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The correlation of urea and creatinine concentrations in sweat and saliva with plasma during hemodialysis: an observational cohort study

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Abstract

Objectives: Urea and creatinine concentrations in plasma are used to guide hemodialysis (HD) in patients with end-stage renal disease (ESRD). To support individualized HD treatment in a home situation, there is a clinical need for a non-invasive and continuous alternative to plasma for biomarker monitoring during and between cycles of HD. In this observational study, we therefore established the correlation of urea and creatinine concentrations between sweat, saliva and plasma in a cohort of ESRD patients on HD.

Methods: Forty HD patients were recruited at the Dialysis Department of the Catharina Hospital Eindhoven. Sweat and salivary urea and creatinine concentrations were analyzed at the start and at the end of one HD cycle and compared to the corresponding plasma concentrations.

Results: A decrease of urea concentrations during HD was observed in sweat, from 27.86 mmol/L to 12.60 mmol/L, and saliva, from 24.70 mmol/L to 5.64 mmol/L. Urea concentrations in sweat and saliva strongly correlated with the concentrations in plasma (ρ 0.92 [$p < 0.001$] and 0.94 [$p < 0.001$], respectively). Creatinine concentrations also decreased in sweat from 43.39 μ mol/L to 19.69 μ mol/L, and saliva, from 59.00 μ mol/L to 13.70 μ mol/L. However, for creatinine, correlation coefficients were lower than for urea for both sweat and saliva compared to plasma (ρ : 0.58 [$p < 0.001$] and 0.77 [$p < 0.001$], respectively).

Conclusions: The results illustrate a proof of principle of urea measurements in sweat and saliva to monitor HD adequacy in a non-invasive and continuous manner. Biosensors enabling urea monitoring in sweat or saliva could fill in a clinical need to enable at-home HD for more patients and thereby decrease patient burden.

Keywords: biosensors for sweat analysis; continuous monitoring; diagnostics of urea in sweat and saliva; hemodialysis at home; non-invasive monitoring

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Introduction

Plasma urea and creatinine concentrations are used to estimate exchange efficiency and guide hemodialysis (HD) in patients with end-stage renal disease (ESRD) [1]. These patients are required to visit the hospital multiple times a week, which affects their quality of life. This burden can possibly be reduced, by performing HD in a home situation. Also, individualizing HD treatment could improve the patients' quality of life [2]. To enable individualized HD, transferable to a home situation, there is a clinical need for non-invasive alternatives to plasma for remote and continuous biomarker monitoring during and between cycles of HD.

In recent decades, the analysis of bio-fluids that are easily and non-invasively collectable, such as sweat and saliva, has gained scientific interest. Sweat is considered a biomarker-rich fluid that shows potential for clinical applications in diagnosis

and monitoring of multiple diseases [3]. For instance, the chloride concentration in sweat is already being quantified in clinical practice to support the diagnosis of cystic fibrosis in young children [4].

In addition to sweat, saliva is a potential non-invasive alternative to plasma analysis during HD as the results of Lasisi et al. suggest a potential supporting role of salivary urea and creatinine in the diagnosis and monitoring of chronic kidney disease [5, 6].

Currently, there are no methods for continuous measurement of urea and creatinine concentrations in sweat and saliva enabling remote readout. However, technological advances in microfluidics make it possible to analyze small volumes of sweat by means of biosensors on the skin [7–10]. In fact, while a variety of interesting biomarkers have been quantified in sweat [3] and saliva [11], the commercialization of technological innovations in the clinic is, among other challenges, hampered by the lack of knowledge about the correlation of biomarker concentrations in sweat and saliva with the patients' plasma concentrations [12]. Sweat and saliva might not simply be an ultra-filtrate of plasma, meaning that sweat and salivary components may not only derive from blood through active transport or passive diffusion, but they may also partly originate from the sweat or salivary glands.

Urea and creatinine are both low-molecular-weight, water-soluble waste products that are normally excreted via urine [1]. To evaluate the potential of sweat and salivary urea and creatinine concentrations to estimate HD adequacy, the association between these and plasma concentrations should be assessed. To our knowledge this association has never been described with sweat and saliva combined, during HD. In this study, we therefore established the correlation and agreement of urea and creatinine concentrations between saliva, sweat and plasma in a cohort of ESRD patients on HD.

