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ORIGINAL RESEARCH

Adherence to Antihypertensive Drugs Assessed by Hyphenated High-Resolution Mass Spectrometry Analysis of Oral Fluids

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BACKGROUND: It is currently unknown if antihypertensive drugs can be monitored in oral fluid (OF) using liquid chromatography coupled to high-resolution mass spectrometry.

METHODS AND RESULTS: We assessed adherence using liquid chromatography coupled to high-resolution mass spectrometry in OF, plasma, and urine of 56 consecutive patients with hypertension referred to a tertiary hypertension unit. Of these patients, 59% were completely adherent (all drugs detectable in urine), whereas 29% and 13% were partially adherent (1 drug not detectable in urine) or nonadherent (>1 drug not detectable in urine), respectively. Adherent patients were on fewer antihypertensive drugs (P=0.001), had fewer daily drug doses (P=0.012), and had lower 24-hour ambulatory systolic (P=0.012) and diastolic (P=0.009) blood pressures than nonadherent or partially adherent patients. Most drugs were detected in urine compared with plasma and OF (181 versus 119 versus 88; P=0.001). Compared with urine and plasma, detection rates of angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and diuretics were lower in OF. There was no difference in the frequency of detecting β blockers (P=1.0) and calcium channel blockers (P=0.063) when comparing OF with urine. There was no difference in the number of calcium channel blockers (P=0.727), β blockers (P=1.000), thiazide diuretics (P=0.125), and α-2 agonists (P=0.125) identified between OF and plasma.

CONCLUSIONS: This study shows the feasibility of drug adherence testing for several antihypertensive drugs, especially those without acidic components, in OF, with a similar recovery compared with plasma. Therefore, drug adherence testing in OF should be further explored as a noninvasive approach, which can easily be performed in an "out-of-office" setting.

Key Words: adherence ■ arterial hypertension ■ compliance ■ liquid chromatography coupled to high-resolution mass spectrometry ■ toxicological analyses

Nonadherence to prescribed drug treatment frequently occurs in hypertension and is associated with increased morbidity and mortality.[1–5] Assessing drug adherence in hypertensive patients can guide specific patient-centered interventions to improve adherence and might reduce the number of unnecessarily prescribed drugs.[6–8] Several indirect (pill count, patient diaries, adherence questionnaires, and prescription record reviews) and direct (drug monitoring in blood and urine and directly observed therapy) methods to evaluate adherence have been introduced.[9] Toxicological analyses of urine and plasma are the most commonly used matrices for assessing drug adherence. However, the collection of both matrices bears potential disadvantages: collecting urine cannot be observed without infringing the patient's privacy, whereas obtaining a blood sample is invasive and requires medical personnel. Furthermore, drug adherence monitoring in urine may cause false-positive results because the washout period for several

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CLINICAL PERSPECTIVE

What Is New?

- This is the first study that showed the feasibility of detecting several antihypertensive drugs, especially those without acidic components, in oral fluid (OF).
- Using liquid chromatography coupled to high-resolution mass spectrometry analysis, most antihypertensive medications were identified in urine, followed by plasma and OF.
- Antihypertensive drugs without acidic components had similar detection rates in plasma and OF, whereas antihypertensive drugs with acidic functions were poorly detected in OF.

What Are the Clinical Implications?

- Although most antihypertensive drugs were detected in urine, drug adherence testing in OF, especially of substances without acidic components, is feasible and should be further explored as a noninvasive approach, which can easily be performed in an "out-of-office" setting.

Nonstandard Abbreviations and Acronyms

LC-HRMS/MS	liquid chromatography coupled to high-resolution mass spectrometry
OF	oral fluid

antihypertensive drugs in urine lasts longer than multiple half-lives, usually exceeding 24 hours.[10] Therefore, there is an unmet need for an easily applicable and reliable method to evaluate drug adherence.[7,9,11] As drugs are in principle distributed to all body compartments, including oral fluids (OFs), the detection of drugs in OF may represent a novel approach in drug adherence testing. This study aimed at investigating liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS/MS) of urine, plasma, and OF to assess drug adherence in patients referred to an outpatient hypertension unit.

METHODS

The data of this investigator-initiated study are available from the corresponding author on reasonable request. Between November 2017 and March 2018, 56 consecutive patients with hypertension who were referred to the outpatient hypertension unit at the Saarland University Medical Center (Homburg/Saar, Germany) were included in this study. All participating patients provided written informed consent, and local ethics committees approved the study. Eligible patients were ≥18 years old, had hypertension, as defined by current guidelines,[12] and were prescribed to stable antihypertensive therapy for at least 2 weeks.

Clinical Assessment

All patients were instructed to take their medication in the morning as prescribed. Medical history, physical examination, routine blood chemistry, and attended office blood pressures were documented for all patients. After 5 minutes of rest in a comfortable, seated position, 3 consecutive blood pressure measurements, 1 minute apart, were taken with a validated automated blood pressure monitor (Omron Health Care, Inc, Lake Forest, IL). If the first 2 readings varied by >10 mm Hg, an additional measurement was performed. The last 2 blood pressure readings were averaged and recorded as the office blood pressure. Also, 24-hour ambulatory blood pressure monitoring (Mobil-O-Graph; I.E.M GmbH, Stolberg, Germany) was done where clinically indicated. Chronic kidney disease was defined as an estimated glomerular filtration rate <60 mL/min per 1.73 m^2. Chronic heart failure comprised patients with a history of chronic heart failure with and without reduced left ventricular ejection fraction.

