storage of genetic information, provide the energy, they take part in signal transduction and translation and form the basis of coenzymes. Cells need stable amount of purines for growth, survival and reproduction. Purine nucleotides are synthesized and degraded in the course of several reactions resulting in the

combination of UA. An increased synthesis of UA is usually a reason for the increase of the UA content in biological fluids. The disorders that may result in UA overproduction are: rise in *de novo* purine synthesis, increased purine nucleotide degradation and enhanced adenosine triphosphate (ATP) degradation or diminished synthesis of ATP [35].
UA is a weak acid (pH=5.8), its ionized form is urate, and it has physiologic pH. The amount of UA in the body depends on the age, sex, diet and synthesis and excretion rate [36]. Hyperuricemia is a condition when the UA concentration in serum exceeds 6.8 mg/dl, at this level urate exceeds its solubility in most biological fluids [1]. From a general standpoint, genetic disturbances resulting in hyperuricemia will lead to an underexcretion of UA (renal handling of UA is influenced) or overproduction of UA [37].
Unlike most mammals, humans are unable to regulate UA levels effectively. The primar reason is the mutational loss of uricase (urate oxidase) which degrades UA to allantoin. The absence of uricase and large-scale reabsorption of filtered urate causes 10 times higher urate levels in human plasma than in most other mammals [36]. It is estimated that the mutation that caused loss of uricase gene occurred in the mid-Miocene epoch about 15 million years ago. Thus, it is presumed that UA level in humans rose during evolution as a protecting mechanism against cancer, aging, and CVD; UA is an influential antioxidant and a destructor of singlet oxygen and radicals. Also, it is suggested that UA has a role in increasing intelligence, reaction time and danger signal to guard humans against starvation [38, 39]. The main outcome of the lack of this enzyme is hyperuricemia developing due to diet, especially from purine rich meats, seafood and beer [40].
Genome scan of 644 participants for the determination of serum UA variability showed that high serum UA levels are significantly heritable. It was also shown that there is a significant genetic influence on the variation of UA and its relationship with different cardiovascular risk markers such as waist circumference, body mass index (BMI), pulse- and systolic blood pressure. During the study, linkage for UA was identified at 133 cM on chromosome 6q22-23. It is essential while the genetic region under discussion has previously been associated with cardiovascular and kidney related phenotypes, diabetes and, for example, IgA nephropathy [41]. Urate transport in the human organism is regulated by SLC2A9 (GLUT9) [42] and ABCG2 [43].

2.3 UA and health risks

It has been reported that increased purine and fructose rich diets that cause hyperuricemia can play an essential role in an epidemic spread of obesity and diabetes [2]. Several studies have shown that there is a significant connection between obesity and developing of chronic- and end-stage renal disease [3].
Recent studies suggest that elevated level of UA could play an essential role in developing hypertension, cardiovascular events and renal damage [4-6]; controversially UA is also a powerful antioxidant in the human organism [4, 6].

However, UA is an antioxidant only in a hydrophilic environment, and this is most probably a major cause for limitations of UA antioxidant powers. Additionally, it has been presumed that UA can react with oxidants and produce radicals that can cause oxidative damage to the cells; also UA itself can be a biologically active proinflammatory factor for oxidative stress [44].
According to the "European Society of Cardiology (ESC) guidelines for the diagnosis and treatment of acute and chronic hearth failure", elevated UA level is one of the conditions of poor prognosis in hearth failure [45].
It was observed that subjects with higher serum UA levels are more at risk of developing type 2 diabetes, and UA is considered as a relevant and independent risk factor for diabetes [46]. Elevated serum UA levels seem to be independently associated with higher probability for cardiovascular mortality but, not for all-cause mortality in type 2 diabetes patients [47]. It has been confirmed by animal studies that UA may cause a metabolic syndrome [48].

UA and gout

UA solves in blood in a small quantity and will crystallize in the case of supersaturation. The UA crystallites are stored on the skin surface, joints, and particularly in the toes, causing gout. Acute gout is typically intermittent and very painful, chronic gout develops during years of recurrent acute gout occasions. Antihyperuricemic treatment is associated with an 80% decrease of risk of recurring gout and it confirms the direct causal between gout and UA levels [36]. Chronic treatment of gout involves antihyperuricemic or urate-lowering therapy and serum UA level below 6.0 mg/dl is an initial target [49]. It is also confirmed that hypertension and alcohol intake are strong risk factors for gout, but consumption of vegetables and fruits (rich in dietary fiber and c-vitamin) helps to prevent gout [50]. An extensive study in Taiwan where 5707 patients from different areas of the country were included showed that 24% of males and 20% of females either had hyperuricemia or were taking medication for it. Hyperuricemia in some areas was not completely explained by obesity or alcohol consumption [51].
UA is a frequent component in urinary stones [52] and high serum UA level is determined on patients with UA urolithiasis [53]. However, it has been found that risk of urolithiasis in patients with asymptomatic hyperuricemia is low compared with normouricemic patients, and occurrence of azotemia attributable to hyperuricemia has no clinical importance until serum UA levels reach 10 mg/dl in women and 13 mg/dl in men [54].

UA and CVD

Association between UA and CVD has been discussed over 100 years, yet there are many studies that confirm and many of those that exclude the role of the UA in cardiovascular events. However, presence of hyperuricemia should warn a clinician

to an overall risk of CVD [55-57]. A study of Chinese population consisting of 1500 subjects detected that hyperuricemia is positively related to many CVD markers and health problems such as BMI, waist hip ratio, blood pressure (BP), level of urea, creatinine, protein, glucose (after fasting and 2 hours after taken orally 75 g of glucose), insulin (measured 2 hours after glucose load) and triglycerides. Relations in men were stronger than in women [58]. Another large 6-year follow-up study in China consisting of 3122 patients showed that patients with high serum UA levels and low ankle-brachial index (ABI) had a higher risk for all-cause and CVD mortality. It has been assumed that these two markers can be used to predict mortality [59].

The findings of recent LIFE study showed an association between serum UA levels and cardiovascular disturbances in hypertensive women. It also showed that about 29% of the treatment advantage of a losartan-based vs. atenolol-based therapy on the primary endpoint (death, stroke or myocardial infarction) may be considered as a result of achieved differences in serum UA levels. It means that lowering serum UA may be a powerful tool to diminish the risk for CVD [60, 61]. However, a large cohort study in Taiwan (including 484 568 subjects followed 13 years) found that UA is a significant, but not an independent risk factor for all-cause and CVD mortality and should not be the target for treatment [62].

The study of *Dunkelgrun* et al. followed 936 open vascular surgery patients to investigate the relationship between the level of UA and early and late cardiovascular outcome. Average follow-up was 3.7 years. It was observed that pre- operational hyperuricemia in vascular patients significantly predicts late (but not early) mortality and cardiac death or myocardial infarction [63].

To reveal the connection between the coronary artery disease (CAD) and hyperuricemia, a study was performed on 540 coronary angiography patients with symptoms of CAD. It was found that hyperuricemia is independently associated with angiographically documented CAD; moreover, severity of CAD in all and in men group was related to hyperuricemia [64]. Additionally, a study of 4.5 years survival of 1140 consecutive patients undergoing scheduled coronary artery bypass grafting showed that higher UA level before surgery is associated with poorer survival afterwards [65].

Japanese researchers studied the connection between UA levels, left ventricular mass index (LVMI) and occurrence of CVD. Altogether 619 hypertensive subjects free of former CVD were studied during 33.5 ± 0.8 months. It was found that serum UA is independently connected with LVMI; the occasion of CVD in subjects with elevated UA level and LVMI was 2.4 fold higher than in subjects with lower levels of UA and LVMI. These results suggest that hyperuricemia in combination with left ventricle hypertrophy is a predictor for CVD [66].

Large vessel disease (atherosclerosis) may appear as coronary or carotid artery disease, renal artery stenosis or abdominal aneurysms; major risk factors for it are family history, high cholesterol and smoking. In contrast, microvascular disease (arteriosclerosis) is noted with primary hypertension and in hypertensive renal disease; major risk factors are hyperuricemia, metabolic syndrome, fetal

programming, and diet. The arteriosclerosis plays a role in the pathogenesis of CKD. The mechanism behind it can be explained as the elevated level of UA (for example due to dietary fructose) that raises the level of angiotensin II (AII), lowers nitric oxide bioavailability, increases BP and oxidative stress. Those stimuli play a role in endothelial cell dysfunction and apoptosis. In vascular smooth muscle cells, the effects of these stimuli are causing a decrease in contractile capacity. Stiffened and thickened microvessels do not have sufficient autoregulatory capacity, resulting in increased BP in target organs as the brain and the kidney. Increased BP in these organs may cause serious diseases such as CKD. Moreover, renal microvascular disease may have an essential part in developing salt-sensitive hypertension [67].
Evaluation of UA levels connections with all-cause and CVD mortality and kidney failure was studied by *Madero* et al. That follow-up study included 840 participants, and it was found that elevated UA levels are a significant and independent predictor for all-cause and CVD mortality but not for the kidney failure [8]. To determine the connection between UA levels in blood and the peripheral arterial disease (PAD) 3987 subjects without clinical history of CVD were studied. It turned out that UA levels and PAD are positively related, and that relation is independent of other common CVD risk factors such as BMI, hypertension, smoking, diabetes, serum cholesterol, and creatinine [68].

UA and hypertension

Hypertension is the most common cardiovascular disease widely spread today; it is known that hypertension causes ESRD [69]. An additional study of 429 untreated patients with essential hypertension demonstrated that hyperuricemia is independently associated with microalbuminuria [70].
About 15 years ago the discussion of the role of UA as a casual risk factor in developing hypertension had resurrected [71].
If blood volume or flow to the kidneys falls, juxtaglomerular cells in the kidneys will release renin into the circulation. As the response renin and angiotensin converting enzyme (ACE) will act with their substrates resulting in the production of active hormone AII. AII can raise BP in two ways: a) AII raises BP by increasing systemic vascular resistance; b) AII stimulates the secretion of aldosterone, which accelerates the reabsorption of water and sodium ions (Na+) by the kidneys. The reabsorption of water increases blood volume and thereby induces hyperuricemia [16].
It has been shown that serum UA independently predicts declined renal vascular responsiveness to AII in humans. Thus, it confirms that UA is associated with an activated intrarenal renin-angiotensin system (RAS). This may explain the relationship between UA levels and the risk of hypertension and nephropathy [72].
It has been recommended that the C-reactive protein (CRP) level in the blood could be the marker for predicting cardiovascular disturbances [73]; risk might be diminished by overweight control while this decreases plasma CRP level [74].

Kang et al. found that UA may affect expression of CPR in vascular endothelial and smooth muscle cells, impede endothelial cell proliferation/migration and damage nitric oxide production. It was also noted that CRP differentially modulates UA-induced cell migration and proliferation in human vascular smooth muscle and umbilical vein endothelial cells. It refers to UA as a true mediator of endothelial dysfunction by increasing CRP production, and this suggests an explanation for the genesis of hypertension and kidney disease in hyperuricemic patients [75]. The effect of UA on the formation of endothelial dysfunction has also been demonstrated by *Khosla* et al. by demonstrating a direct linear relation between serum UA and serum nitric oxide levels in rats. Reducing serum UA levels by allopurinol reversed the decrease in nitric oxide. It has been recommended that the UA may accect endothelial dysfunction via inhibiting the production of nitric oxide [76]. *Zoccally* et al. conducted a study to determine the relationship between UA, renal function and endothelial function. They used endothelium-dependent vasodilatory reaction to acetylcholine in the forearm for estimating the endothelial function. Totally 217 never-treated subjects with uncomplicated essential hypertension who had normal levels of serum creatinine were included to the study. The results showed that serum UA levels relation with endothelial dysfunction is independent of other traditional risk factors [77].

Chinese researchers studied 3520 patients to reveal the relationship between UA concentration and BP. It was found that serum UA levels are significantly related to high BP; however, this relation may be explained by the strong relation of BMI to both UA level and BP [78]. A large cohort study in China (7220 participants) where BMI and other CVD markers were adjusted showed a significant relation between higher UA levels and risk for developing hypertension [79]. A study of 2145 patients in Taiwan showed very high rate of occurrence of hyperuricemia among hypertension patients (average in men 35% and in women 43%); also serum creatine level and diuretic usage were significantly and independently related to serum UA levels. A study of 2394 hypertensive patients in China revealed that serum UA and creatinine are predictors of mortality [80]. To manage hypertension diuretics are used (because of the antihypertensive effect), unfortunately it has been found that diuretic users have higher serum creatinine and UA levels than those on non-diuretic therapy [81, 82]. Therefore, use of losartan, a drug which has antihypertensive and UA lowering ability, has been recommended [83].

A study of 95 children with hypertension *Feig* et al. confirmed the hypothesis that elevated serum UA levels may have a part in the development of hypertension [84]. Studies on rats performed by *Mazalli* et al. demonstrated that rats develop high BP after 3-5 weeks after raising the UA level by feeding them the inhibitor of uricase, an oxonic acid [85].

UA and chronic kidney disease

Chronic kidney disease (CKD) is a widely spread health problem that affects 10-15% of the adult population, it has high economic cost and is related to high prevalence of CVD. The general causes of CKD are diabetes [86] and aging [87]. It has been found that using allopurinol for slowing the progression of renal disease and decreasing the BP on patients with kidney disease has a good effect, and it lowers the level of UA in the blood [88]. Controversially, no significant difference has been found in UA levels between users and non-users of allopurinol among CKD (stage 5) patients [9]. It has been observed that UA level in blood was higher in patients with CKD than in non-CVD patients; however, UA did not predicted independently processing of CKD on non-diabetic patients [89].