Materials and methods

Subjects

This paper is based on the observational clinical trial (UMIS Study) that is registered in the International Clinical Trials Registry Platform with main ID NL9831. The study was approved by the local Ethical Review Board and the Medical research Ethics Committees United (MEC-U), and

written informed consent was obtained from each patient before participation. This study was carried out in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013) and in accordance with Dutch law. Forty hemodialysis patients were recruited at the Dialysis Department of the Catharina hospital in Eindhoven, The Netherlands. Exclusion criteria were as follows: aged <18 years, presence of an implanted device (e.g. defibrillator, neuro-stimulator, pacemaker, or ECG monitor), pregnancy, known allergy to any ingredient of the sweat test, damaged or denuded skin or other recent scar tissue at the sweat stimulation site, or cardiac conditions or suspected heart problems. The exclusion criteria were based on the contraindications of the Macroduct® Advanced for sweat gland stimulation, as described in the user manual.

Study design

A schematic overview of this observational cohort study design is shown in Figure 1. All 40 patients participated during one hemodialysis cycle, where plasma, sweat and salivary samples were collected at the start and at the end of the procedure.

Sample collection and analysis

Plasma: Blood was drawn from each patient while connected to the HD machine. The dialysate flow was temporarily reduced from 300 to 100 mL/min during blood collection. All samples were centrifuged within 2 h after collection. Biomarker concentrations were determined using the Cobas Pro analyzer (Roche Diagnostics, Rotkreutz, Switzerland) with a kinetic method for urea (measuring range: 0.5–40 mmol/L, Limit of Quantitation [LoQ]: 0.5 mmol/L) and an enzymatic method for creatinine (measuring range: 5–2,700 µmol/L, LoQ: 10 µmol/L), according to standard operating procedures.

Sweat and saliva: Sweat samples were collected on the forearm with the Macroduct Advanced sweat collection system (Elitechgroup, Logan, Utah, USA), according to manufacturer's instructions. In short, two electrodes (one anode and one cathode) were placed over agarose gel discs containing pilocarpine, that were in contact with the skin. A mild current (1.5 mA) allows the pilocarpine to enter the skin under the anode, where it activates the sweat glands. After 5 min of stimulation, the electrodes were removed, the arm was washed successively with alcohol and deionized water, and sweat was collected for 30 min with the Macroduct advanced sweat collector.

Saliva samples were obtained with the Salivette saliva collection method (Starsted, Nümbrecht, Germany). In short, patients were asked to refrain from eating, drinking or brushing their teeth for 30 min before saliva was collected. Patients were then asked to chew a cotton swab for 1 min and then spit the swab with all accumulated saliva into the collection tube. Samples were centrifuged at 2,495 rpm for 10 min and stored at –80 °C in cryogenic vials for a maximum of 3 weeks before analysis.

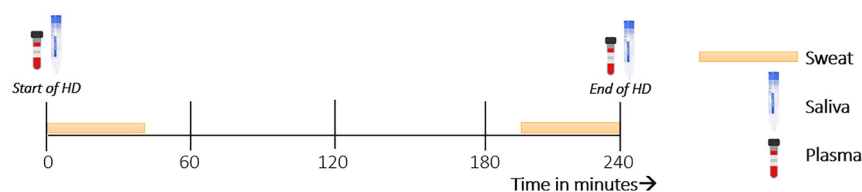


Figure 1: Schematic timeline of study design. All patients (n=40) participate for one hemodialysis cycle, where plasma, sweat and salivary samples were collected at the start and the end of the treatment.

Analysis of sweat and saliva samples: Concentrations of urea and creatinine were determined with the Cobas Pro analyzer (Roche Diagnostics, Rotkreutz, Switzerland), using the plasma method. This method was validated for sweat and salivary samples with solutions in distilled H₂O in 5 separate batches in the expected range of urea (0.5–40 mmol/L) and creatinine (5–250 µmol/L), with a maximum Coefficient of Variation (CV) of 10.48 % (data not shown). Sweat samples were diluted with distilled H₂O to increase the collected volume to a higher sample volume (70 µL) that was sufficient as input for the Cobas analyzer.