Sampling of OF, Plasma, and Urine

OF was sampled in the morning using the Quantisal OF collection device (Immunalysis Corporation, Pomona, CA). The collection device consists of a collection pad that collects 1 mL (±10%) of OF and a transport tube containing liquid buffer. The device pad was placed under the patient's tongue until the volume adequacy indicator turned blue. Of note, no chemical stimuli, such as acidic stimulation (citric acid), were used to increase salivation, as this might influence drug concentrations.[13] After sampling, the collection pad was inserted in the transport tube and was shaken for 2 hours at room temperature to allow the extraction of the OF from the collection pad. Afterward, the collection pad was removed. Venous EDTA blood and spot urinary samples were drawn, blood was centrifuged, and the supernatant was separated. Then, OF, plasma, and urine samples were stored at –20°C until the analysis was performed at the Department of Experimental and Clinical Toxicology at Saarland University (Homburg/Saar, Germany).

Drug Adherence Analysis by LC-HRMS/MS

The detailed bioanalytical method of the targeted adherence monitoring method has been described previously.[14] In brief, samples were separated on a ThermoFisher Accucore PhenylHexyl column

(ThermoFisher, Dreieich, Germany) with mobile phase A consisting of 2 mmol/L aqueous ammonium formate containing 0.1% formic acid (v/v, pH 3) and mobile phase B consisting of 2 mmol/L aqueous ammonium formate with acetonitrile:methanol (50:50, v/v, 1% water) containing 0.1% formic acid. Drugs and metabolites were analyzed by a ThermoFisher Scientific Dionex UltiMate 3000 (ThermoFisher) interfaced to an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland) and a TF Q-Exactive system with a heated electrospray ionization-II source set to positive/negative switching. Mass spectrometry was performed using full-scan data and a subsequent data-dependent acquisition mode with an inclusion list containing masses of interest. TraceFinder 4.1 software (ThermoFisher) was used for data processing. People assessing drug adherence were blinded to patients' characteristics.

Definition of Adherence

Despite recent efforts to establish a standardized classification of medication adherence,[15] the definitions used in the literature remain inconsistent.[16] We applied 2 commonly used classifications: In the first set of analyses, nonadherence was defined according to the absolute number of nondetectable drugs. If all prescribed drugs were detectable in urine, a patient was classified as "adherent." If 1 or at least 2 of the prescribed drugs were not detectable, the patient was considered to be "partially adherent" and "nonadherent," respectively. In the second set of analyses, provided in Tables S1 and S2, adherence was defined dichotomously on the basis of a threshold of 80%. If ≥80% of the prescribed drugs were detectable in urine, the patient was classified as "adherent." Otherwise, the patient was considered to be "nonadherent." For analyses of clinical characteristics, adherence to all substances of a single-pill combination was assumed, if at least one component of a single-pill combination was detectable (referred to as "adherence corrected for single-pill combinations").

Statistical Analysis

Data are presented as mean±SD and numbers (percentages). Because of the relatively low sample size, we used the Wilcoxon matched-pairs signed-rank test and the Mann-Whitney test to compare continuous variables between 2 paired and unpaired groups, respectively. The Kruskal-Wallis test was used for comparisons of continuous data between adherent, partially adherent, and nonadherent patients. If the Kruskal-Wallis test was significant, we used the Dunn-Bonferroni post hoc method for pairwise comparisons. For categorical variables, comparisons between independent groups were performed using Pearson's χ^2 or Fisher's exact test, whereas McNemar's or Cochran's Q test was used for paired groups. For nominal variables, we used Fleiss' K to assess reliability. Interrater reliability was interpreted as initially suggested, with a K <0 tentatively considered to be poor; 0 to 0.2, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 1.00, almost perfect.[17] A 2-sided $P<0.05$ was considered to be statistically significant. All statistical analyses were performed with IBM SPSS Statistics, version 23.0 (IBM Corp, Armonk, NY), and graphs were created with GraphPad Prism, version 8.4.2 (GraphPad Software, La Jolla, CA).

RESULTS

Patients' Characteristics

The patients' characteristics are summarized in Table 1. The patients' mean age was 60.0±13.1 years, 54% were women, with a body mass index of 29.2±6.8 kg/m^2. Mean 24-hour ambulatory blood pressures were 133.8/81.4±16.1/10.4 mm Hg, despite the prescription of 3.8±1.4 antihypertensive drugs. In total, 59% of the patients were completely (all drugs detectable in urine, 66% after correcting for single-pill combinations) and 29% partially adherent (1 drug not detectable urine, 23% after correcting for single-pill combinations) to their prescribed antihypertensive medication. Adherence rates were highest when using urine as the matrix for LC-HRMS/MS (Figure 1). Adherent patients were prescribed to fewer antihypertensive drugs and daily doses (Figure 2) and had lower 24-hour ambulatory systolic and diastolic blood pressures compared with nonadherent or partially adherent patients (Table 1). Except for mineralocorticoid receptor antagonists (P=0.042), α-1 blockers (P=0.008), and α-2 agonists (P=0.008), there was no difference in prescribed drug classes between completely adherent, partially adherent, and nonadherent patients (Table S3). Figure 3 depicts the adherence rates for each antihypertensive drug class.