Usually serum creatinine level is used to describe renal function, and higher creatinine levels in blood refer to nephron loss and thereby decreased renal function [90]. However, it has been shown that creatinine level underestimates the renal involvement in elderly hypertensive patients whereas serum UA level is a more sensitive marker for hypertensive target organ damage [91]. A large-scale study in Japan revealed that serum creatinine level correlates very well with the serum UA level, and UA may be an early indicator of renal dysfunction [92].

It has been demonstrated that UA acts as a stimulator of RAS in mesangial cells (cells that are regulating ultrafiltration coefficient (Kf)); it causes a rise in the intracellular calcium level and it could be (one of) the reason of glomerulosclerosis in the case of hyperuricemia [93].

To determinate the effect of UA on GFR a 5-year follow-up study of healthy and normotensive individuals was performed by *Bellomo* et al., and it was discovered that GFR decreases over time, UA being an independent risk factor for it [94].

A large cohort study with 148 217 subjects was conducted by *Neri* et al., and it was found that increased serum UA levels are related to decrease in GFR, and increase in CVD and morbidity [95].

The results of a study of type 1 diabetes patients show that high serum UA level occurs with impaired renal function [96]. During a 6-year follow-up study of the same patients it was found that serum UA is an independent and significant predictor of the change of early GFR loss [97]. A similar result was achieved by *Obermayr* et al. who found that an elevated UA level is an important and probably an independent risk factor for new-onset kidney disease [98].

Hyperuricemia and gout are extremely common problems among RT patients. Higher UA levels may be the response to medicaments (for example, Cyclosporine) given to transplant patients to avoid edema or hypertension but which will influence nephronal handling of UA [99]. It is advised to use Tacrolimus instead of Cyclosporine to achieve efficient and safe immunosuppressive therapy [100]. However, it has been shown that both of those drugs cause hyperuricemia in RT patients and there is no difference between them from that point of view [101]. In the study of 350 kidney transplant patients in Germany, it was observed that elevated serum UA level has a significant role in

reduced transplant survival [102]. In contrast, the follow-up study of kidney allograft recipients did not observe a relationship between elevated UA levels and decline in renal transplant function during 30 months [103]. It has been suggested that UA levels should be evaluated as a possible threat for renal allograft nephropathy or renal malfunction [104].

A study of 116 children with CKD revealed that hyperuricemia is highly prevalent among these patients and seems to be connected with increased BMI, elevated BP, albuminuria and reduced estimated GFR [105].

As aresult of a Cardiovascular Health Study among 5888 adults aged 65 or older, it was found that UA levels were strongly associated with kidney dysfunction and prevalent CKD; however, UA levels had a significant but much weaker relation with progression of kidney disease [106]. Similarly, a study of 800 elderly subjects (age≥65) in Taiwan revealed that UA independently predicts the progression of kidney disease [107].

Studies show that UA does not only cause but also aggravates renal disease in rats by activating RAS and cyclooxygenase-2 (COX-2) system in progressive renal disease [108-110]. An analysis of the influence of mild increase in UA level on glomerular hemodynamics of the rat revealed a correlation between UA and glomerular pressure that is probably so due to insufficient vasoconstriction of the afferent arteriole [111]. To investigate the casual role of UA in renal disease *Nakagawa* et al. performed studies in rats with induced hyperuricemia. It was found that UA is not only a risk marker but also the reason and accelerator of renal disease [112].

A large-scale study in Japan examined 48 177 subjects to find the relationship between serum UA levels and risk for developing ESRD. It was witnessed that hyperuricemia has a significant effect on developing of ESRD, especially in women [113].

Mortality among dialysis patients is noticeably higher compared with the general population worldwide. However, it has been discovered that mortality rates differ in regions, and mortality among dialysis patients is in good correlation with mortality among the general population in a specific region. The latter reveals the importance of the role of genetic and environmental factors in dialysis patients outcome [114].

It has been found that higher UA levels are connected with a higher risk for mortality in non-CKD population [115]. Similar studies of CKD patients have reported contradictory results. To investigate the relationship between UA levels and all-cause mortality *Suliman* et al. studied 294 patients with stage 5 CKD. They found that there is J-shaped relationship between UA levels and mortality, patients with UA level of 9.0 mg/dl or higher had almost 2.0 fold increased risk for mortality, also patients with UA levels of 5.2 mg/dl or less had 40% increased risk for mortality. They also observed that the serum UA level correlates positively with levels of triglycerides, CRP and phosphate and negatively with the level of calcium [9]. Similar results have been achieved by *Hsu* et al. [7]. Significant effect of low UA level on mortality among dialysis patients has been proved by *Lee* et al.

[116]. The study by *Madero* et al. observed that a higher UA level seems to act as an independent threat factor for CVD and all-cause mortality [8], similarly *Chung* et al. have demonstrated that hyperuricemia is an independent risk factor for all-cause mortality among CKD patients [10]. In contrast, a large-scale study identified high serum UA level as a factor which lowers the risk for all-cause and CVD mortality among CKD patients [11]. *Cohen* et al. found that incident of gout is an independent mortality risk factor in dialysis patients [117].

2.4 Standard biochemical methods for UA measurement

To detect UA in clinical chemistry laboratories the following methods are used [118, 119]:

- Enzymatic and colorimetric method (one reagent, absorbance at 520 nm, caused by quinoneimine dye, is proportional to the UA concentration):

  $$UA + 2H_2O + O_2 \xrightarrow{UA} CO_2 + H_2O_2$$

  $$H_2O_2 + \text{2,4,6-Tribromo-3-hydrobenzoic acid} + \text{4-Aminoantipyrine} \xrightarrow{Peroxidase} \text{Quinoneimine Dye} + 4H_2O$$

- Enzymatic and colorimetric method (two reagents):

  $$UA + 2H_2O + O_2 \xrightarrow{uricase} \text{Allantoin} + CO_2 + H_2O_2$$

  $$2H_2O_2 + \text{3,5-Dichloro-2-hydroxybenzenesulfonate} + \text{4-Aminoantipyrine} \xrightarrow{Peroxidase} \text{Quinoneimine Dye} + 4H_2O$$

- Enzymatic and ultraviolet (decrease of the absorbance at 293 nm is proportional to the UA concentration)

  $$UA + 2H_2O + O_2 \xrightarrow{uricase\ \&\ pH\ 9.4} \text{Allantoin} + CO_2 + H_2O_2$$

These methods need blood (urine) sampling, expensive lab equipment, disposables or chemicals, an extra effort from medical personnel and as the most crucial part - it is possible to estimate dialysis adequacy only after the procedure when corrections or adjustments are impossible. Therefore, these methods are unsuitable for on-line dialysis monitoring.

3 OPTICAL METHOD FOR UREMIC SOLUTES ASSESSMENT

3.1 Light and matter

If light interacts with biological fluid, it can be reflected from the surface or transmitted to the medium; inside the medium it can be absorbed, scattered or internally reflected; transmission can occur without effect, after attenuation by scattering/absorbance (diffuse transmission) or as a reflection from the medium (diffuse reflection) [120].
According to *Welch* et al., in tenuous media (like spent dialysate) all the incident light is either reflected, absorbed or transmitted and scattering can be ignored [121]. Therefore, it is possible to monitor successfully the spent dialysate with concentration calculation methods utilized in absorbance spectroscopy [122, 123].

3.2 Bouguer−Beer−Lambert Law and absorbance

Absorption is annihilation of photonic energy in the course of interaction with electrons, atoms or molecules and transformation into heat or photons with decreased energy, e.g. fluorescence or phosphorescence [124].

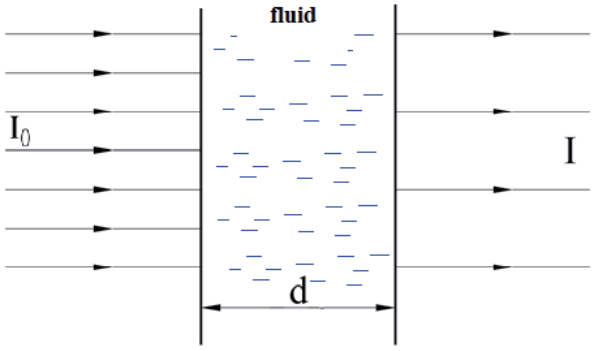

Figure 2. Entering I_0 and passing I light through a cuvette filled with fluid

The absorption of light by a sample is generally expressed by the Bouguer-Beer-Lambert law (often abbreviated to Beer-Lambert law) that states that the incident light intensity decreases exponentially with the thickness of the sample and absorbance of entering light is proportional to the amount of material that absorbs the incident light (i.e. the concentration of the compound).

$$I = I_0 e^{-\alpha d C} \qquad \textbf{(1)}$$

where I_0 is the intensity of incident radiation, and I is the intensity of light passing the sample, α is a molar absorption coefficient [m^{-1} $(mol/L)^{-1}$], d is the optical path length [m] and C is the concentration of the absorbing compound [mol/L]. If (1) is given in logarithmic form:

$$\log_{10}\frac{I}{I_0} = -\alpha d C \log_{10}(e) \qquad \textbf{(2)}$$

Absorbance (A) is a logarithmic measure of the amount of light absorbed (at a certain wavelength) as the light passes through biological fluid (i.e. spent dialysate) (Figure 2).

$$A = \log_{10}\frac{I_0}{I} \qquad \textbf{(3)}$$

Absorbance is connected with transmittance T as follows [125, 126]:

$$A = -\log_{10} T = -\log_{10}\frac{I}{I_0}$$
$$T = \frac{I}{I_0} \qquad \textbf{(4)}$$

The molar extinction coefficient (ε) is defined as follows:

$$\varepsilon = 0.4343\alpha \qquad \textbf{(5)}$$

Accordingly, the Beer-Lambert law can be written

$$A = \varepsilon C d \qquad \textbf{(6)}$$

It states that the amount of e.g. the UV-light absorbed (A) [dimensionless] when pervade a cuvette (made of UV-transparent material such as quartz) containing the fluid under investigation, is linearly related to the concentration C of the absorbing compound, the optical path length (width of the cuvette) and the extinction coefficient ε, sometimes called the molar absorptivity at a certain wavelength.
If ε is known and A is obtained from the spectroscopic measurement, it is possible to reveal the concentration as

$$C = \frac{A}{\varepsilon d} \qquad \textbf{(7)}$$

When several different absorbing compounds are presented in the spent dialysate, the overall extinction coefficient is the cumulation of the impact of each compound.

$$A = \log_{10}\left[\frac{I_0}{I}\right] = (\varepsilon_1 C_1 + \varepsilon_2 C_2 + ... + \varepsilon_n C_n)d \quad \textbf{(8)}$$

or

$$A^{\lambda} = \sum_{i=1}^{n} A_i^{\lambda} = d\sum_{i=1}^{n} \varepsilon_i^{\lambda} C_i \quad \textbf{(9)}$$

The Beer-Lambert law could be used in the case of dilute solutions on the assumption that:

- light is attenuated only by absorption, not by scattering or reflections;
- concentration (and resulting absorbance) of the solution is not very high;
- an analyte does not dissociate, associate or react with a solvent;
- absorbing substance is distributed homogenously in a solvent;
- radiation is monochromatic and collimated [122, 123, 125, 126].

Spent dialysate can be considered as a biological fluid, which is transparent and weakly scattering [127] and therefore the Beer−Lambert law is applicable.

3.3 Optical monitoring of uremic solutes in the spent dialysate

Pathfinders in the field of optical monitoring of dialysis efficacy were *Gal and Grof* who published a paper how UV-transmittance of the spent dialysate at 254 nm could be used for this purpose [128].

In 1998, a patent application concerning waste products determination in dialysate was filed in Sweden [129]. In 2001, the results of optical tool development for monitoring different uremic substances in the spent dialysate by using UV-absorbance of the spent dialysate were presented [130, 131]. Soon afterwards in a successful clinical study it was demonstrated that it is feasible to estimate the dialysis dose by using UV-absorbance technology [132]. UV-absorbance of several uremic solutes and good correlation between absorbance of the spent dialysate and solute concentration have been demonstrated [133, 134].

Ways of monitoring UA and other biological compounds in fluids using optical tools were also reported by other groups [135-139].

Since then, numerous studies have been conducted in collaboration with researchers at Tallinn University of Tehnology and Linköping University. Many possibilities to use optical information for dialysis adequacy monitoring have been introduced, including urea and related dialysis quality measures [33, 122, 140, 141], creatinine quantification [142-144], uric acid concentration evaluation [145-151], estimation of nutrition status [152, 153], whole dialysate studies [14, 15, 154-156] and also use of optical methods to detect protein bound toxins [157, 158], and middle molecules [159] removal during hemodialysis. Discussions of whether UV techniques could be used for dialysis monitoring have reached the conclusion that this technique can be supportive for medical personnel by ensuring delivery of the

dialysis dose and providing real time feedback being a relatively inexpensive method [160]. Moreover, it has been found that it might be a valid alternative to other on-line urea monitoring devices (ionic dialysance calculated on the basis of dialysate conductivity) [161].

4 EXPERIMENTAL STUDIES: METHODS, RESULTS AND DISCUSSION

4.1 Methods

Patients and experimental set-up
In all experiments, hemodialysis patients from Tallinn, Estonia and Linköping, Sweden were included. Summary of patients who participated in the studies, conditions of studies, and sampling times are given in Table 1. The studies were conducted after acceptance of the protocol by the Regional Ethical Review Board, Linköping, Sweden and the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. Informed consent was obtained from all participating patients.