Statistical analysis

All analyses were carried out using software R (version 4.2.3 or higher) with RStudio (version 2023.03.0 or higher) and a p-value of less than 0.05 was considered significant. The Shapiro-Wilk test combined with visual inspection of the QQ-plot were carried out to assess the normality of the data. When data was normally distributed, a student t-test was conducted to test for statistical differences. Otherwise, the difference between biomarker concentrations in different bio-fluids and at different time-points was tested for significance with the non-parametric Wilcoxon signed rank test for paired samples. Spearman's correlation analysis was conducted to assess the strength of the association between biomarker

concentrations in different bio-fluids. Linear regression analysis was used to estimate plasma urea and creatinine concentrations based on sweat and saliva measurements of urea and creatinine.

This concerns a study in which diagnostic methods were compared and was analyzed according to the EP9 protocol, as generally applied in clinical laboratories [13]. In this protocol, a number of 40 patients is recommended for an adequate method comparison study to determine and quantify the bias with sufficient statistical substantiation.

Results

Inclusion

Main patient characteristics are described in Table 1. Seventy-four patients met the inclusion criteria and were asked to participate. Thirty-three patients were not willing to participate and one patient was excluded based on health issues preventing participation. Forty patients were included in this study (27 males and 13 females). The median age was 71 (min: 22, max: 90) years, with no significant difference between genders (p=0.29).

Table 1: Main patient characteristics.

Variable	All patients (n=40)
Gender, men, n (%)	27 (67.5 %)
Age, years (min.–max.)	68 (22–90)
BMI, n (%)	
<18	2 (5.0 %)
18–25	13 (32.5 %)
25–30	17 (42.5 %)
>30	8 (20 %)
Postdialysis weight, mean ± SD in kg	79.3 ± 19.3
Access via AV shunt, n (%)	26 (65.0 %)
Diabetes Mellitus, n (%)	17 (42.5 %)
GFR (mL/min/1.73 m ²) (median [IQR])	5.95 (4.98–8.15)
Type of dialyzer, n (%)	
Fx Cordiax 1000	26 (65 %)
Fx Cordiax 80	8 (20 %)
Nephral 500	6 (15 %)

GFR, glomerular filtration rate; AV shunt, arteriovenous shunt.

Urea and creatinine concentrations

Table 2 summarizes the results of the urea and creatinine concentrations in plasma, sweat and saliva before and after HD. For six sweat samples at the start and nine sweat samples at the end of HD, there was not enough volume for accurate quantification of urea concentration and these were therefore excluded from further analysis. Remaining urea concentrations in the collected saliva and sweat samples were well above the detection limits of the laboratory tests (0.5 mmol/L). In plasma, the urea concentration decreased from 22.43 (18.75–26.25) mmol/L (median [IQR]) to 5.75 (4.08–7.55) mmol/L in the studied HD cycle. This decrease was also observed in sweat, from 27.86 (22.02–33.25) mmol/L to 12.60 (9.10–15.11) mmol/L, and saliva, from

Table 2: Biomarker concentrations pre- and post-dialysis. p-Values give the difference between the group median vs. plasma group median. All p-values are determined for paired measures only.

Biomarker	Biofluid	Start of HD			End of HD			p-Value for difference start-end
		n	Concentration (median [IQR])	p-Value for difference with plasma	n	Concentration (median [IQR])	p-Value for difference with plasma	
Urea, mmol/L	Plasma	40	22.50 (18.75–26.25)	–	40	5.75 (4.08–7.55)	–	<0.001
	Sweat	34	27.86 (22.02–33.25)	<0.001	31	12.60 (9.10–15.11)	<0.001	<0.001
	Saliva	39	24.70 (20.75–29.85)	<0.001	40	5.64 (4.11–7.51)	0.299	<0.001
Creatinine, µmol/L	Plasma	40	676.50 (509.20–853.50)	–	40	211.50 (145.00–274.80)	–	<0.001
	Sweat	26	43.39 (23.41–50.42)	<0.001	16	19.69 (17.22–27.34)	<0.001	<0.001
	Saliva	39	59.00 (39.75–126.50)	<0.001	39	13.70 (10.55–25.70)	<0.001	<0.001