Comparison of Detection Methods

Figure 4 shows an example of an LC-MS spectrum for the same sample in urine, plasma, and OF. In total, 215 antihypertensive drugs were prescribed, of which 182 drugs (parent and/or their metabolites) were detected. Using LC-HRMS/MS analysis, most antihypertensive medications were identified in urine, followed by plasma and OF (181 versus 119 versus 88; P<0.001; Figure 5). For all substance classes but alpha-1 blockers, detection rates of LC-HRMS/MS were highest in the urine. The overall detection rates of drugs with acidic functions, such as angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and

Table 1. Patients' Characteristics

Characteristic	Nonadherent Patients		Partially Adherent Patients		Fully Adherent Patients		*P* Value
	Value	N	Value	N	Value	N	
Age, y	64.0±8.7	6	56.2±13.4	13	59.1±13.7	37	0.400
Women, n (%)	5 (83)	6	8 (62)	13	17 (46)	37	0.249*
Body mass index, kg/m^2	26.1±8.2	4	30.0±6.5	11	29.3±6.9	36	0.695
Current smoker, n (%)	2 (33)	6	4 (31)	13	9 (24)	37	0.726*
Type 2 diabetes mellitus, n (%)	1 (17)	6	3 (23)	13	10 (27)	37	0.908*
Coronary artery disease, n (%)	3 (50)	6	2 (15)	13	8 (22)	37	0.242*
Chronic heart failure, n (%)	3 (50)	6	3 (23)	13	14 (38)	37	0.434*
eGFR <60 mL/min per 1.73 m^2, n (%)	0 (0)	6	2 (17)	12	8 (23)	35	0.575*
Office systolic BP, mm Hg	155.5±37.3	6	143.9±24.7	12	142.3±24.7	36	0.666
Office diastolic BP, mm Hg	90.2±22.5	6	90.5±19.1	12	83.1±12.3	36	0.507
24-h Systolic BP, mm Hg	147.0±17.1	5	141.7±14.1	10	128.4±14.4	27	0.012
24-h Diastolic BP, mm Hg	81.6±9.3	5	90.0±9.3	10	77.7±9.2	27	0.009
Heart rate, bpm	80.5±15.6	6	79.6±18.3	12	78.0±11.8	35	0.950

Values are mean±SD or number (percentage). *P* values are given for between-group comparisons. BP indicates blood pressure; bpm, beats per minute; and eGFR, estimated glomerular filtration rate.

*Fisher's exact test. Adherence rates were corrected for single-pill combinations.

thiazides, were low in OF. However, there was no statistical difference in the frequency of detecting calcium channel blockers (P=0.727), β blockers (P=1.000), thiazides (P=0.125), and α-2 agonists (P=0.125) between LC-HRMS/MS analyses of OF and plasma samples. Compared with urine, there was no significant difference in the detection of calcium channel blocker (P=0.063) and β blockers (P=1.000) in OF. Of note, detection rates of drugs in OF were not different in patients with or without concomitant medication, known to possibly (eg, domperidone; P=0.651) or commonly (eg, amiodarone; P=0.702) cause hyposalivation.

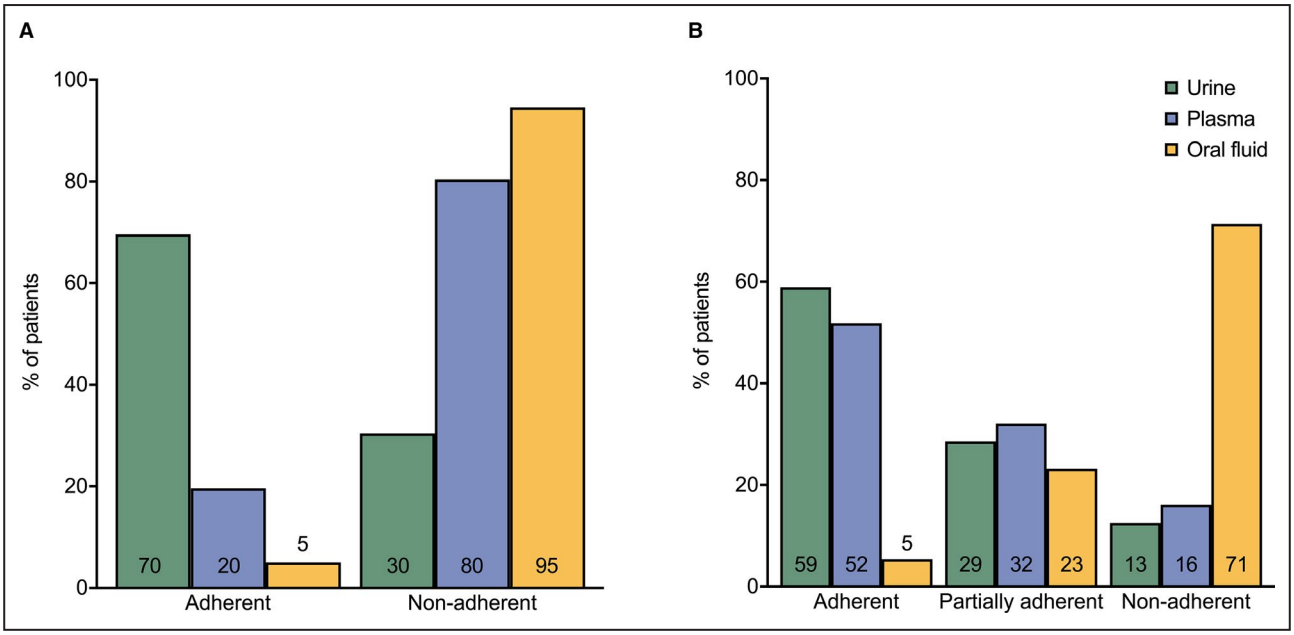

Figure 1. Assessment of adherence rates using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS/MS) in urine, plasma, and oral fluid.