Table 1. Patients, conditions and sampling times

	Publication I	**Publication II**	**Publication III**	**Publication IV**
Total number of patients (M/F)	10 (7/3)	16 (13/3)	60 (41/19)	51 (43/8)
-from Tallinn, mean age ± SD	10 (7/3), 63 ± 19	10 (7/3), 63 ± 19	10 (7/3), 63 ± 19 7(4/3), 56 ± 13	10 (7/3), 63 ± 12
-from Linköping, mean age ± SD		6 (6/0), 64 ± 19	10 (6/4), 63 ± 21 7 (4/3), 57 ± 23 10 (6/4), 60 ± 19 8 (7/1), 77 ± 7 8 (7/1), 77 ± 7	33 (29/4), 71 ± 12 8 (7/1), 77 ± 7
Number of sessions	30	56	188	51
Dialysis machine	Fesenius 4008H	Fesenius 4008H Gambro AK200	Fesenius 4008H Fesenius 5008H Gambro AK200	Fesenius 4008H Fesenius 5008H
Blood flow ml/min	245-350	200-350	200-350	250-390
Session's duration (min)	180-240	180-270	180-270	180-270
Sampling time - dialysate	10,60,90,120, 180,210,240,tank	5,10,15,30,60, 90,120,180,240, 270,tank	5,10,15,30,60, 90,120,180,240, 270,tank	10,240 or 270, tank
Sampling time - blood				pre-and post-dialysis

Clinical set-up is given in Figure 3.

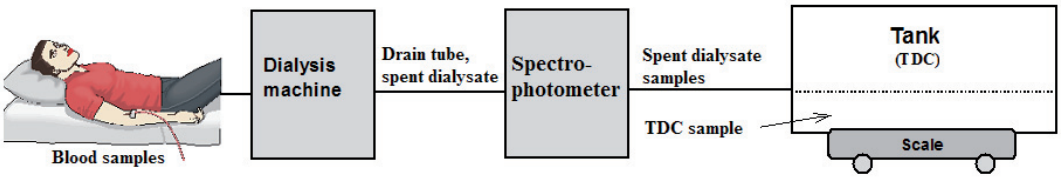

Figure 3. Set-up of the studies for monitoring UA concentration, removal and influence on patient's survival

To detect UV-absorbance spectra of the collected spent dialysate samples the following double-beam spectrophotometers were used: UVIKON 943 (Kontron, Italy), JASCO V-570 (Jasco, Japan) and SHIMADZU UV-2401 PC (Shimadzu, Japan). Spectrophotometric measurements over a wavelength range of 190–380 nm were taken; an optical cell with a path length of 1 cm was used.
For on-line UV-absorbance registration, spectrophotometers Uvikon 943 (Kontron, Italy) and HR2000 (Ocean Optics Inc., USA) were used. UV-absorbance of the spent dialysate at 285 nm was registered during dialysis; rectangular and circular optical flow cuvette with an optical path length of 1 cm was utilized.
The concentrations of UA and urea in the samples were determined in the Clinical Chemistry Laboratories at the North Estonian Medical Centre and Linköping University Hospital using standardized methods. The estimation accuracy of the methods for UA and urea in the dialysate was to ± 3-5%.

Statistics and signal processing

In publication II UV-absorbance spectra were processed with the Savitzky-Golay algorithm using Panorama Fluorescence 1.2 (Shimadzu, Japan). The Savitzky-Golay algorithm used for smoothing and differentiation of data [162] is considered an efficient method for eliminating baseline effects in spectra [163]. Publication III describes multiple linear regression for determining the best wavelengths for the UA models [164-168]. Statistic software Statsoft 9.0 (Statsoft Inc., US) was used.
Publication IV presents Kaplan-Meier survival analysis with a log-rank test to compare survival in different groups. The Kaplan-Meier method calculates the probability of surviving a given length of time; to compare survival in two groups the most commonly used log-rank test was employed. The log-rank method tests the null hypothesis that the compared groups are from the same population as regards survival experience [164]. Logistic regression analysis was used to create models for survival estimation; survival was set as a dependent parameter and UA and/or urea based clinical parameters as independent variables. Statistic software Statsoft 9.0 (Statsoft Inc., US) was used. Logistic regression analysis is an extension of multiple regression analysis, and it can be used if an outcome variable is categorical [169]. Modelling survival is also possible perform by using Cox regression model, also known as proportional hazards regression analysis. In its

capability Cox method is equivalent to multiple regression analysis except that the Cox regression model defines hazard at a given time [164].
Student t-test was used to determine differences between the values from the chemical laboratory and UV models, a p value lower than 0.05 was considered significantly different.

4.2 UV-absorbance of spent dialysate and online monitoring of UA (Publication I and II)

A need for continuous on-line monitoring systems for the dialysis process and their efficiency has been raised due to the rising quantity of dialysis patients and quality requirements of the treatment. A technique using optical properties of the spent dialysate that would offer aforenamed functionality in a reliable, simple and cost-effective way would be valuable. In publication I, correlations between five uremic retention solutes (urea, uric acid, creatinine, phosphate and potassium) and UV-absorbance in the spent dialysate were detected. For that purpose, ten uremic patients were followed during their three consecutive dialysis sessions, and spent dialysate samples were collected during the procedures. The uremic solutes' content of the samples was investigated in a chemical laboratory and UV-absorbance was registered over a wavelength range of 190-380 nm. Correlation values were calculated for each substance at each wavelength, and the wavelength dependence between the UV-absorbance and the solutes removed during the procedure was investigated. Relatively high correlation coefficient values (r=0.74-0.92) were detected for all studied substances. Moreover, a good correlation coefficient can be achieved even for non UV-absorbing solutes (e.g. urea) when its reduction ratio during the dialysis is similar to some UV-absorbing solute. The linear relationship detected offers a possibility to calculate the concentration of the substance using the measured UV-absorbance values. It confirms the possibility to use the UV-absorbance technology for dialysis monitoring. The correlation maximums for urea, creatinine, phosphate and potassium were obtained in the wavelength region 227-240 nm while the correlation maximum for UA was obtained at 294 nm. It indicates that using different wavelengths for estimating different substances could be advantageous.
Hyperuricemia is widely spread among CKD patients [115], and it is presumed that high UA levels may aggravate CKD progression and have a part in CVD morbidity and mortality in dialysis patients [8, 94, 95, 107]. Moreover, recent reviews suggest that elevated UA levels should be lowered to prevent progression of CKD [170]. This confirms that monitoring of UA levels among dialysis patients could be beneficial. In current practice, dialysis patients' serum UA levels are determined from blood samples in clinical chemistry laboratories. In reasonable excuses, blood sampling is not performed too often. It causes a situation where the procedure performance in terms of removed UA remains unknown in most dialysis treatments. One solution could be the optical monitoring of the spent dialysate while it was demonstrated in Publication I that UA concentration is in good linear

correlation with the overall UV-absorbance of the spent dialysate [134]. It has been demonstrated that UA is an UV-absorbing solute contributing essentially to UV-absorbance in the spent dialysate at certain wavelengths (Figure 4).

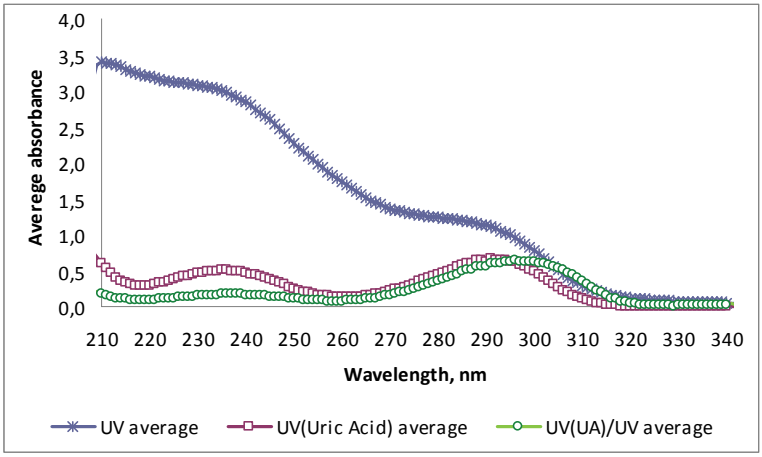

Figure 4. Average value of measured UV-absorbance and contribution of UA to UV-absorbance

Around 90% of the cumulative UV-absorbance measured during a dialysis treatment originates from the ten main peaks, one of which is UA [13]. An additional study where the HPLC analysis was used revealed that the main solute producing UV-absorbance around 280 nm is UA [14, 15]. Moreover, by monitoring the UV-absorbance during the dialysis does not only allow one to detect the concentration of several removed solutes but immediately indicates the disturbances in treatment [33]. It has been discussed that the optical on-line method could make it possible to reduce the number of blood samples, and thereby reduce blood loss and costs for the laboratory tests [34].

Publication II presents an online monitoring possibility of removed UA. For that purpose, 16 patients from Estonia and Sweden were followed during their dialysis sessions (N = 56). The spent dialysate samples were collected during the treatment, and their UA content was determined in the clinical laboratory. On-line UV-absorbance at 285 nm was recorded during the sessions. Absorbance values were set against concentration values and the achieved regression model was used to estimate UA concentration and the total removed amount of UA (TR_UA). Two regression models were created: a patient specific (UV1) which used values from the first session of each patient's treatment, and a general model which used data from all sessions (UV2).

A slope and an intercept from the abovementioned regression lines were used for TR_UA calculation for all dialysis sessions as follows:

$$TR\_UA = (\alpha\overline{A} + \beta)(Q_D T + UF) \qquad \textbf{(10)}$$

where α is a slope, β is an intercept, $\bar{A}$ is a average value of all absorbance values from the beginning up to the end of the treatment, Q_T is the dialysate flow rate, T is the duration of the procedure [min] and UF is the ultrafiltrated volume [l]. The first part of the equation expresses the sessions mean UA concentration value in the spent dialysate.
All the dialysate was collected in a dialysate collection tank, which gave the reference for TR_UA.
The total removed amount of a UA in the total dialysate collection (TDC) was calculated as follows:

$$TR\_TDC = C_t W_t \qquad \textbf{(11)}$$

where C_t is the UA concentration in the total dialysate collection tank and W_t is the weight of the dialysate collection tank (kg). It was assumed that 1 kg =1 litre of the dialysate. The results from individual and general models were compared with the reference values from the TDC center specifically and in the case of an entire group. It was observed that it is feasible to estimate TR_UA by using UV-absorbance. The results from individual model UV1 and TDC were not statistically different, whereas results from UV2 for the entire group were different from TDC. Results from UV2 remained indifferent from the reference in case the results from different centers were compared separately. The models created on the basis of Linköping data (UV2 Linköping) remained reliable in long-term patient monitoring in Linköping.
Statistical difference from the reference, in case data from two centers were united, reflected a need for more specific considerations for creating universal models. In the current case cuvettes with different geometry were used and the proportion of utilized filters (low- vs. high-lux filters) in centers was different. Wavelength for optical monitoring of the UV-absorbance of the spent dialysate was selected according to previous studies [132, 141]. However, maximum UV-absorbance of UA is achieved around 294 nm (Figure 4). All these factors may affect model performance and all of them may explain significant differences in the general model for the entire material (UV2).
Therefore, a need for more reliable models for determining UA content in the spent dialysate arises.

4.3 UA concentration estimation algorithms (Publication III)

Publication III presents several algorithms for determining the concentration of UA from the spent dialysate by using original or processed UV-absorbance spectra values of the spent dialysate from one or several wavelengths. Data from 7 studies conducted during 10 years in Linköping and Tallinn were used. Altogether 60 patients and 188 dialysis sessions were followed. During each session dialysate samples were collected, their UA content was determined in the chemistry laboratory and UV-absorbance spectra over a wavelength range of 180-390 nm were registered. Registered spectra were processed with the Savitzky-Golay

algorithm to obtain the first derivate spectra. On the basis of the UA concentrations estimated in the clinical laboratory and measured and processed UV-absorbance spectra from 75 randomly selected sessions (calibration set of material), multiple linear regression (MLR) analysis was performed applying the forward stepwise regression method to reveal the best wavelengths for the algorithms. UA concentration was chosen as a dependent variable, and UV-absorbance values from the wavelength range 190–380 nm were set as independent variables. The algorithms obtained for the UA concentration (Y) calculation are in the form

$$Y = a + b_1 x_1 + b_2 x_2 + ... + b_i x_i \qquad \textbf{(12)}$$

where a is an intercept, b is a slope and x is an independent variable (the value of original or derivate of UV-absorbance at a certain wavelength).

Three models for both, original and derivate of UV-absorbance, were composed; each of them used original or derivate of absorbance data from one to three wavelengths. Three steps were considered sufficient while the root mean squared error (RMSE) did not decrease markedly while adding an additional wavelength. Created models were applied on the validation set of material (data of 113 sessions) and the performance of each model was estimated in terms of systematic, standard and root mean squared errors. UA concentration values from all models were not significantly different from the values determined in the clinical chemistry laboratory. By using simple signal processing tools as smoothing and the first derivate calculation and/or absorbance or processed absorbance values from different wavelengths in concentration calculation algorithms, more reliable results were achieved. The Savitzky-Golay algorithm for spectra processing is an efficient method for eliminating baseline effects in the spectra [163]. This could explain the enhancement in accuracy. Utilization of original or processed UV spectral information from several wavelengths ensures more accurate results than a single wavelength approach. The wavelengths used in the models are characteristic for UA: maximal UV-absorbance of UA is around 294 nm, minimal around 264 nm and the relative importance of UA in the overall UV-absorbance signal of the dialysate is the largest in the region around 300 nm. The best result was achieved by applying the model which is using derivate spectra values at three wavelengths. The mean concentration (μmol/l) of UA calculated utilizing this model was 48.9 ± 22.4; corresponding result from the chemical laboratory was 49.7 ± 23.0. Considering the improvement in the accuracy, signal processing and utilizing the information from several wavelengths should be the future practice while monitoring removed amount of UA. Developed models created on the basis of results from both HD and HDF sessions, validation of the models revealed that performance of them was good, irrespective of the type of treatment.