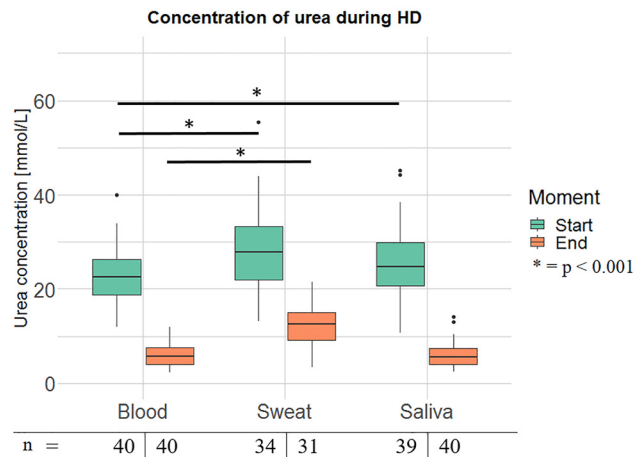


Figure 2: Boxplot of urea concentrations at the start and at the end of hemodialysis in plasma, sweat and saliva. Concentrations in sweat were significantly higher than in plasma at the start and end of HD ($p < 0.001$). Salivary urea concentrations were also significantly higher at the start ($p < 0.001$), but not at the end of HD.

24.70 (20.75–29.85) mmol/L to 5.64 (4.11–7.51) mmol/L. Concentrations in sweat were significantly higher than in plasma at the start and end of HD ($p < 0.001$). Salivary urea concentrations were also significantly higher at the start ($p < 0.001$), but not at the end of HD (Figure 2).

In plasma, the median creatinine concentrations decreased from 676.50 (509.20–853.50) $\mu\text{mol/L}$ to 211.50 (145.00–274.80) $\mu\text{mol/L}$ in the studied HD cycle. A decrease was also observed in sweat, 43.39 (23.41–50.42) $\mu\text{mol/L}$ to 19.69 (17.22–27.34) $\mu\text{mol/L}$, and saliva, from 59.00 (39.75–126.50) $\mu\text{mol/L}$ to 13.70 (10.55–25.70) $\mu\text{mol/L}$. Concentrations of creatinine in sweat could not be accurately quantified before HD in 14 samples and after HD in 24 samples due to analytical limitations in the combination with the sparse sample volume. Concentrations of creatinine in plasma were significantly higher than in sweat and saliva at the start and end of HD ($p < 0.001$), with the median sweat and salivary creatinine concentrations being 6.0 and 8.3 % of the plasma concentration, respectively.

Associations of sweat and saliva with plasma

As shown in Figures 3 and 4, as well as Table 3, the urea concentrations in sweat correlated well with the corresponding plasma concentrations (Spearman's correlation coefficient ρ : 0.92 [CI: 0.88–0.95, $p < 0.001$]). The saliva concentration of urea showed a similar correlation coefficient of 0.94 [CI: 0.90–0.96, $p < 0.001$]. Consistently higher urea concentrations were found in sweat than in plasma, reflected in the intercept of the linear regression line ($y = 1.04x + 5.19$ [R^2 : 0.80]). The absolute differences

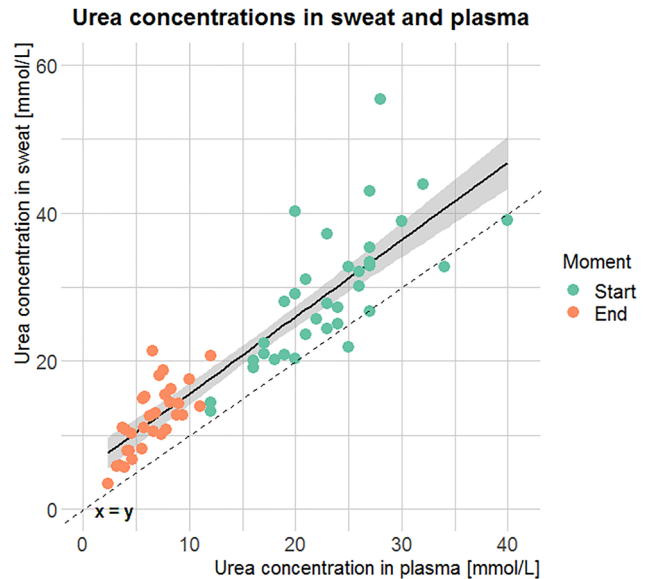


Figure 3: Urea concentrations in sweat and plasma before (start, $n=34$) and after (end, $n=31$) hemodialysis. Spearman's correlation coefficient $\rho=0.92$ (95 % CI: 0.88–0.95), formula of linear regression line: $y=1.04x + 5.19$ ($R^2=0.80$). The dashed line represents the $x=y$ line.