A, Patients were considered "adherent" if ≥80% of the prescribed drugs were detectable in the matrices. **B**, Patients were classified as "adherent" if all prescribed drugs were detected. If 1 or at least 2 of the prescribed drugs were not detectable, the patient was considered to be "partially adherent" and "nonadherent," respectively.

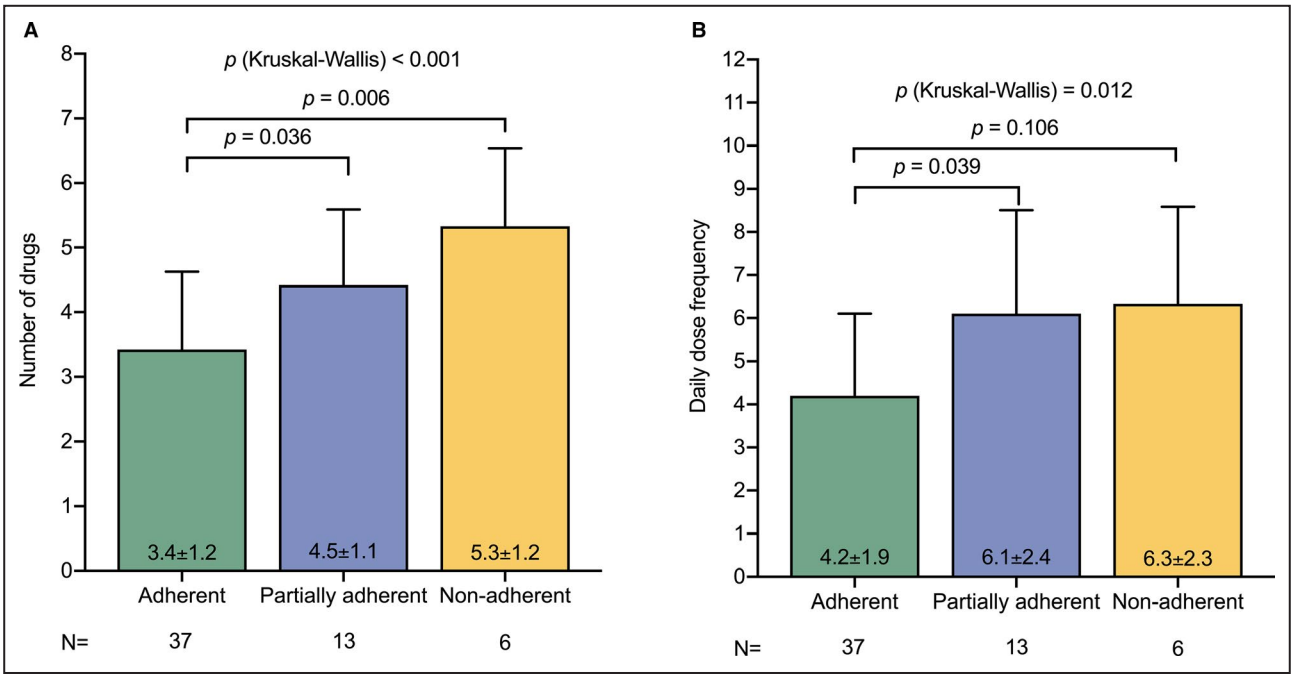

Figure 2. Impact of dosing regimen on adherence.
The impact of the total number of antihypertensive drugs (**A**) and dosing frequency (**B**) on adherence to antihypertensive drugs. Adherence rates were corrected for single-pill combinations. *P* values are given for between-group comparisons.

Furthermore, there were no differences in detection rates in patients with conditions known to impact salivation, such as diabetes mellitus (P=0.626) or chronic kidney disease (estimated glomerular filtration rate <60 mL/min per 1.73 m^2; P=0.059). Interrater agreement was assessed for urine, plasma, and OF samples of 56 patients. For drug detection, Cohen's κ ranged from 0.14 to 1.00 for urine and plasma samples, from −0.14 to 0.87 for urine and OF samples, and from 0.31 to 1.00 for plasma and OF samples (Table 2).

DISCUSSION

Detection rates by LC-HRMS/MS for all substance classes but α-1 blockers were highest in the urine. Antihypertensive drugs without acidic components had similar detection rates in plasma and OF, whereas antihypertensive drugs with acidic functions were poorly detected in OF.

In this study, 59% of the patients were completely (66% after correcting for single-pill combinations)