The results from Publication I-III confirm the possibility to monitor the UA content in the spent dialysate, which makes it possible to monitor one real uremic toxin and its removal during dialysis treatments.

4.4 Clinical importance of UA (Publication IV)

In Publication IV, clinical importance of UA in terms of dialysis patients' survival was discussed. Possibilities to use UA and urea levels and removal amounts for a patient's status estimation and long-term survival prediction were investigated. Importance of finding more indicators for the dialysis quality to improve dialysis patients' survival has been pointed out by *Vanholder* et al. [25]. Novel biomarkers have the potential for indicating risks and guide therapy in patients on HD; additionally, multimarker approach might be beneficial [171]. It has been found that UA may be the prognostic biomarker for heart failure [172] and vascular calcification in HD patients [173].

During the current study it was found that dialysis patients' survival was significantly higher when the UA level was below 5.7 mg/dl (342 micromol/l). It can be explained by the fact that high UA level has been found to be significantly related to the occurrence of CVD [55-57].

Similar survival tests have shown that higher serum urea levels predict better survival. Still, this result was not significant (p=0.095). A beneficial trend of higher urea level in HD patients' survival may be caused by better nutrition status while the urea level, among other things, reflects protein intake of patients [27].

Logistic regression analysis was used to create models for dialysis patients' 3-year survival estimation - survival was set as a dependent parameter and UA and/or urea based clinical parameters were independent variables. Logistic regression models for estimating the survival probability (z) were created in the following form:

$$z = \frac{\exp(a + b_1 x_1 + b_2 x_2 + b_3 x_3)}{1 + \exp(a + b_1 x_1 + b_2 x_2 + b_3 x_3)} \qquad \textbf{(13)}$$

where a is an intercept, b_i-s are a slopes (regression coefficients), and x_i-s are the variables (e.g. concentration, TR and RR value).

The reduction ratio of the substance was calculated as follows:

$$RR = \frac{C_0 - C_t}{C_0} 100\% \qquad \textbf{(14)}$$

where C_0 and C_t are the substance concentrations at the beginning and at the end of the dialysis, respectively.

The best model for predicting patients' 3-year survival status included both UA and urea based clinical parameters, indicating that survival is determined by a set of causal factors. Algorithms for dialysis patients' survival estimation have been proposed earlier. *Cohen* et al. used univariate Cox proportional hazards regression model for dialysis patients' 6- month survival estimation. Parameters included to the model were: age, dementia, peripheral vascular disease, albumin level and personal question from a patient's doctor ("Would I be surprised if this patient died next year?") [174].

Current survival prediction models reflect the levels and a combination of two important uremic substances assigned with survival probability. Since the number of participating patients was small, it is reasonable to include more patients in the future studies. No differences in the performance of best prediction models were detected whether blood or spent dialysate sample values were used in the models.
Whereas concentration and removal of small molecules is feasible to monitor optically from the spent dialysate, it is possible to obtain continuous and valuable information about each dialysis procedure and a patient's outcome. By using optical tools, information could be accessed automatically without any extra effort from the clinical staff. It may also have a predictive importance on patient survival.

4.5 Epilogue: UA and health risks monitoring

For decades, focus has been on discussions if high UA level is a risk factor for health. It has been speculated that elevated UA levels have a role in the formation and development of several health problems. Naturally the question: "Is elevated UA level a root or fruit of health issues?" remains, but in the course of numerous studies it has been suggested that often UA levels do rise before other symptoms appear and UA influences crucial processes in the formation of health issues. Definitely, control of this single substance can not be a solution for all suggested health problems. It is also confirmed by the fact that the models developed for dialysis patients' long-term outcome prediction have used urea levels besides UA. To sum up, it seems that UA is an essential marker to be monitored and taken seriously in this specific patient group. Moreover, a recent review study points out that elevated UA levels should be lowered for preventing the onset and progression of CKD [170]. Certainly, UA is not the only uremic retention solute the concentration of which is higher in dialysis patients compared to general population. Dialysis can effectively remove small molecules but it is not so effective in the removal of protein-bound and middle molecules; new modalities such as HDF show a trend towards a benefit in this issue [20]. Another problem of dialysis patients is related to nutrition - patients are losing proteins and amino acids in the course of the dialysis, which leads to muscle mass loss [175]. Therefore, monitoring several parameters and combining them in models for estimating a patient's status is reasonable.
Evidence from the literature assures that lifestyle influences the entire organism and homeostasis. With medications, operations and cures we only can solve problems locally, but it is impossible to reach everywhere and treat everything simultaneously. It can happen that cure of a single problem may result in severe problem(s) somewhere else. Prevention is always better than treatment, therefore wider information of possible influence or benefits of several activities and substances (also content of our food) should be provided. Responsible production and sufficient information from food companies would be an asset.

CONCLUSIONS

The study introduces the correlations between UV-absorbance and uremic retention solutes in the spent dialysate, monitoring possibilities of UA during dialysis treatments, detects relation between dialysis patients' survival and UA levels and suggests survival prediction models for indicating a possible outcome of the patient.
Since UA is a uremic toxin itself and seems to influence survival of the dialysis patients, it might be essential to monitor its level and removal.
As a result of the current research, the following was found:

- UV-absorbance method can be used for monitoring of the spent dialysate whereby different wavelengths could be used for monitoring different uremic solutes.
- On-line monitoring of removed UA during the dialysis is achievable. UV-absorbance at 285 nm was detected by on-line spectrophotometer and transformed to UA concentration, total removed amount of UA was calculated, and the results were compared to the reference method. The results demonstrate the possibility to monitor removed amount of UA optically and on-line by using UV spectral information of the spent dialysate. Moreover, models were reliable in the long-term patient monitoring.
- Accuracy of the optical UA concentration estimation is improved if multi-spectral information of the UV-absorbance of the spent dialysate is taken into account. Introduced multi-wavelength algorithms enable reliable and more accurate calculation of the concentration of UA from the spent dialysate. Algorithms utilize original or processed UV spectral information from one or several wavelengths. The algorithms had similar performance on data achieved in the HD and also in the HDF sessions.
- Kaplan-Meier survival analysis confirms that higher UA level in studied ESRD patients indeed predicts mortality, whereas higher level of urea has an opposite effect. The latter, however, was not statistically significant. It may give a hint to nephrologists that levels on UA and urea are important to monitor in dialysis patients.
- Survival prediction models combining the parameters of the two molecules - UA and urea, seem to be more accurate than models based on a single molecule. Logistic regression analysis was used to develop the models; three UA and urea based parameters were included and prediction accuracy of 100 % was achieved.
- The survival prediction can be performed either on the parameters detected from serum or dialysate samples, indicating that prediction of patient outcome could be monitored continuously, and the information could be assessed during each treatment. The results could give valuable

information for medical personnel and thereby a possibility to prolong life of dialysis patients.

- Via optical monitoring tools the abovementioned parameters are easily accessible during each dialysis procedure without the need of additional effort from the medical personnel.

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KOKKUVÕTE

Optiline meetod kusihappe eemaldamise määramiseks dialüüsiravi käigus

Alates ajast, mil hakati pakkuma dialüüsiravi, on selle kvaliteeti parandatud. Arenenud on dialüüsimasinad, dialüsaatorid (filtrid), veesüsteemid ning markerid ja meetodid protseduuri kvaliteedi hindamiseks. Hoolimata tehtud edusammudest, on dialüüsipatsientide suremus endiselt kõrge. Sellest tingituna jätkub individuaalsema dialüüsi, uute markerite ning monitooringumeetodite otsimine ja juurutamine. Levinuim marker dialüüsiravi kvaliteedi hindamiseks on uurea, mis iseenesest ei ole ureemiline toksiin ning mille bioloogiline ja toksiline mõju pole (laialdaselt) kinnitust leidnud. Samas on leitud, et kõrge kusihappe tase organismis võib olla seotud kõrgvererõhutõve, neeruhaiguse ja südame-veresoonkonna haiguste tekke ning süvenemisega. Kuna kusihape on ureemiline toksiin, võiks temast kujuneda uudne marker dialüüsiravi kvaliteedi hindamiseks. Siiski, uuringud kusihappe taseme ning dialüüsipatsientide suremuse vahelise seose hindamiseks on jõudnud vastakate tulemusteni – mõned uuringud kinnitavad ning mõned lükkavad ümber kõrge kusihappe taseme kahjuliku mõju dialüüsipatsientide elulemusele.
Käesoleva töö eesmärgiks oli töötada välja algoritmid kusihappe eemaldamise jälgimiseks dialüüsiprotseduuri jooksul, kasutades kulunud dialüsaadi ultravioletkiirguse absorptsiooni. Samuti uuriti kahe väikese molekulkaaluga ureemilise jääkprodukti taseme mõju dialüüsipatsientide elulemusele. Ka töötati välja mudelid hindamaks patsientide elulemuse tõenäosust lähtuvalt kahe molekuli, uurea ja kusihappe, tasemest ning eemaldamisest.
Töö esimeses osas antakse ülevaade neerude rollist organismis, neerupuudulikkusest ning dialüüsiravist.
Teine osa on pühendatud kahele väikesemolekulaarsele ureemilisele jääkainele, uureale ja kusihappele, kusjuures keskendutakse viimase mõjule inimese tervisele.
Kolmandas osas antakse ülevaade dialüüsiprotseduuri kvaliteedi hindamise meetoditest, tutvustatakse optilisi jälgimismetoodikaid ning nende aluseks olevaid põhiprintsiipe.
Töö neljas osa keskendub tehtud eksperimentaalsele uurimistööle; antakse ülevaade toimunud kliinilistest uuringutest nii Eestis (Tallinnas) kui ka Rootsis (Linköpingis), mille käigus kogutud optilised ja biokeemilised andmed võimaldasid välja töötada algoritmid kusihappe hulga ja eemaldamise määramiseks. Teostati suremusanalüüs tuvastamaks kusihappe ja uurea taseme mõju patsientide elulemusele ning pakuti välja meetodid, kuidas kahe molekuli parameetreid kombineerides võiks dialüüsiravi patsientide elulemustõenäosust hinnata.
Töö peamised tulemused on järgmised:

1. Kasutades optilisi meetodeid (kulunud dialüsaadi UV-kiirguse absorptsiooni), on võimalik mõõta kusihappe kontsentratsiooni dialüsaadis ja kindlaks teha neeruasendusravi protseduuri jooksul eemaldatud kusihappe hulk reaalajas.

2. Kulunud dialüsaadi absorptsioonispektrite töötlemine (silumine ning esimese tuletise arvutamine) kõrvaldab baasjoone efektid ning võimaldab välja töötada töökindlamad algoritmid kusihappe kontsentratsiooni määramiseks kui originaalspektrit kasutades. Originaal- või töödeldud spektri väärtuste kasutamine mitmelt lainepikkuselt võimaldab kusihappe kontsentratsiooni veelgi täpsemini hinnata. Algoritmid töötavad hästi nii HD kui HDF protseduuride korral.
3. Kõrge kusihappe tase dialüüsipatsientidel on statistiliselt oluliselt seotud patsientide kõrgema suremusega ning kõrge uurea tase lubab loota paremat elulemust (viimane sõltuvus ei olnud antud uuringu käigus statistiliselt oluline).
4. Kahe väikese molekulkaaluga ureemilise toksiini parameetreid kombineeriv mudel võimaldaks hinnata dialüüsipatsiendi 3 aasta elulemustõenäosust. Mudelite töökindlus ei sõltunud sellest, kas ennustamiseks kasutati vere- või kulunud dialüsaadiproovide väärtusi.
5. Kasutades optilisi monitooringumeetodeid, on ülaltoodud ureemilisi markereid ja parameetreid võimalik hinnata reaalajas iga dialüüsiprotseduuri jooksul.

Võtmesõnad: kusihape, uurea, dialüüs, optiline meetod, reaalajas monitooring, suremusanalüüs, elulemuse ennustusmudelid.

ABSTRACT

Optical Method for Uric Acid Removal Assessment during Dialysis

From the start of delivering dialysis treatment, its performance has been improved on an ongoing basis. Developments in dialysis machines, filters, water systems etc. have been spectacular, and great efforts have been made to find proper dialysis quality measures. Nevertheless, patients' survival has not been improved accordingly. Therefore, more individual dialysis, finding new markers, monitoring technologies could help to achieve better procedure quality and survival of dialysis patients.

Urea is the most common marker for the estimation of dialysis quality; however, there is shortage of evidence of its direct biologic and toxic effects. It has been suggested that a high level of uric acid (UA) may play an influencial part in the development of cardiovascular problems, hypertension and renal disease. Being a uremic toxin, UA might develop into a novel marker for dialysis quality assessment. However, results from the studies of connection between dialysis patients' survival and UA levels are contradictory – some prove, and others disprove the harmful effect of high UA level to a dialysis patient outcome.

The aim of this work was to develop reliable algorithms for determining the concentration and removal of UA optically and on-line. Additionally, the impact of levels of two small molecules, UA and urea, on a dialysis patient's survival was studied, and methods for combining the parameters into a single model for predicting a patient's survival were proposed.

Section 1 summarizes the role of kidneys, kidney failure and dialysis.

Section 2 is dedicated to two small uremic solutes, urea and UA, whereat focus is mainly on the influences of UA levels on human health.