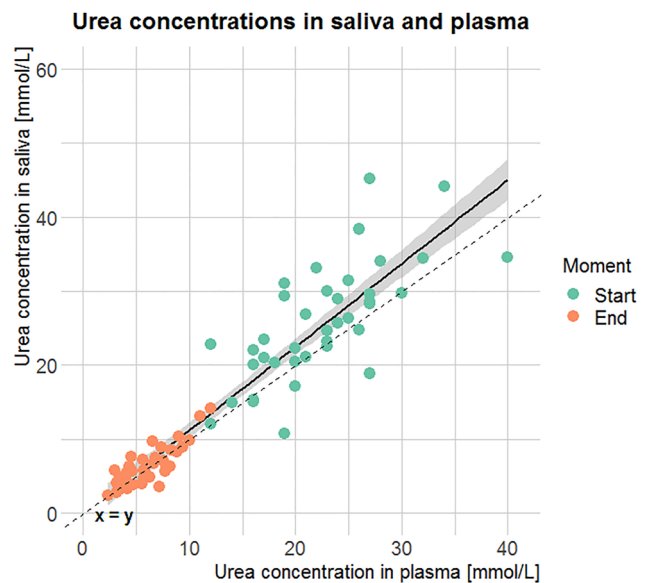


Figure 4: Urea concentrations in saliva and plasma before (start, $n=39$) and after (end, $n=40$) hemodialysis. Spearman's correlation coefficient $\rho=0.94$ (95 % CI: 0.90–0.96), formula of linear regression line: $y=1.13x - 0.06$ ($R^2=0.87$). The dashed line represents the $x=y$ line.

between sweat and plasma urea concentrations were similar at the start and at the end of HD within individual patients ($p=0.89$), but not between individual patients, ranging from a mean difference of 0.98 mmol/L to 17.69 mmol/L (Figure 5). This inter-individual variation was not observed with urea in saliva and plasma ($y=1.13x - 0.06$ [R^2 : 0.87]) (Figure 6). A linear regression

Table 3: Correlation coefficients for associations between sweat or saliva with plasma concentrations of urea and creatinine. Correlations and R^2 values for urea are higher than for creatinine.

Biomarker	Compared biofluids	Spearman correlation coefficient (ρ) with 95 % CI	p-Value	R^2	Number of samples, n
Urea	Plasma/sweat	0.92 (0.88–0.95)	<0.001	0.80	65
	Plasma/saliva	0.94 (0.90–0.96)	<0.001	0.87	79
Creatinine	Plasma/sweat	0.58 (0.34–0.75)	<0.001	0.38	42
	Plasma/saliva	0.77 (0.66–0.85)	<0.001	0.35	78

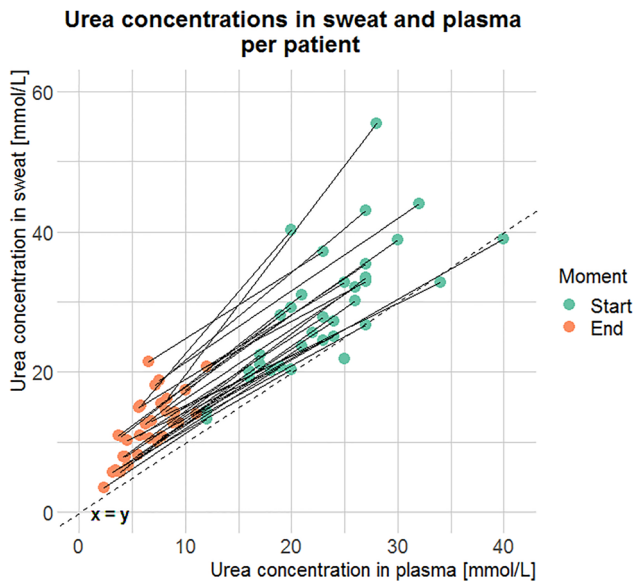


Figure 5: Urea concentrations in sweat and plasma before (start, $n=34$) and after (end, $n=31$) hemodialysis. Every line represents one patient, the dashed line represents the $x=y$ line.