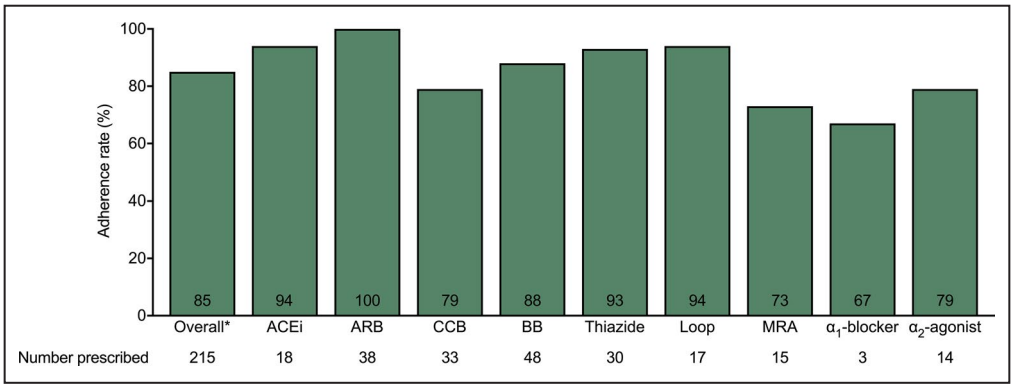

Figure 3. Adherence rates for different substance classes.
Adherence rates were corrected for single-pill combinations. *The overall number of drugs detected includes triamterene. As only one patient was prescribed to triamterene, potassium-sparing diuretics are not explicitly depicted in the figure. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BB, β blocker; CCB, calcium channel blocker; and MRA, mineralocorticoid receptor antagonist.

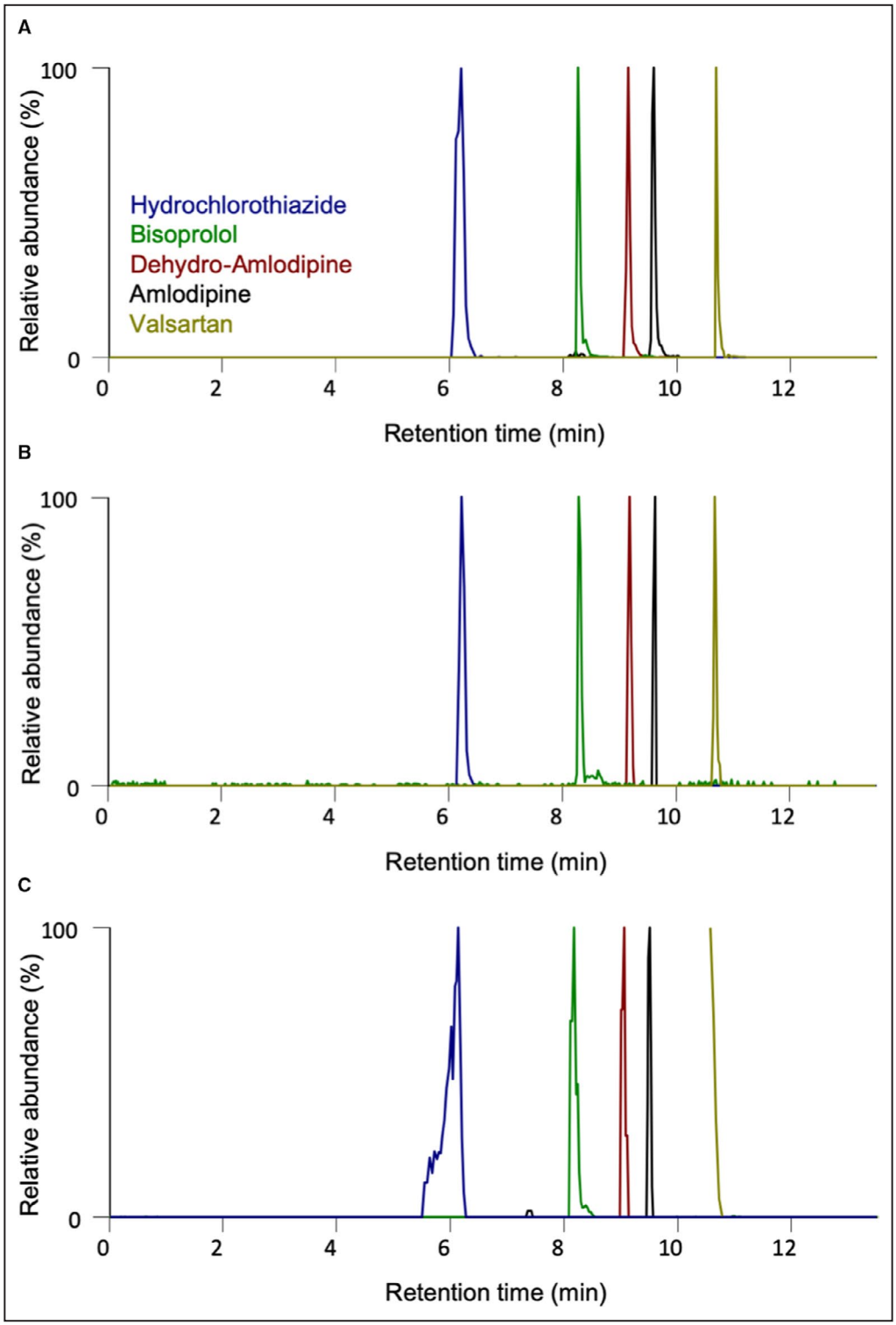

Figure 4. Example of liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS/MS) chromatograms.
The figure shows reconstructed LC-HRMS/MS chromatograms (BCD protonated molecule; AE1E2 deprotonated molecule; E3 fragment ion) of analytes in one patient's urine (**A**), plasma (**B**), and oral fluid (**C**).

adherent to their prescribed antihypertensive medication, whereas 29% (23% after correcting for single-pill combinations) were partially adherent and 13% (11% after correcting for single-pill combinations) were non-adherent. The relatively high rate of poor adherence to antihypertensive medication in this study is in line