Section 3 reviews optical monitoring options of UA and basic principles behind it.

Section 4 focuses on the results of the author's experimental studies. An overview of clinical studies performed in Tallinn, Estonia and in Linköping, Sweden is given. Optical and biochemical data collected during the studies created a basis for developing algorithms for UA concentration and removal estimation. To investigate the influence of the levels of UA and urea on a patient's outcome, a survival analysis was performed. Models combining the parameters of UA and urea for predicting a patient's 3-year survival were developed.

The main results of the thesis are:

1. It is convincingly demonstrated that the UA concentration in the spent dialysate and the amount of UA removed during the dialysis can be optically and on-line monitored using UV-absorbance of the spent dialysate.
2. Processing (using the Savitzky-Golay algorithm for smoothing and differentiation) of the absorbance spectra removes the baseline effects, and thereby leads to more reliable UA concentration calculation algorithms than usage of original spectra. Utilization of original or

processed UV spectral information from several wavelengths ensures more accurate results than a single wavelength approach. Algorithms are reliable in the HD and HDF sessions.

3. Higher UA level in ESRD patients is significantly related to higher mortality in the dialysis patients whereas higher level of urea has an opposite effect (which was insignificant).
4. Survival prediction models combining the values of two molecules, UA and urea, could predict dialysis patients' 3-year survival. Performance of the models was found independent of whether values from the blood or the dialysate samples were used.
5. Via optical monitoring tools, abovementioned parameters are easily accessible during each dialysis procedure.

Keywords: uric acid, urea, dialysis, optical method, on-line monitoring, survival analysis, survival prediction models.

APPENDIX 1

PUBLICATIONS

Publication I

Jerotskaja (Holmar) J, Lauri K, Tanner R, Luman M, Fridolin I (2007) "Optical dialysis adequacy sensor: wavelength dependence of the ultraviolet absorbance in the spent dialysate to the removed solutes", *In: Proceedings of 29th Annual International Conference of the IEEE EMBS, Lyon, France August 23-26*, 2960-63 (DOI 10.1109/IEMBS.2007.4352950).

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Cité Internationale, Lyon, France
August 23-26, 2007.

Optical dialysis adequacy sensor: wavelength dependence of the ultraviolet absorbance in the spent dialysate to the removed solutes

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Abstract— **A need for dialysate-based, on-line, continuous monitoring systems for the control of dialysis efficiency and the prevention of dialysis-associated complications is arisen due to increasing number of dialysis patients and related treatment quality requirements. The aim of this study was to investigate the wavelength dependence between the the ultra-violet (UV) absorbance in the spent dialysate and the retained solutes removed during the hemodialysis in order to explain possibilities to estimate removal of the solutes by the optical dialysis adequacy sensor.**

Ten uremic patients, during 30 hemodialysis treatments, were followed at the Department of Dialysis and Nephrology, North-Estonian Regional Hospital. The dialysate samples were taken and analyzed with spectrophotometer to get absorbance spectra. The results confirm previous studies considering similarity for the UV-spectrum on the spent dialysate samples during a single dialysis session indicating presence of the same type of chromophores in the spent dialysate removed from the patient's blood for different patients groups. At the same time the highest correlation in the spent dialysate for urea, creatinine, potassium, and phosphate was obtained at the wavelength 237 nm that is a new finding compared to earlier results. The highest correlation between the UV-absorbance and uric acid in the spent dialysate was obtained at the wavelength 294 nm. Presence of at least two different wavelength ranges may add selectivity for monitoring several compounds.

Our study indicates that the technique has a potential to estimate the removal of retained substances.

I. Introduction

A NEED for dialysate-based, on-line, continuous monitoring systems for the control of dialysis efficiency as well as for haemodynamic surveillance and the prevention of dialysis-associated complications is arisen due to increasing number of dialysis patients and related treatment quality requirements [1]. Regular dialysis dose assessment is recommended based on the quality parameters determined from the solute urea removal [2]. Several spectrophotometrical sensors for on-line monitoring of total ultra-violet (UV) absorbance or urea in the spent dialysate have been presented, aiming to follow continuously a single hemodialysis session [3], [4], [5].

At the same time, there are several molecules or molecular groups having an impact on survival of the dialysis patients, and for this reason urea, the traditional marker for dialysis quality, should not be the only solute used to model the dialysis therapy [6], [7]. In this context, development of techniques, which can offer a tool to monitor several compounds retained in uremic patients and with potential clinical significance, is important.

Earlier relationship between the UV-absorbance measured by the optical dialysis adequacy sensor in dialysate and the concentration of some solutes both in the spent dialysate and in the blood of the dialysis patients was investigated on a patient group in Sweden [8], [9]. However, more data is needed to validate if the relationship is general and if more parameters should be considered for the further analysis.

The aim of this study was to investigate the wavelength dependence between the the ultra violet absorbance in the spent dialysate and the retained solutes removed during the hemodialysis in order to explain possibilities to estimate removal of the solutes by the optical dialysis adequacy sensor.

II. Materials and Methods

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

Ten uremic patients, three females and seven males, mean age 62.6 ± 18.6 years, on chronic thrice-weekly hemodialysis were included in the study at the Department of Dialysis and Nephrology, North-Estonian Regional Hospital, using the clinical set-up of the experiments as described earlier [17]. Three different polysulphone dialysers were used: F8 HPS (N=14), F10 (N=3), and FX80 (N=13) (Fresenius Medical Care, Germany) with the effective membrane area of 1.8 m2, 2.2 m2, and 1.8 m2, respectively. The dialysate flow was 500 mL/min and the blood flow varied between 245 to 350 mL/min. The type of dialysis machine used was Fresenius 4008H (Fresenius Medical Care, Germany).

UV-absorbance was determined by a double-beam spectrophotometer (SHIMATSU UV-2401 PC, Japan)

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with an accuracy of ±1% on the dialysate samples taken at pre-determined times during dialysis. Spectrophotometric analysis over a wavelength range of 190 - 380 nm was performed by an official cuvette with an optical path length of 1 cm. The obtained UV-absorbance values were processed and presented on the computer screen by a PC incorporated in the spectrophotometer using UV-PC software (UV-PC personal spectrophotometer software, version 3.9 for Windows). The final data processing was performed in EXCEL (Microsoft Office Excel 2003)

Seven dialysate samples were taken during the dialysis: in the beginning, 10, 60, 120 and 180 minutes after the start of the dialysis session, and immediately at the end of the treatment (210 or 240 minutes) (Fig. 1). Also sample from the total dialysate collection, marked as "Mixture" was included into analysis. Pure dialysate was collected before the start of a dialysis session, used as the reference solution, when the dialysis machine was prepared for starting and the conductivity was stable.

The concentrations of substances such as urea, creatinine, uric acid, phosphate and potassium were determined at the Clinical Chemistry Laboratory at North-Estonian Regional Hospital using standardized methods.

TABLE I
SUMMARY OF OBSERVED SUBSTANCES

Substance	MW, D	No. of dialysate samples
Potassium	39,10	185
Urea	60,06	168
Phosphate	96,17	168
Creatinine	113,12	166
Uric acid	168,11	158

The accuracy of the methods for the determination of different solutes in dialysate were: urea, creatinine, uric acid ±5% and phosphate, ±2%. All samples collected during both studies were analysed within 2-4 hours after collection using the standard methods at the Clinical Chemistry Laboratory and within the 24 hours using the spectrophotometer.

Some of the measured values (absorbance or concentration) were excluded from data before the calculation of correlation coefficient r. The exclusion criteria were incorrect or illogical values of the measured concentration or absorption.

III. Results

Fig. 1 shows an example of the absorbance spectrum obtained on the spent dialysate samples at different time moments over a wavelength range of 190 - 380 nm during a single dialysis session. A lower UV-absorbance value is measured at all wavelengths as time increases, due to decreased concentration of the UV-absorbing compounds in the blood when transported through the dialyser into the dialysate and removed from the blood during the dialysis treatment.

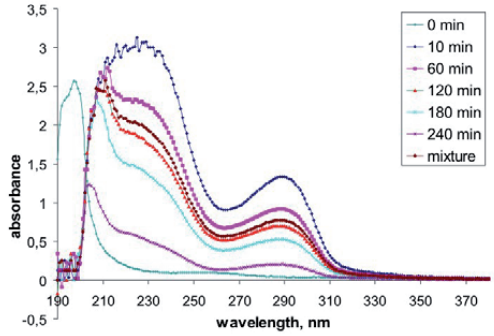

Fig. 1. An example of the absorbance spectrum obtained over a wavelength range of 190-380 nm on the spent dialysate samples at different times during a single dialysis session.

Fig. 2 presents the maximum, mean +/-SD and minimum values of the absorbance spectra calculated for all dialysis treatments (see Table 1 for number of samples). As seen in the figure, the absorbance spectrum has maxima in the range of 210 - 250 nm, one minimum around 260 nm, and one maximum around 290 nm.

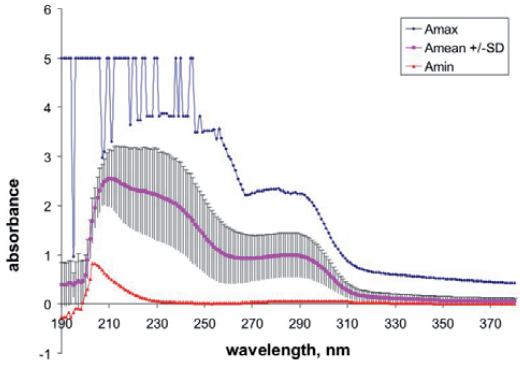

Fig. 2. Maximum, mean +/- SD and minimum values of the absorbance spectra calculated for all dialysis treatments

Fig. 3 presents the correlation coefficient r between UV-absorbance over a wavelength range of 180 - 380 nm and certain substances with different molecular weights in the spent dialysate.

The highest r value for uric acid, creatinine, urea, potassium, and phosphor was obtained at wavelengths from 227 nm to 294 nm. The highest correlation coefficients were: for uric acid 0,9215 at the wavelength 294 nm, for creatinine 0,8739 (237 nm), for potassium 0,8928 (227 nm), for phosphate 0,7382 (237 nm), and for urea 0,8752 (237 nm).

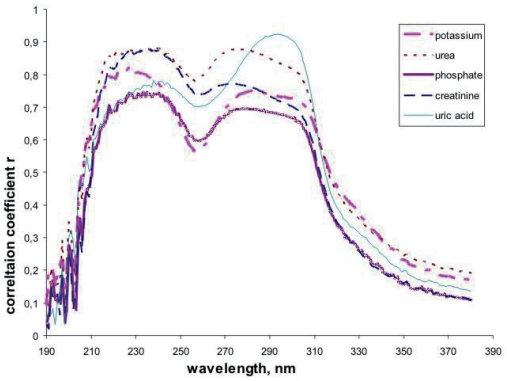

Fig. 3. Value of correlation coefficient r between UV-absorbance over a wavelength range 190-380 nm and certain substances with different molecular weights in the spent dialysate.

IV. Discussion

Fig. 1 shows an example of the absorbance spectrum obtained on the spent dialysate samples at different times over a wavelength range of 190 - 380 nm from a single dialysis session. A lower UV- absorbance value is obtained at all wavelengths versus time due to a decreased concentration of UV-absorbing compounds in the blood when transported through the dialyser into the dialysate and removed from the blood during the dialysis treatment. Similar absorbance or transmittance spectra in this UV-region have also been reported by other researchers both for the serum of dialysis patients [10], [11] and in one healthy subject [10] and for the spent dialysate [11], [12], [9]. This similarity for the UV spectrum in the spent dialysate samples during a single dialysis session indicates presence of the same type of chromophores in the spent dialysate removed from the patient's blood despite possible local differences in the diet, climat, medicine, etc. Note very low absorbance over 220 nm in the pure dialysate (0 min) containing electrolytes and acetate.

Fig. 2 shows that at wavelengths higher than 300 nm the absorbance maximum and minimum values remain approximately within the range of 0.1 – 2.0. However, the mean absorbance value is low (< 0.1) at wavelengths higher than about 330 nm indicating that the hardware noise level might be significant.

Extremely high maximum absorbance (> 3.5) can be obtained in the wavelength range of 220 – 260 nm. Generally, wavelengths at which the absorbance for the particular chromophores is characteristic, such as regions of flat maxima and minima, are preferable. The wavelength corresponding to the maximum value of the absorbance for the species of interest is advantageous because the largest slope (and thus highest sensitivity) is obtained and the measurement will be relatively insensitive to errors in reproducing the wavelength setting because the maximum band is often quite flat at the maximum [13]. However, this very large dynamic range sets high demands on the hardware design (radiation source intensity, detector sensitivity and output signal amplification from the detector over the whole dynamic range) making the technical solution more expensive and complicated. The noise appearing on the maximum absorbance curve on Fig. 2 is a good example of the instrumental error due to abovementioned reasons. This stresses importance of the sensitivity and dynamic range when assessing the wavelength dependence of the UV-absorption.

The highest r value in the spent dialysate was obtained for uric acid at the wavelength 294 nm (Fig. 3), which contributes mostly to the mean absorbance value in this wavelength region [14], [9]. Similar relation between UV-absorbance and uric acid in the spent dialysate has been confirmed during an earlier study [9]. At the same time, the highest r value in the spent dialysate for urea, creatinine, and phosphate was obtained at the wavelength 237 nm (Fig. 3) and for potassium at 227 nm, which is a new outcome. The highest r value in the spent dialysate for retained solutes like urea, creatinine, and phosphate was obtained in the wavelength region from 290 to 330 nm in an earlier study [9]. The reason for this difference is not clear but may be explained by instrumental error during the earlier experiments due to very large dynamic range in the region below 260 nm or by some difference in chromophore content and behaviour in different patient groups. Still, a correlation minima around 260 nm is clearly present in both studies.