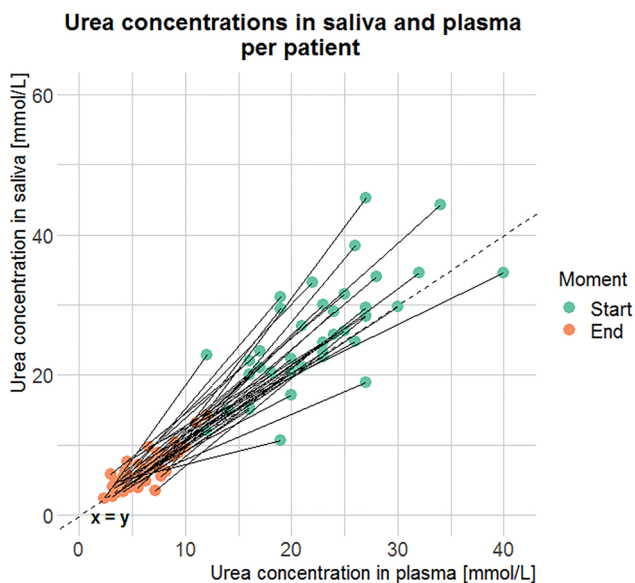


Figure 6: Urea concentrations in saliva and plasma before (start, $n=39$) and after (end, $n=40$) hemodialysis. Every line represents one patient, the dashed line represents the $x=y$ line.

model could be used to estimate plasma urea concentrations based on the sweat and/or saliva urea concentrations that were not significantly different from the actually measured plasma urea concentrations ($p=0.35$ and $p=0.29$, respectively). However, for creatinine (Figures 7 and 8 and Table 3), correlation coefficients were lower for both sweat and saliva compared to plasma (0.58 [CI: 0.34–0.75, $p<0.001$] and 0.77 [CI: 0.67–0.85, $p<0.001$], respectively) and the data could not be described with a linear regression model ($R^2<0.4$).

Discussion

In this study, the association between urea and creatinine concentrations in sweat and saliva compared to those in plasma was investigated to evaluate the feasibility of sweat and saliva for non-invasive and continuous monitoring of HD patients. Urea concentrations in sweat and saliva were found to be highly correlated with plasma urea concentrations. For creatinine, the correlation coefficients were lower.

Urea in sweat

Previous literature on urea and creatinine concentrations in sweat and saliva and the correlation with plasma concentrations during HD is limited. However, consistent with the literature, the urea concentrations in sweat were found to be higher than the urea concentrations in plasma [14, 15]. Based on size and solubility in water, Komives et al. [16] hypothesized that urea passively diffuses through cell membranes and tight junctions. However, as sweat concentrations are higher than in plasma, this could imply an additional source of urea outside plasma, the occurrence of additional active transport mechanisms from plasma to sweat, or water reabsorption in the sweat gland duct [15]. Other studies suggested the epidermis as source for urea [17, 18] or the sweat gland itself by arginase cleavage of arginine to ornithine and urea [19, 20]. More recently, Xie et al. [21] found evidence of increased expression of urea transporters in sweat glands from uremic patients,