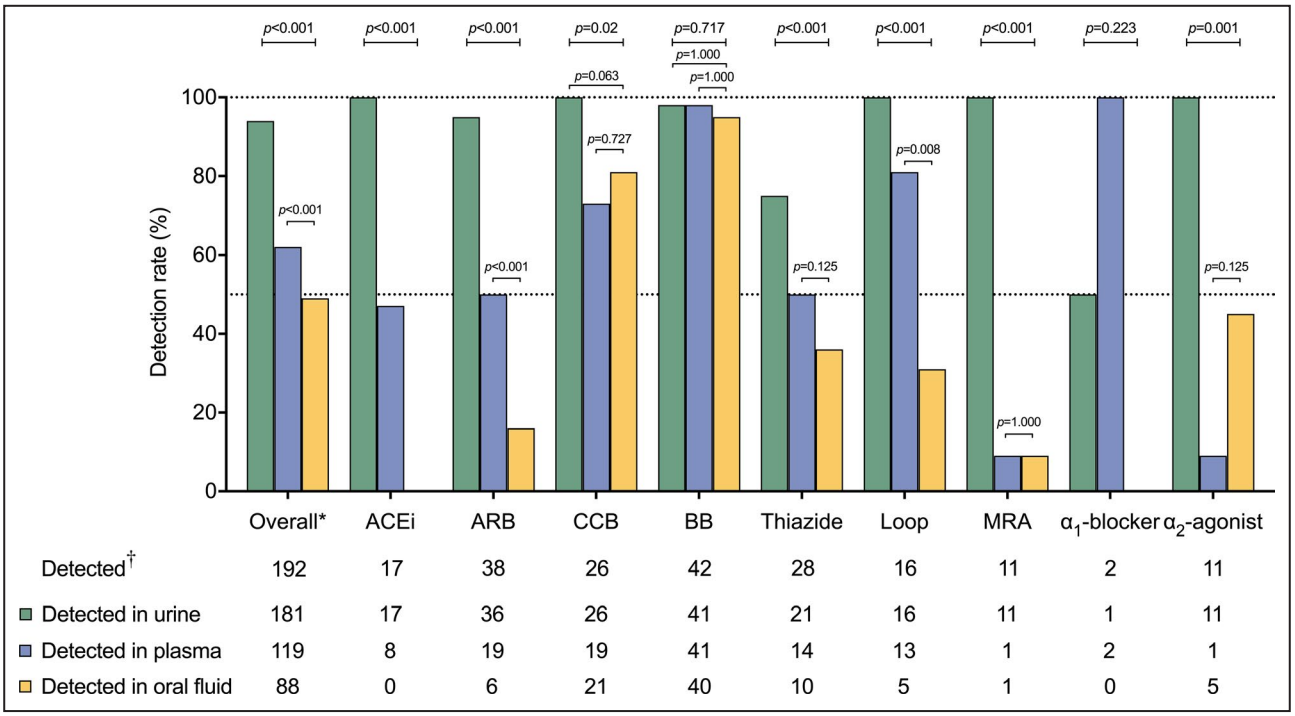

Figure 5. Comparison of detection rates between matrices.
The figure depicts the detection rates of antihypertensive drugs in urine, plasma, and oral fluids. †The number of drugs detected was corrected for single-pill combinations. *The overall number of drugs detected includes triamterene. As only one patient was prescribed to triamterene, potassium-sparing diuretics are not explicitly depicted in the figure. *P* values were calculated for the comparison between all matrices (Cochran's Q test) and between-group comparisons of oral fluid with plasma or urine (McNemar's test). ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BB, β blocker; CCB, calcium channel blocker; and MRA, mineralocorticoid receptor antagonist.

with previous studies using LC-HRMS/MS urine analysis.[18–20] In hypertension, evidence suggests that nonadherence represents an important problem in routine care. Approximately one third of the hypertensive patients do not initiate a new prescription of antihypertensive drugs,[2] and almost half of the patients who were prescribed an antihypertensive medication become nonadherent within 1 year.[16] Nonadherence to the antihypertensive medication has also been shown to be associated with increased cardiovascular risk.[1–4]

The 2018 European Society of Cardiology/European Society of Hypertension guidelines for the management of arterial hypertension[12] have put a strong emphasis on evaluating potential nonadherence as a major cause of insufficient blood pressure control and recommend drug monitoring to improve blood pressure control.[12,21,22] Although toxicological analyses using LC-HRMS/MS of urine and plasma are regarded as the most accurate method for the assessment of adherence, their use in clinical practice is often limited. There is an unmet need to develop an easily applicable, reliable method of drug detection, which can be implemented in the management of patients with hypertension without requiring invasive sampling and sophisticated instrumentation on site.[10–12] In this context, we evaluated the feasibility of adherence monitoring of antihypertensive drugs in OF when compared with urine and plasma. In contrast to venous plasma, sampling of OF is performed noninvasively and, therefore, neither requires medical personnel nor causes discomfort, which may negatively impact patient compliance, especially in the setting of clinical studies. Unlike plasma sampling, OF collection can also easily be done in an "out-of-office" setting as the shipping and processing of OF samples does not require special capabilities.