It is worth to mention that relatively good correlation between UV-absorbance and a particular solute may be achieved when the removal rate of a non-absorbing solute, e.g. urea, is similar to UV-absorbing substances during haemodialysis. A good correlation between UV-absorbance and a certain substance both in the dialysate and in the blood indicates that UV-absorbance has a linear relationship with the substance in the dialysate. This linear relationship offers possibility to calculate the concentration values for the given substance from the measured UV-absorbance values

In summary, the most interesting wavelength regions for further investigations regarding instrumental design seem to be around 227 and 290 nm. The final algorithm should probably take into account several parameters relating to the diffusive and convective transport over the membrane, as they differ depending on the type of dialyser. Earlier study by our group [15] indicated that approximately 90 % of the cumulative and integrated UV-absorbance measured by the optical dialysis adequacy sensor originates from the 10 main peaks for a particular dialysis treatment.

V. Conclusion

This study investigated the wavelength dependence between the the ultra-violet absorbance in the spent

dialysate and the retained solutes removed during the hemodialysis in order to explain possibilities to estimate removal of the solutes by the optical dialysis adequacy sensor.

The study confirms previous studies considering similarity for the UV spectrum on the spent dialysate samples during a single dialysis session indicating presence of the same type of chromophores in the spent dialysate removed from the patient's blood. At the same time the highest r value in the spent dialysate for urea, creatinine and phosphate was obtained at the wavelength 237 nm, and for potassium at the wavelength 227 nm, that is a new finding compared to earlier results. The highest correlation between the UV-absorbance and uric acid in the spent dialysate was obtained at the wavelength 294 nm. Moreover, presence of at least two different wavelength ranges may add selectivity for monitoring several compounds.

This study indicates that the technique has a potential to develop an optical dialysis adequacy sensor that provides continuous, on-line measurements with no need for repeated blood samples or disposables-chemicals that may immediately identify and alert to any deviations in dialysis treatment due to changes in the dialyser performance or the solute's removal from the blood.

More detailed analysis to use the ultra-violet absorbance in the spent dialysate for estimation of removal of separate compounds during hemodialysis will be issue of the next studies.

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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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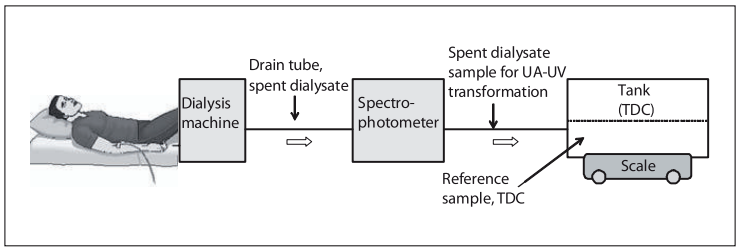

Fig. 1. Schematic clinical setup of the experiments.

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).

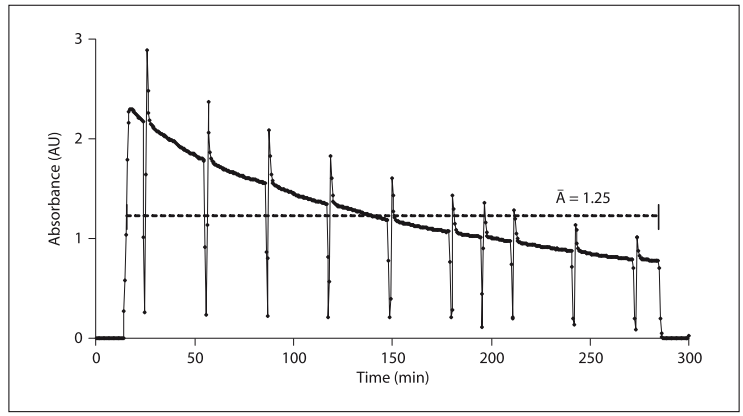

Fig. 2. A typical online monitoring curve during a dialysis treatment lasting 270 min where UV absorbance is plotted against time. AU = Arbitrary units. The spikes correspond to the self-test of the dialysis machine when dialysate automatically has been set in bypass. The mean absorbance value (Ā) is 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 ($p > 0.05$).

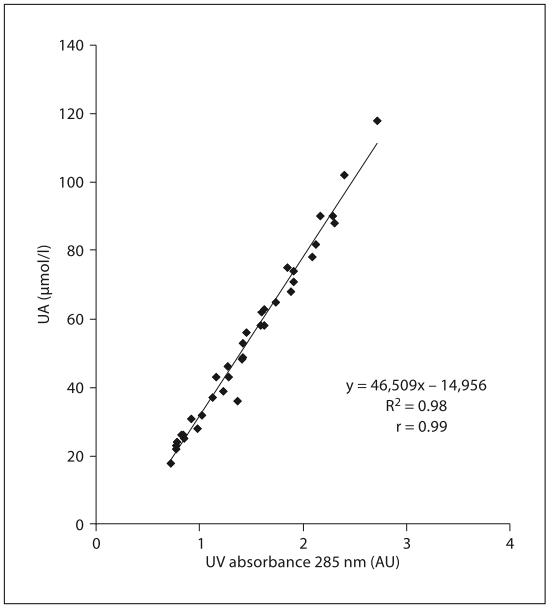

Fig. 3. An example of the regression line between concentration of UA in spent dialysate and UV absorbance in 4 sessions in 1 patient. This relationship was utilized when UV1 was calculated. AU = Arbitrary units.

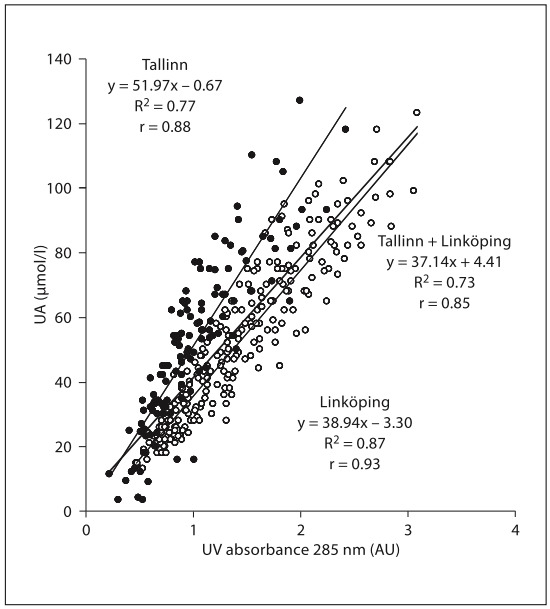

Fig. 4. Regression lines between concentration of UA in spent dialysate and UV absorbance in all sessions (n = 56) and also with the two centers, Linköping (n = 28) and Tallinn (n = 28), with separately calculated TR_{UA} from the general model UV2.

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

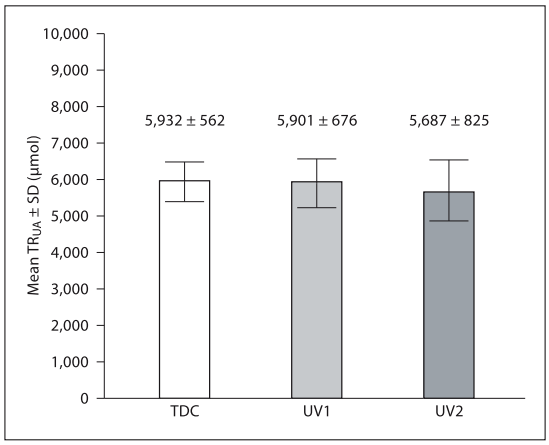

Fig. 5. Mean and SD values of TR_{UA} in micromoles for the 1-year follow-up sessions (n = 6). No significant difference ($p > 0.05$) was seen between TDC and UV1 and between TDC and center-specific (Linköping) UV2.

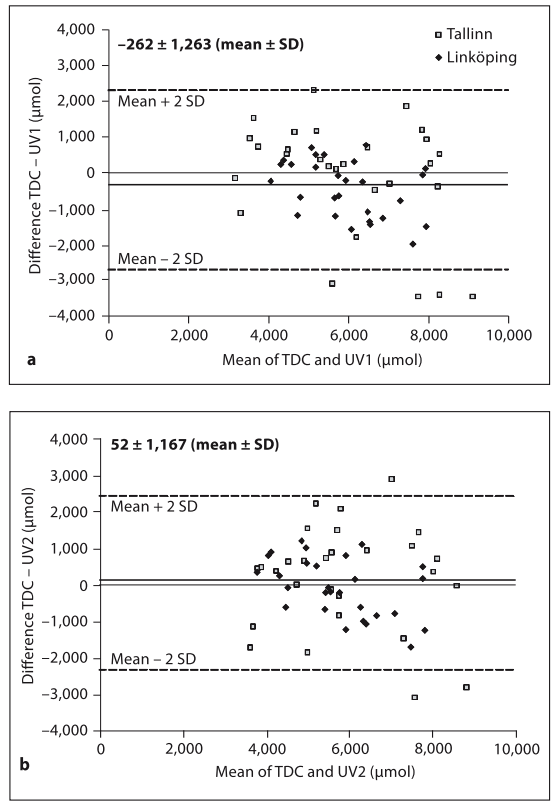

Fig. 6. Bland-Altman plot. **a** The difference between the TDC and UV1 method is plotted against the mean value of TDC and UV1 method (n = 56). **b** The difference between the TDC and UV2 method is plotted against the mean value of the TDC and UV2 method (n = 56).

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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Research Article

Optical Method for Cardiovascular Risk Marker Uric Acid Removal Assessment during Dialysis

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The aim of this study was to estimate the concentration of uric acid (UA) optically by using the original and processed ultraviolet (UV) absorbance spectra of spent dialysate. Also, the effect of using several wavelengths (multi-wavelength algorithms) for estimation was examined. This paper gives an overview of seven studies carried out in Linköping, Sweden, and Tallinn, Estonia. A total of 60 patients were monitored over their 188 dialysis treatment procedures. Dialysate samples were taken and analysed by means of UA concentration in a chemical laboratory and with a double-beam spectrophotometer. The measured UV absorbance spectra were processed. Three models for the original and three for the first derivate of UV absorbance were created; concentrations of UA from the different methods were finally compared in terms of mean values and SD. The mean concentration (micromol/L) of UA was 49.7 ± 23.0 measured in the chemical laboratory, and 48.9 ± 22.4 calculated with the best estimate among all models. The concentrations were not significantly different ($P \geq 0.17$). It was found that using a multi-wavelength and processed signal approach leads to more accurate results, and therefore these approaches should be used in future.

1. Introduction

Uric acid (UA), a final product of the metabolism of purine, is a very important biological molecule present in body fluids. It is mostly excreted from the human body through the kidneys in the form of urine. The concentration of UA in blood increases when the source of UA increases or the kidneys malfunction. Hyperuricemia is a symptom when the UA concentration is above 7 mg/dL. UA is hard to dissolve in blood and will crystallise when supersaturated. The UA crystallites are deposited on the surface of the skin, in joints, and particularly in the toes, resulting in gout. Analysis of the UA concentration in blood helps to diagnose gout. In addition to gout, hyperuricemia is connected with lymph disorders, chronic haemolytic anaemia, an increase in nucleic acid metabolism, and kidney malfunction. Elevated serum UA contributes to endothelial dysfunction and increased oxidative stress within the glomerulus and tubulointerstitium, with associated increased remodelling fibrosis of the kidney [1]. A high level of serum UA, hyperuricemia, has been suggested as an independent risk factor for cardiovascular and renal diseases [2] especially in patients with heart failure, hypertension, and/or diabetes [3–5], and has been shown to cause renal disease in a rat model [6]. UA is mostly associated with gout, but studies have implied that UA affects biological systems [7] and could also influence the risk of higher mortality among dialysis patients [8], although the pathogenic role of hyperuricemia in dialysis patients has not been fully established [9]. High caloric foods and alcohol as well as disorders of the organs and tissues are the main causes of hyperuricaemia, obesity, kidney stone formation, and even gout [10]. It is likely that high UA levels in the blood are the reason for the emergence of renal microvascular disease, which may be a key mechanism

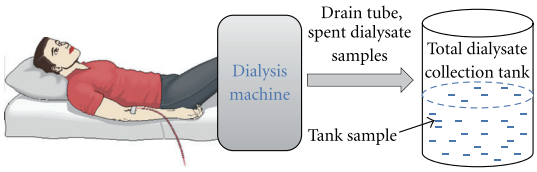

Figure 1: Schematic clinical setup of the experiments.

in inducing salt-sensitive hypertension [11]. Harm can be prevented and reduced by early diagnosis and monitoring, especially by screening obese patients [12].

It would be advantageous to measure the concentration of UA during dialysis online. For creating this opportunity it is necessary to create accurate and reliable models. UA may be the novel marker molecule for estimating the quality of dialysis procedure, since the UA is uremic toxin itself, removal pattern and amount of this compound during the dialysis are informative for patients and medical personnel.

Ways of monitoring UA, dialysate, and other biological fluids with optical tools have been shown previously by our and other groups [13–15]. If you use a simple signal processing tool for smoothing and calculating the first derivate of UV absorbance and/or absorbance or processed absorbance values from several wavelengths, more reliable results are achieved [16–20]. An effective way of estimating UA concentrations using the UV technique has been shown in previous studies by our group. Current paper, involving larger amount of patients from different countries, presents more general and accurate models making it possible to apply the technique in the large patient community.