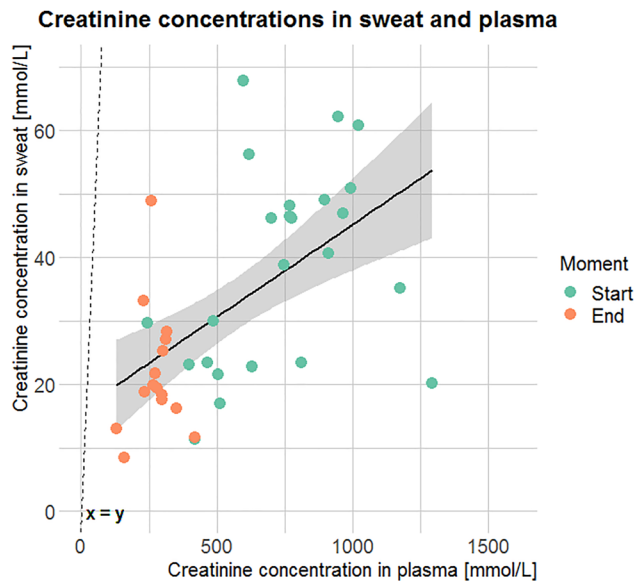


Figure 7: Creatinine concentrations in sweat and plasma before (start, $n=26$) and after (end, $n=16$) hemodialysis. Spearman's correlation coefficient $\rho=0.58$ (95 % CI: 0.34–0.75) and $R^2=0.38$, formula of regression line: $y=0.03x + 14.4$ ($R^2=0.38$). The dashed line represents the $x=y$ line.

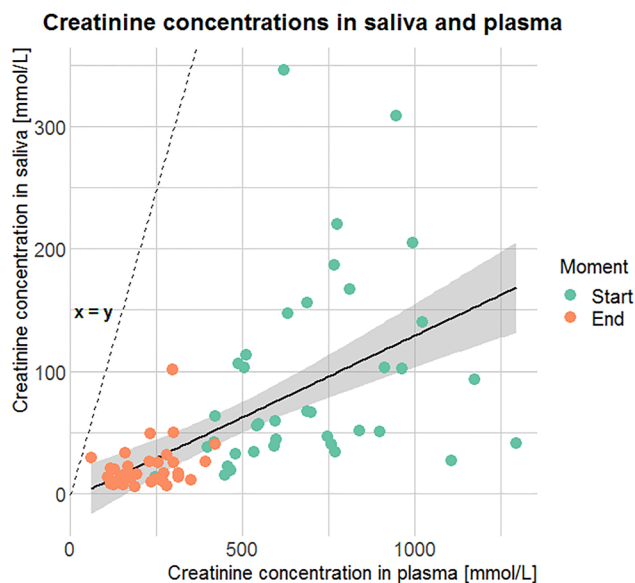


Figure 8: Creatinine concentrations in saliva and plasma before (start, $n=39$) and after (end, $n=39$) hemodialysis. Spearman's correlation coefficient $r=0.77$ (95 % CI: 0.66–0.85), formula of regression line: $y=0.13x - 4.18$ ($R^2=0.35$). The dashed line represents the $x=y$ line.

suggesting an alternative excretion pathway via the sweat glands, occurring when kidney function decreases. The variation in the expression of the urea transporters between individuals could explain the inter-individual variation in the variability that we described.

The outcome of the high correlation between sweat and plasma urea contradicts the results of Al-Tamer et al. [14], who found no significant association. This discrepancy could be attributed to the different method used for sweat stimulation and collection. The sweat collection method with unstimulated sweat that Al-Tamer et al. employed, might be more prone to evaporation as systemic heat stimulation was applied, causing the high urea concentrations found in sweat.

Urea in saliva

The high correlation found for urea in saliva and plasma ($\rho: 0.94$) is consistent with the findings of Bilancio et al. [22] who also found a correlation of 0.94. In addition, the results of five HD patients in that study showed higher salivary urea compared to plasma at the start of HD, but not at the end of HD, which is also in line with our findings. In addition, our findings also confirm those of Bader et al. [5], who found a correlation of 0.98 in 30 ESRD patients undergoing regular HD treatment.

Creatinine in sweat and saliva

Sweat concentrations of creatinine could only be quantified in a subset of samples due to a too high limit of quantitation (LoQ) of the applied assay. Therefore, the median and IQR values might be biased and need to be interpreted with caution. Despite this limitation, the findings of the creatinine concentration being lower in sweat than in plasma is in accordance with other reported studies [10, 23]. The literature is inconclusive about the correlation coefficient between sweat and plasma creatinine concentrations [14]. Saliva concentrations of creatinine showed a significant positive correlation with concentrations in plasma, in line with the research of Bader et al. [5]. However, the correlation coefficients are slightly higher than in our study ($\rho=0.89$ vs. $\rho=0.77$). The difference could be caused by a difference in the saliva collection method (passive drooling vs. actively stimulated saliva while chewing a swab), impacting and changing the contribution of the three main salivary glands, which in turn could alter the concentration of the biomarker and thus the correlation with plasma [24].