Using LC-HRMS/MS analysis, most antihypertensive drugs were detected in urine, followed by plasma and OF. Most drugs appear to enter the saliva by passive diffusion. The saliva/plasma ratio of drugs depends, among others, on the concentration gradient of the free (unbound) fraction of the drug in the blood, the pH of OF and blood, the protein binding of the drug, and its acid dissociation constant.[23,24] For acidic and highly protein-binding drugs, the equilibrium generally favors blood.[25,26] Consequently, detection rates for angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and thiazides were low in OF and high in plasma and urine. For calcium channel blockers, however, detection rates in OF were comparable to urine (P=0.063) and plasma (P=0.727). As single-pill combinations comprising

Table 2. Interrater Reliability for Drug Detection Between Urine, Plasma, and OF Samples

Antihypertensive Medication	No. of Prescriptions	Urine and Plasma		Urine and OF		Plasma and OF		Fleiss' K (95% CI)
		% Agreement	κ (95% CI)	% Agreement	κ (95% CI)	% Agreement	κ (95% CI)	
Angiotensin-converting enzyme inhibitor	18	84	0.55 (0.31; 0.79)	70	*	86	*	0.20 (0.05; 0.35)
Angiotensin II receptor blocker	38	73	0.50 (0.19; 0.31)	50	−0.14 (0.03; 0.26)	77	0.38 (0.15; 0.61)	0.27 (0.12; 0.42)
Calcium channel blocker	33	88	0.74 (0.57; 0.91)	91	0.82 (0.67; 0.97)	86	0.69 (0.49; 0.89)	0.75 (0.60; 0.90)
Thiazide	30	88	0.71 (0.52; 0.90)	80	0.53 (0.31; 0.75)	93	0.79 (0.59; 0.98)	0.67 (0.52; 0.82)
Loop diuretic	17	95	0.86 (0.71; 1.01)	80	0.39 (0.14; 0.65)	86	0.49 (0.21; 0.77)	0.59 (0.44; 0.75)
Triamterene	1	100	1.00 (1.00; 1.00)	98	*	98	*	0.49 (0.34; 0.65)
β Blocker	48	96	0.91 (0.79; 1.03)	95	0.87 (0.72; 1.01)	98	0.96 (0.87; 1.04)	0.91 (0.76; 1.06)
Mineralocorticoid receptor antagonist	15	82	0.14 (−0.11; 0.38)	82	0.14 (−0.11; 0.38)	100	1.00 (1.00; 1.00)	0.17 (0.02; 0.32)
α-1 Blocker	3	98	0.66 (0.04; 1.28)	98	*	96	*	0.32 (0.17; 0.47)
α-2 Agonist	14	82	0.14 (−0.11; 0.38)	89	0.57 (0.28; 0.87)	93	0.31 (−0.16; 0.79)	0.35 (0.19; 0.50)

OF indicates oral fluid.
*Computation of Cohen's κ not possible, as the drug was not detected in OF.

a renin-angiotensin system inhibitor in conjunction with a calcium channel blocker or diuretic are recommended as initial therapy in most patients, the detection of one of these substances may be sufficient enough to screen for adherence. Drug adherence monitoring in urine alone may cause false-positive results because the washout period for several antihypertensive drugs in urine lasts longer than multiple half-lives, usually >24 hours.[10] Less is known on the detection time of drugs in OF. However, for drugs of abuse, detection times are far shorter in OF than in urine.[27] Urine may still be the matrix of choice for qualitative analyses, such as adherence screening in general, but an additional LC-HRMS/MS analysis of plasma or OF should be considered to assess recent drug intake. Although the present study analyzed the feasibility of qualitative analyses of antihypertensive drugs, recent data indicate that OF can be used for quantitative analyses similarly.[28,29]

The OF is secreted by 3 pairs of salivary glands (parotid, submandibular, and sublingual glands), the gingival cervicular sulci, and hundreds of minor accessory salivary glands.[30] The sympathetic and parasympathetic nervous system regulates salivation and follows a circadian pattern.[30] A higher salivatory flow rate is associated with higher bicarbonate concentrations and pH.[24,30,31] The pH of OF highly depends on physiological conditions and can range from 6.0 to 8.0.[24,30,31] Under resting conditions, the pH of OFs is stabilized at ≈7.0.[30] When collecting OF, several factors have to be considered, which may influence salivation. These include the patient's diet, emotional states (eg, hunger and anxiety), medications (antidepressants), and medical conditions (cystic fibrosis, diabetes mellitus, and end-stage renal disease).[25,30,32,33] Therefore, sampling of OF should be performed under standardized conditions (at the same daytime and during rest). However, herein, there was no difference in detection rates for patients with or without diabetes mellitus (P=0.626), chronic kidney disease (P=0.059), or drugs known to influence salivation (P>0.651).

LIMITATIONS

This study was primarily designed to assess the feasibility of adherence monitoring of antihypertensive drugs in OF as a noninvasively accessible matrix. In this feasibility study, the sample size was relatively low and was not based on a priori power calculation. The results should be regarded as hypothesis generating and need validation in larger cohorts. As with all cross-sectional studies, measuring drug adherence at one occasion only incompletely reflects the dynamic process of a patient's drug-taking behavior, which encompasses the initiation of a newly

prescribed drug, the implementation of the dosing regimen, and its discontinuation.[34] The study protocol did not require directly observed drug intake. As most drugs enter saliva by passive diffusion, the saliva/plasma ratio does not only depend on the concentration gradient but also the pH of OF, which can be influenced by drugs, medical conditions, and the autonomic nervous system.

CONCLUSIONS

Although more drugs were detectable in urine, this study showed the feasibility of detecting several antihypertensive drugs or their compounds in OF. Therefore, drug adherence testing in OF should further be investigated as a noninvasive approach, not requiring medical personnel, and can be directly observed without interfering with the patient's privacy.