The aim of this study was to estimate the concentration of uric acid (UA) optically by using the original and processed ultraviolet (UV) absorbance spectra of spent dialysate. Data from different dialysis centres and over a long period was used to build models to increase general validity and reliability.

2. Materials and Methods

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

During the period 1999–2009 seven studies were carried out in the Department of Dialysis and Nephrology at the Linköping University Hospital in Sweden and at the North Estonian Medical Centre in Estonia. Clinical setup of the experiments is presented in Figure 1. A summary of the studies and information about the participating patients are presented in Table 1.

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

For all of the studies, samples of spent dialysate were taken at discrete times for analysis (Table 3). The numbers under "sampling time" correspond to the number of minutes after the start of hemodialysis. The dialysate samples were taken at 255, 270, and 300 minutes when the duration of sessions was long enough. Also, the sample from the total dialysate collection tank was included in the analysis in most cases. Pure dialysate was collected before the start of a dialysis session and used as the reference solution when the dialysis machine was prepared and conductivity was stable.

Table 1: Summary of the of the studies and patients participated.

Study	No. of sessions	No. of patients (male/female)	Mean age
1	40	10 (6/4)	63 ± 21
2	19	7 (4/3)	57 ± 23
3	40	10 (6/4)	60 ± 19
4	30	10 (7/3)	63 ± 19
5	11	7 (4/3)	56 ± 13
6	24	8 (7/1)	77 ± 7
7	24	8 (7/1)	77 ± 7

Table 2: Summary of the conditions of the studies.

Study	Dialyser	Area, m^2	N	Dialysis machine	Blood flow, mL/min
1	AF180	1.8	40	AK200 Fresenius 4008 H	250–300
2	AF180	1.8	7	AK200	
	Polyflux17S	1.7	12	Fresenius 4008 H	300–350
	Polyflux17L	1.7	18	AK200	
3	TCA150G	1.5	3	Fresenius 4008 H	
	Nephral300	1.3	9		200–350
4	F8	1.8	14	Fresenius 4008 H	
	F10	2.2	3		245–350
	FX80	1.8	13		
5	FX80	1.8	11	Fresenius 4008 H	245–350
6	FX80	1.8	24	Fresenius 5008	280–350
7	FX800	1.8	24	Fresenius 5008	280–350

Table 3: Summary of the samples taken during the studies.

Study	Sampling time, min.
1	5, 15, 30, 60, 90, 120, 180, 240, 270, 300, tank
2	5, 15, 30, 60, 90, 120, 180, 240, 255, 270, 300, tank
3	5, 60, 120, 180, 240, tank
4	10, 60, 120, 180, 240, tank
5	10, 60, 120, 180, 240
6	10, 240, tank
7	10, 30, 60, 120, 180, 240, 270, tank

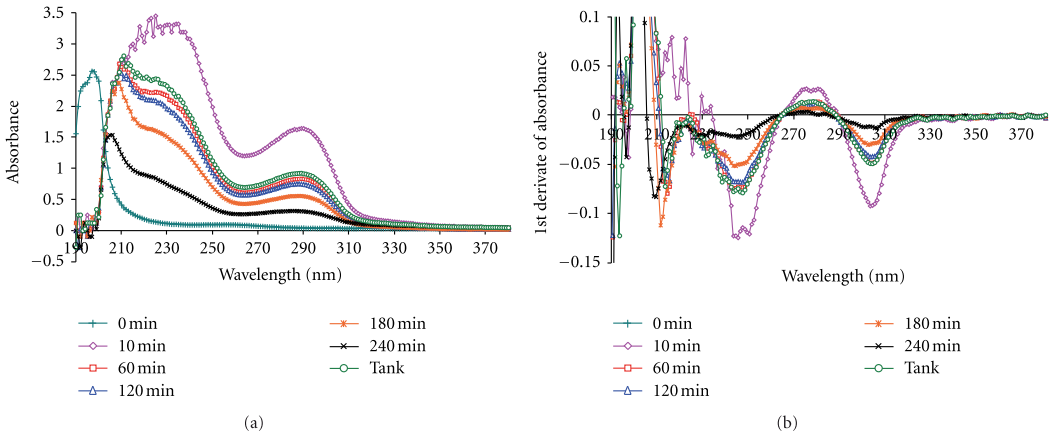

Figure 2: Example of absorbance spectrum (a) and first derivate of absorbance spectrum (b) obtained over wavelength range of 190–380 nm on spent dialysate samples at different times during dialysis session.

The concentration of UA was determined in the Clinical Chemistry Laboratories at the North Estonian Medical Centre and at Linköping University Hospital using standardised methods. The accuracy of the methods for the determination of UA in dialysate was ±5%.

Double-beam spectrophotometers (UVIKON 943, Kontron, Italy, and JASCO V-570, UV/VIS/NIR spectrophotometer, Japan, in Linköping and SHIMADZU UV-2401 PC, Japan, in Tallinn) were used for the determination of UV absorbance. Spectrophotometric analysis over a wavelength range of 190–380 nm was performed by an optical cell with an optical path length of 1 cm. A lower UV absorbance value is obtained at all wavelengths versus time due to a decreased concentration of UV-absorbing compounds in the blood when transported through the dialyser into the dialysate and removed from the blood during the dialysis treatment. The treatments were also monitored with a single wavelength online, and thereby all interruptions, self-tests, alarms, and so forth could be identified directly on a screen. Some of the measured values (absorbance or concentration) were excluded from data before analysis. The exclusion criteria were incorrect or illogical values of measured concentration or absorption, for example, sampling coexisting with self-tests of the dialysis machine.

The obtained UV spectra were processed with a signal-processing tool using a Savitzky-Golay algorithm for smoothing, and the first derivative calculation wherein a smoothing window with nine points was used (Figure 2). Panorama Fluorescence 1.2 was used for signal processing, and multiple stepwise regression analysis was performed with Statistica 9.0. Final data processing was performed in EXCEL (Microsoft Office Excel 2007).

On the basis of the UA concentrations measured in the laboratory, measured UV absorbance spectra and processed UV absorbance spectra, multiple regression analysis was carried out on the calibration set of material (data from 75 randomly selected dialysis procedures). UA was set as a dependent variable, and UV absorbance values between 190–380 nm were set as independent variables. Multiple linear regression (MLR) analysis using the forward stepwise regression method was employed to determine the best wavelengths for the models [21–25]. Using the stepwise regression method helps us avoid mistakes in the models due to the possible collinearity of the independent variables [26]. In both UV absorbance (UVa) and the first derivate of UV absorbance (UVd), the number of steps was increased until no relevant improvements were achieved by means of model performance. At each step the model for estimation of UA was saved, resulting in different models for both UVa and UVd.

Models for the calculation of the concentration of UA (Y) are in the form

$$Y = a + b_1 * x_1 + b_2 * x_2 + \cdots + b_i * x_i, \quad (1)$$

where a is intercept, b is slope and x is an independent variable (the value of original or derivate UV absorbance at a certain wavelength).

The obtained models were used on the data from the remaining 113 dialysis procedures (validation set) to calculate the concentration of UA and compare these values with the laboratory results and validate different models.

Systematic error was calculated for the models as follows [26]:

$$\text{BIAS} = \frac{\sum_{i=1}^{N} e_i}{N}, \quad (2)$$

where e_i is the residual and N is the number of observations.

Standard error was calculated for the models as follows:

$$\text{SE} = \sqrt{\frac{\sum_{i=1}^{N} (e_i - \text{BIAS})^2}{N-1}}. \quad (3)$$

Table 4: Summary of achieved models.

Model for	a	$b_1 * x_1$	$b_2 * x_2$	$b_3 * x_3$
original UV absorbance spectra at 294 nm (UVa_1WL)	−2.28	$51.69*A_{294}$		
original UV absorbance spectra at 294 and 312 nm (UVa_2WL)	−1.67	$60.56*A_{294}$	$-60.75*A_{312}$	
original UV absorbance spectra at 294, 312 and 266 nm (UVa_3WL)	−1.55	$75.38*A_{294}$	$-62.27*A_{312}$	$-7.36*A_{266}$
derivative spectra at 300 nm (UVd_1WL)	−1.44	$-1038.84*D_{300}$		
derivative spectra at 300 and 270 nm (UVd_2WL)	−2.12	$-1111.09*D_{300}$	$128.67*D_{270}$	
derivative spectra at 300, 270 and 222 nm (UVd_3WL)	−3.56	$-1128.73*D_{300}$	$120.74*D_{270}$	$-32.54*D_{222}$

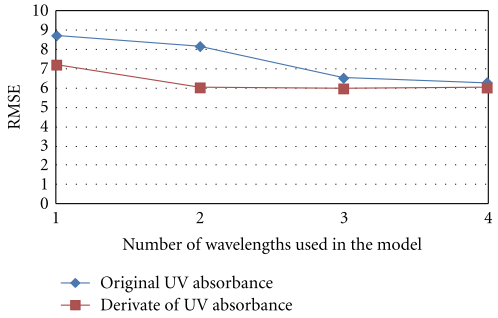

Figure 3: Behavior of RMSE with different models including 1–4 independent variables.

Root mean squared error was calculated for the models as follows:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} (e_i)^2}{N}}. \quad (4)$$

3. Results

During regression analysis, three steps were considered sufficient after estimation of the behaviour of the root mean squared error (RMSE). From Figure 3 it was concluded that adding one additional wavelength to the models did not markedly improve the results in terms of RMSE. This was also confirmed by a t-test for residuals, which were significantly different (at P level 0.05) between models that used an absorbance or first derivate of absorbance value from one, two, or three wavelengths and which were not different in the case of models which used four wavelengths.

As a result of regression analysis, three models for UV absorbance and three models for derivate of UV absorbance were found wherein each used an absorption or derivate of absorption value from one, two, or three wavelengths, respectively (Table 4). The models were marked as UVa_1WL for the model which used a UV absorbance value from one wavelength, UVa_2WL for the same information from two wavelengths, and so on. UVd_1WL-UVd_3WL marks models which used a derivative value of UV absorbance from one, two, or three wavelengths.

Figures 4 and 5 show the wavelengths of original UV absorbance and first derivate of UV absorbance included in the models for estimating UA concentration.

The models presented in Figures 4 and 5 were applied to the material to calculate UA concentrations, R^2, BIAS, SE, and RMSE. The results are presented in Table 5.

The concentrations achieved by the models were not significantly different (P = 0.17–0.48) from the observed concentrations in the laboratory for any model.

The systematic and root mean squared errors were significantly different (at P level 0.05) in the following cases (validation group):

(a) UVa_1WL versus UVd_1WL,

(b) UVa_1WL versus UVa_2WL,

(c) UVa_1WL versus UVa_3WL,

(d) UVd_1WL versus UVd_3WL,

(e) UVd_2WL versus UVd_3WL.

The differences between individual values of the UA concentration from the laboratory and UA values from two models (UVa_3WL and UVd_3WL) are presented in Figure 6.

The root mean squared error decreased as wavelengths were added to the models in the case of both the UVa and UVd models, and the decrease was slightly greater in the case of UVd models.

These results demonstrate that using UV absorbance from several wavelengths provides more accurate results in the estimation of the concentration of UA. Also, using information from the first derivate of spectra instead of original UV absorbance spectra produces a notable effect.

4. Discussion

The results in Table 5 show that it is possible to estimate UA concentration in spent dialysate using UV absorbance data. The presented models were built on the calibration set of material which contained absorbance values from Tallinn, Estonia, and Linköping, Sweden. The data included in the study were collected during seven studies from 1999 to 2009.

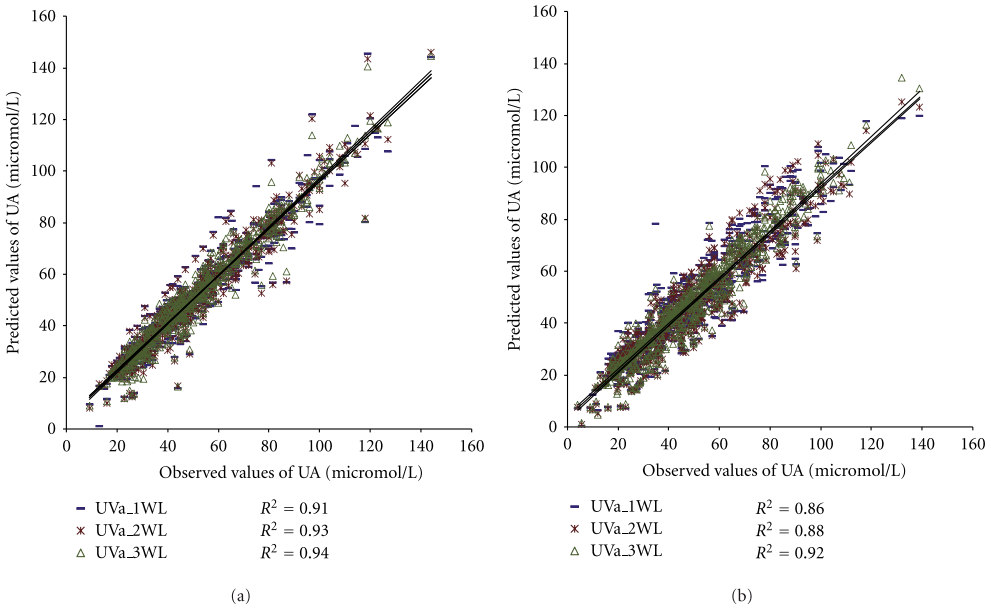

Figure 4: Models using UV absorbance values from one, two, or three wavelengths to estimate concentration of UA: (a) calibration group ($N = 579$) and (b) validation group ($N = 639$).