Innovation and future perspective

Although biosensors are proposed for urea and creatinine in sweat and saliva [7, 10], there is a lack of fundamental knowledge about the underlying transport mechanisms of

urea and creatinine between sweat, saliva, and plasma. To our knowledge, this study is the first to elucidate the association of urea and creatinine in sweat and saliva with the corresponding plasma concentrations in 40 ESRD patients during one HD cycle. This research adds fundamental knowledge about the kinetics of urea and creatinine towards sweat and saliva at the start and at the end of HD, needed for clinical interpretation of these biomarkers. Our findings confirm an excellent correlation of urea in sweat and saliva with plasma, implying that biosensors that can adequately monitor urea concentrations in sweat or saliva may enable non-invasive and continuous remote HD monitoring. These sensors should however still be developed further to enable accurate and robust analysis [12]. The clinical need for continuous creatinine monitoring is a subject for further exploration.

Additional potential clinical applications

Some modern HD machines are able to quantify the HD adequacy by clearance of small solutes or electrolytes via conductivity measures, ion-selective electrodes or optical measurements [25]. However, the on-line measurements are limited to the duration of the HD treatment, whereas sweat and/or saliva measurements could be performed between the HD cycles at any moment. This would allow further individualization of the HD treatment schedule, as the patient or medical specialist can evaluate the urea in real-time. In addition, the findings of this study could potentially be translated to the non-invasive and continuous urea monitoring of other patients, e.g., requiring peritoneal dialysis or in the pre-dialysis stage. However, this requires additional testing in the respective patient populations.

Limitations

The main limitation of sweat analysis is the relatively low volume of sweat, which in some patients leads to unquantifiable urea and creatinine concentrations. In our experience (results not shown), several ESRD patients on HD produce relatively low sweat rates after iontophoresis, specifically women. This could be caused by relative dehydration during the HD cycle. Despite the fact that this had no impact on the outcome of our study, this shows that handling of low sample volumes by and microfluidics in biosensors are essential in further development.

The physiology underlying the excretion of urea and creatinine by means of sweating and saliva production is, to

our knowledge, largely unknown. Although Kao et al. [26] suggested decreased saliva production in ESRD patients on HD, it is unclear how alternative excretion pathways change in the process of renal failure. Our study did not address these knowledge gaps. It would therefore be of great interest to further explore excretion of urea and creatinine through sweat and saliva production in the healthy population and in renal failure patients in the pre-dialysis stage. Also, we did not address the intra-individual variability or reproducibility of urea and creatinine in sweat and saliva, since this would require the measurements at the start and end of multiple HD cycles. This study was designed to assess the feasibility of urea and creatinine measurements in sweat and saliva and clarify the correlation between the analyses in these different matrices. The intra-individual variability remains to be established during further development.

Conclusions

In conclusion, urea and creatinine concentrations in sweat and saliva showed a positive correlation with the corresponding concentrations in plasma in a cohort of 40 ESRD patients requiring HD. As in plasma, urea and creatinine concentrations in sweat and saliva decreased extensively during a dialysis cycle. Based on these findings, urea in sweat or saliva is a promising biomarker that can easily be translated into plasma concentrations at the start and at the end of HD. This would allow the estimation of HD adequacy by sweat or saliva monitoring, but requires further investigation. The results illustrate a proof of principle of urea measurements in sweat and saliva to monitor HD adequacy in a non-invasive and continuous manner. This suggests that biosensors enabling urea monitoring in sweat or saliva remotely could fill in a clinical need to improve HD in a home situation, individualize the treatment schedule and thereby decrease patient burden.

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Research ethics: The study was approved by the local Ethical Review Board and the Medical research Ethics Committees United (MEC-U). This study was carried out in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013) and in accordance with Dutch law.

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: Research idea and study design: SA, CJAMK, LC, EB, RAB, DHK; data acquisition: SA; data analysis/interpretation: SA, CJAMK, LC, EB, MM, RAB, DHK; statistical analysis: SA; supervision or mentorship: MM, RAM, DHK. The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest.

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Data availability: The raw data can be obtained on request from the corresponding author.

Trial registration: This paper is based on the observational clinical trial (UMIS Study) that is registered in the International Clinical Trials Registry Platform with main ID NL9831.

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