PERSPECTIVES

Assessing drug adherence in hypertensive patients might reduce the number of unnecessarily prescribed drugs. In patients with apparently treatment-resistant hypertension, therapeutic drug monitoring was shown to improve blood pressure control. LC-HRMS/MS of urine and plasma is regarded as the most accurate method for the assessment of adherence. However, there is an unmet need for an easily applicable, cost-effective, and reliable method to evaluate drug adherence, with no need for invasive sampling at best. Drug adherence testing in OF is feasible for several antihypertensive drugs and should further be investigated as a noninvasive approach, which can also easily be done in an "out-of-office" setting.

ARTICLE INFORMATION

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Supplementary Materials

Tables S1–S3

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Supplemental Material

Table S1. Patients' characteristics.

	Non adherent		Adherent		p-value
	Value	**N**	**Value**	**N**	
Age, years	59.9±8.8	13	58.7±14.3	43	0.930
Female, n (%)	8 (62)	13	22 (51)	43	0.545
Body mass index, kg/m^2	26.2±5.9	10	30.0±6.8	41	0.145
Current smoker, n (%)	5 (38)	13	10 (23)	43	0.302
Diabetes mellitus type II, n (%)	2 (15)	13	12 (29)	41	0.475
Coronary heart disease, n (%)	5 (38)	13	8 (20)	41	0.262
Chronic heart failure, n (%)	5 (38)	13	15 (37)	41	1.000
eGFR <60 mL/min/1.73 m^2, n (%)	1 (8)	12	9 (23)	40	0.420
Office systolic BP, mmHg	150.1±31.4	12	142.4±24.4	42	0.466
Office diastolic BP, mmHg	89.8±18.5	12	84.3±14.3	42	0.338
24-hour systolic BP, mmHg	146.1±14.4	9	130.4±15.0	33	0.010
24-hour diastolic BP, mmHg	86.3±9.1	9	79.7±10.4	33	0.075
Heart rate, bpm	82.1±17.2	12	77.7±12.5	41	0.807

Values are mean ± standard deviations (SD) or numbers (%). *p-values for between group comparisons. Adherence rates were corrected for single-pill combinations. BP, blood pressure; eGFR, estimated glomerular filtration rate.

Table S2. Prescribed antihypertensive medication for non-adherent and adherent patients.

	Non adherent		Adherent		p-value
	Value	**N**	**Value**	**N**	
Antihypertensive drugs prescribed, n	4.4±1.3	13	3.7±1.3	43	0.128
Daily doses of antihypertensive drugs, n	5.6±1.9	13	4.7±2.3	43	0.114
Single-pill combinations, n (%)	5 (38)	13	15 (35)	43	1.000
Angiotensin-converting enzyme inhibitor, n (%)	6 (46)	13	12 (28)	43	0.310
Angiotensin II receptor blocker, n (%)	8 (62)	13	28 (65)	43	1.000
Calcium channel blocker, n (%)	9 (69)	13	24 (56)	43	0.525
Thiazide, n (%)	7 (54)	13	23 (53)	43	1.000
Loop diuretic, n (%)	3 (23)	13	14 (33)	43	0.733
Triamterene, n (%)	0 (0)	13	1 (2)	43	1.000
Beta blocker, n (%)	12 (92)	13	36 (84)	43	0.665
Mineralocorticoid receptor antagonist, n (%)	5 (38)	13	10 (23)	43	0.302
Alpha-1 blocker, n (%)	2 (15)	13	1 (2)	43	0.131
Alpha- 2 agonist, n (%)	6 (46)	13	8 (19)	43	0.067

Values are mean ± standard deviations (SD) or numbers (%). p-values for between group comparisons. Adherence rates were corrected for single-pill combinations.

Table S3. Prescribed antihypertensive medication for non-adherent, partially and fully adherent patients.

	Non-adherent		Partially adherent		Fully adherent		p-value*
	Value	**N**	**Value**	**N**	**Value**	**N**	
Antihypertensive drugs prescribed, n	5.3±1.2	6	4.5±1.1	13	3.4±1.2	37	<0.001
Daily doses of antihypertensive drugs, n	6.3±2.3	6	6.1±2.4	13	4.2±1.9	37	0.012
Single-pill combinations, n (%)	3 (50)	6	4 (31)	13	13 (35)	37	0.829†
Angiotensin-converting enzyme inhibitor, n (%)	2 (33)	6	5 (39)	13	11 (30)	37	0.906†
Angiotensin II receptor blocker, n (%)	5 (83)	6	8 (62)	13	23 (62)	37	0.788†
Calcium channel blocker, n (%)	5 (83)	6	8 (62)	13	20 (54)	37	0.500†
Thiazide, n (%)	5 (83)	6	7 (54)	13	18 (49)	37	0.329†
Loop diuretic, n (%)	1 (17)	6	5 (39)	13	11 (30)	37	0.676†
Triamterene, n (%)	0 (0)	6	1 (8)	13	0 (0)	37	0.339†
Beta blocker, n (%)	6 (100)	6	12 (92)	13	30 (81)	37	0.601†
Mineralocorticoid-receptor antagonist, n (%)	3 (50)	6	6 (46)	13	6 (16)	37	0.042†
Alpha-1 blocker, n (%)	2 (33)	6	1 (8)	13	0 (0)	37	0.008†
Alpha-2 agonist, n (%)	4 (67)	6	5 (39)	13	5 (14)	37	0.008†

Values are mean ± standard deviations (SD) or numbers (%). p-values for between group comparisons. †Fisher's exact test. Adherence rates were corrected for single-pill combinations.