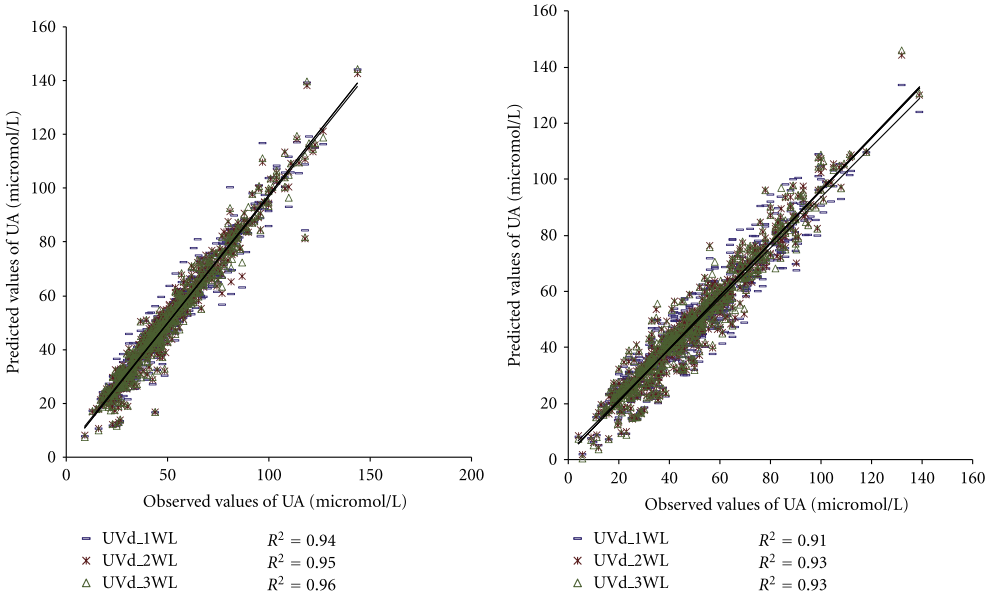

Figure 5: Models using values of first derivate of UV absorbance from one, two, or three wavelengths to estimate concentration of UA: (a) calibration group ($N = 579$) and (b) validation group ($N = 639$).

Table 5: Summary of results of different methods of measuring concentration of uric acid.

Method	Set	N	Concentration of UA ± SD (micromol/L)	R^2	BIAS	SE	RMSE
Lab	Cal.	579	52.1 ± 23.3	—	—	—	—
	Val.	639	49.7 ± 23.0	—	—	—	—
UVa_1WL	Cal.	579	52.1 ± 22.3	0.91	0.00	6.83	6.83
	Val.	639	48.9 ± 21.8	0.86	−0.88	8.70[a,b,c]	8.74[a,b,c]
UVa_2WL	Cal.	579	52.1 ± 22.5	0.93	0.00	6.19	6.19
	Val.	639	48.1 ± 21.6	0.88	−1.70	8.00	8.18
UVa_3WL	Cal.	579	52.1 ± 22.6	0.94	0.00	5.52	5.52
	Val.	639	48.4 ± 21.8	0.92	−1.39	6.39	6.54
UVd_1WL	Cal.	579	52.1 ± 22.6	0.94	0.00	5.64	5.64
	Val.	639	48.2 ± 21.8	0.91	−1.57	7.05[d]	7.22[d]
UVd_2WL	Cal.	579	52.1 ± 22.8	0.95	0.00	4.95	4.95
	Val.	639	48.7 ± 22.3	0.93	−1.07	5.94[e]	6.04[e]
UVd_3WL	Cal.	579	52.1 ± 22.8	0.96	0.00	4.83	4.83
	Val.	639	48.9 ± 22.4	0.93	−0.89	5.92	5.99

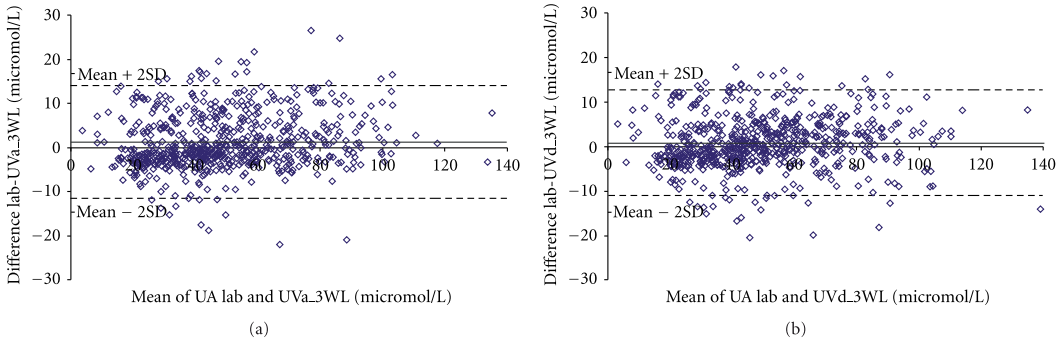

Figure 6: The Bland-Altman plots. (a) The difference between UA Lab and UVa_3WL is plotted against the mean value of UA Lab and UVa_3WL ($N = 639$). (b) The difference between UA Lab and UVd_3WL is plotted against the mean value of UA Lab and UVd_3WL ($N = 639$).

The coefficient of determination, R^2, between the laboratory and calculated values of UA are higher or equal in the case of the UVd (single/two/three) compared to the UVa (single/two/three) (0.86/0.88/0.92 versus 0.91/0.93/0.93) (Figures 4 and 5). Also, the systematic error and RMSE are lower if we use several wavelengths and/or derivate spectra (Table 5). This indicates that using several wavelengths instead of a single one produces a significant effect, which is larger when we use processed spectra instead of original absorbance spectra. However, it seems that adding a third wavelength to the UVd model does not improve results in terms of R^2, although the results of systematic error and RMSE improve. For describing the differences between individual values of the UA concentration from the laboratory and UA values from models, a Bland Altman plot for two models (UVa_3WL and UVd_3WL) was created (Figure 6); differences in UA values were somewhat smaller in the case of the model using derivate spectral values.

Considering the improvement in the accuracy of the model, systematic error and RMSE, the signal processing and information from several wavelengths should be used in the future. In this study the best result was achieved with the model using derivate spectra values at three wavelengths.

It was found that haemodialysis adequacy can be quantified using UV absorbance of spent dialysate. By using this method, it is possible to reduce costs by reducing the number of blood samples and amount of laboratory analyses [27].

A good way of estimating UA concentrations using the UV technique has been shown in previous studies [13, 14, 16–20], but if we use signal processing tools and absorbance information from several wavelengths, we can essentially improve the accuracy and reliability of the results.

A previous study by our group [28] indicated that app. 90% of the cumulative and integrated UV absorbance measured by the optical dialysis adequacy sensor originates from the ten main peaks of a particular dialysis treatment,

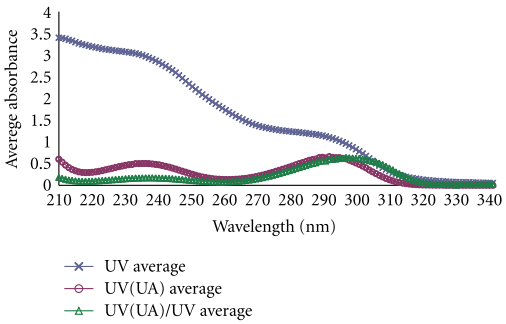

Figure 7: Average values of measured UV absorbance for total material and contribution of UA to UV absorbance.

one of which is UA. Another study where HPLC analysis was used indicated that the main solute responsible for UV absorbance of around 280 nm is UA [29].

As can be seen from Figure 7, the contribution of UA to total UV absorbance (UV (UA)/UV average presents an average absorbance sourced from UA in the dialysate divided by average UV absorbance of the whole dialysate) is relatively large in the wavelength region of 280–310 nm. This explains the wavelengths appearing in the models. UA absorbance spectra have one minimum around 265, and this explains why the wavelength is also included in the models.

The high correlation between UV absorbance and UA could be explained by the characteristic absorbance around 294 nm for UA in combination with the relatively high molar extinction coefficients of UA in this wavelength region compared to other chromophores among uremic retention solutes eliminated from blood into spent dialysate during dialysis [30]. This makes it possible to determine UA concentration even when the technique does not solely measure UA.

The use of a Savitzky-Golay algorithm for smoothing and first derivate calculation is an effective method of correcting baseline effects in spectra, which could explain the improvement in accuracy. Using UV absorbance and processed UV absorbance information from several wavelengths reduces randomness and is probably the reason why better results have been achieved.

In this study, multiple linear regression (MLR) analysis using the forward stepwise regression method was used to determine the best wavelengths for models. Using the stepwise regression method helps us to avoid mistakes in the models due to the possible collinearity of independent variables. It seems that models developed with MLR are relevant and work well in a validation set of material, although using other approaches like partial least squares regression (PLS-R) or principal component regression (PCR) to create models should be considered in the future [26].

The clinical aim in the future is to develop an online monitoring system that offers an estimation of the removal of clinically important solute and marker UA during haemodialysis.

Also, regarding the optical properties of UA, it is possible to develop an optical system to measure the UA concentration in blood and/or urine. This makes it possible to rapidly detect hyperuricemia widely and at an early stage. This is very important in preventing serious clinical issues caused by hyperuricemia [2–6, 8, 11, 12, 31].

An accurate optical method makes it possible to measure UA rapidly online without the need for blood samples and disposables or chemicals. Using a simple signal-processing tool and UV absorbance values from several wavelengths could be very helpful in achieving more accurate and reliable results.

5. Conclusion

This study investigated the effect of using several wavelengths and a simple signal processing to estimate the concentration of UA in dialysate using an optical method. The data analysed were collected over 10 years: 60 patients participated and 188 dialysis sessions were monitored in various centres in different countries. It was found that using a multi-wavelength and processed signal approach leads to more accurate results. This approach enables us to develop an advantageous, reliable, and cost-effective method of measuring the concentration of UA, an independent risk marker of cardiovascular and renal diseases and also a novel risk factor for type 2 diabetes mellitus. Developed algorithms could be used in optical dialysis quality monitors; these monitors should be integrated to dialysis machines and with these several parameters; UA among them is possible to monitor during the dialysis. No blood will be monitored; removal on substances is possible to estimate only by monitoring the spent dialysate. A future method evaluates the treatment dose and makes it possible to control treatments against set target values.

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PUBLICATIONS

Publication IV

Holmar J, Uhlin F, Fridolin I, Luman M, Fernström A (2013) "Can multicomponent on-line monitoring of small molecule uremic markers be beneficial for dialysis patients?", *Manuscript submitted*

APPENDIX 2

ELULOOKIRJELDUS

1. Isikuandmed

Ees- ja perekonnanimi	Jana Holmar (Jerotskaja)
Sünniaeg ja -koht	09.03.1983, Põlva, Eesti
Kodakondsus	eestlane

2. Kontaktandmed

Aadress	Kirsi 3-53, 10616 Tallinn, Eesti
Telefon	+372 620 22 05
E-posti aadress	jana@cb.ttu.ee

3. Hariduskäik

Õppeasutus (nimetus lõpetamise ajal)	Lõpetamise aeg	Haridus (eriala/kraad)
Tallinna Õismäe Humanitaargümnaasium	2001	Keskharidus
Tallinna Tehnikaülikool	2005	Elektroonika ja biomeditsiinitehnika, bakalaureusekraad
Tallinna Tehnikaülikool	2007	Biomeditsiinitehnoloogia, magistrikraad

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

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

5. Täiendusõpe

Õppimise aeg	Täiendusõppe läbiviija nimetus
08.2012-01.2013	Linköpingi Ülikool, Rootsi
11.2009-04.2012	5 Rahvusvahelise doktorikooli iBioMEP täiendkursust

6. Teenistuskäik

Töötamise aeg	Tööandja nimetus	Ametikoht
2005-2007	Tallinna Tehinkaülikool	Projektijuht
2007-k.a.	Tallinna Tehinkaülikool	Teadur

7. Teadustegevus

   Ureemiliste toksiinide ja kardiovaskulaarsete markerite uurimine ning nende dialüüsiravi käigus eemaldamise hindamine reaalajas optiliste meetoditega.

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

   ETF8621, Uudne optiline meetod ureemiliste toksiinide - alatoitumuse ja kroonilise põletiku ning SVH riski potentsiaalsete markerite, monitooringuks, 2011-2014

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

APPENDIX 3

CURRICULUM VITAE

1. Personal data

Name	Jana Holmar
Date and place of birth	09.03.1983, Põlva, Estonia

2. Contact information

Address	Kirsi 3-53, 10616 Tallinn, Estonia
Phone	+372 620 22 05
E-mail	jana@cb.ttu.ee

3. Education

Educational institution	Graduation year	Education (field of study/degree)
Tallinn Õismäe Gymnasium of Humanities	2001	Secondary education
Tallinn University of Technology	2005	Electronics and biomedical technology /Bachelor degree
Tallinn University of Technology	2007	Biomedical technology/Master degree

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

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

5. Special Courses

Period	Educational or other organization
08.2012-01.2013	Linköping University, Sweden
11.2009-04.2012	5 graduate courses in International Doctoral Programme – iBioMEP

6. Professional Employment

Period	Organization	Position
2005-2007	Tallinn University of Technology	Project manager
2007 -	Tallinn University of Technology	Research scientist

7. Scientific work

Research of uremic toxins and cardiovascular markers and optical on-line estimation of their elimination during dialysis

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

ETF8621, A novel optical technology for monitoring of uremic toxins - potential markers for malnutrition–inflammation syndrome and CVD risk, 2011-2014

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

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.

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