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Letter to the Editor

Urine Concentration Does Not Affect Biochemical Testing for Non-adherence

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Abstract

Hypertension is one of the most important modifiable risk factor causing cardiovascular disease. Unfortunately, non-adherence to antihypertensive medications is frequently observed in hypertensive patients and can lead to an increase in morbidity and mortality. Until recently, there was no robust clinical method to objectively diagnose non-adherence. Recently, the detection of medications in urine or blood by mass spectrometry techniques such as liquid chromatography-tandem mass spectrometry (LC–MS-MS) has been accepted as the diagnostic method of choice for the detection of non-adherence. Despite this, it is unclear whether the concentration of urine can affect the detection of medications in urine. Therefore, this study aimed to assess the effect of urine concentration on detection of antihypertensive medications by LC–MS-MS in which urine creatinine is used as an independent marker of urine concentration. Biochemical adherence results for 22 different medications (1,709 prescriptions) in 463 different subjects were converted to an adherence score. The adherence score was defined as the ratio of the total number of subjects in which the drug was detected to the total number of subjects to whom the drug was prescribed. The adherence scores for each medication were correlated with urine creatinine concentration for each medication. Non-adherence was observed in 47.1% of samples with a mean urine creatinine concentration of these samples of 9.4 ± 7.1 mmol/L. There was no significant difference between the urine creatinine concentrations in the detected *vs* non-detected groups for each of the 22 medications. Furthermore, there are no differences in adherence scores across the urine creatinine concentration. This is the first study to demonstrate that urine creatinine concentration does not affect the results of the adherence screening by LC–MS-MS.

Introduction

Hypertension is a global health epidemic that is predicted to affect 1.56 billion adults worldwide by 2025 (1). It is a dominant and modifiable risk for cardiovascular disease with the mainstay of therapy being antihypertensive medications. Drug therapies are well-documented as an effective treatment in the disease but, despite their availability, control of hypertension is only reached in ∼50% of patients (1). The World Health Organization define adherence as 'the extent to which a person's behaviour – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider'.

Adherence is believed to be a major factor controlling chronic and frequently clinically asymptomatic conditions such as hypertension and is considered to be an important factor in achieving optimal blood pressure control.

Until recently, there has been a lack of clinically useful, objective and robust techniques to diagnose non-adherence (2). The development of new biochemical tests employing liquid chromatography-tandem mass spectrometry (LC–MS-MS) has provided a direct and reliable measure of adherence with biochemical non-adherence detected in 30–50% of patients with hypertension (2, 3). Adherence testing using such methods is now an established diagnostic test in patients with hypertension, with our laboratory and others demonstrating the test's clinical utility (4–7). Biochemical testing is now stated as the preferred method to detect non-adherence in the 2018 European Society of Cardiology/European Society of Hypertension Guidelines for the management of hypertension (8).

The urinary biochemical adherence test developed by our laboratory is a qualitative (yes/no) detection method for antihypertensive medications and requires a spot/random urine sample. Spot urine samples (i.e., urine samples that are collected at any single time point) are preferred to 24-h urinary collections in adherence testing. This is because there are several well-documented issues associated with 24-h collections such as under- and over-collection, the convenient nature of spot collections, particularly in the outpatient setting where adherence testing is typically requested, and the fact that the same spot urine sample is collected for other analyses such as urine protein measurement. However, there may be disadvantages of using spot urines such as the variation in dilution effect, sample volume and the rate of urine production. Urine creatinine concentration (UCrt) has frequently been used as an independent marker of urine concentration. It is used to corrects for dilute spot urine samples and correlates well with urine osmolality (9), and It has been used in biochemistry to normalize the concentration of biochemical analytes such as urine albumin and total protein. Furthermore, UCrt is often used to test the integrity of urine samples in toxicology, with low UCrt being considered a marker of dilute urine (10). To further assess the robustness of urine biochemical screening by LC–MS-MS methods, it is important to establish whether urine concentration in adherent patients has an independent impact on the detection of medications or whether dilute urine samples could be potentially falsely interpreted as non-adherence. We decided to use UCrt as an independent marker of urine concentration to investigate its influence on detection rates of medications in patients.

Methods

Urine adherence screening and creatinine analysis

Data from 2011 to 2014 of 463 unselected consecutive subjects whose urine samples were received for the biochemical screening of adherence in the Department of Chemical Pathology and Metabolic Diseases, University Hospitals of Leicester National Health Service (NHS) Trust, were retrospectively analyzed. The data available included subjects' demographics (age, sex and prescribed medications) and adherence test results. The list of currently prescribed medications was obtained by the details provided on the request form. This was assumed to be accurate and up-to-date. Adherence screening was performed as described previously (4). The samples were stored as described previously (11) and we did not anticipate sample instability to be an issue in this study.

Briefly, this involved two sample preparations, which were injected onto the LC–MS-MS system separately. The first sample preparation was a simple 1:10 dilution in deionized water, that is, 100 µL of the sample was added to 1 mL of deionized water and

injected into the LC–MS-MS. This sample preparation technique was used to detect strong polarity antihypertensive medications such as nicorandil and lisinopril. The second sample preparation was used to detect weakly acidic, basic and neutral medications, for instance, diltiazem, bisoprolol and amiloride, by using the supported liquid extraction (SLE) column (Kinesis, Cheshire, UK). The SLE technique involved adding 3 mL of urine with 50 µL of 200 µM hexobarbital (Cerilliant, TX, USA) to the SLE column, and the samples were then eluted by adding 5 mL of 9:1 dichloromethane:2-propanol. The samples were evaporated using nitrogen at 40°C and were then reconstituted using 5% methanol before running on the LC–MS-MS.

LC–MS-MS was performed on MS Agilent Technologies 1290 series High-Pressure Liquid Chromatograph interfaced with an Agilent Technologies 6460 Triple Quadrupole Mass Spectrometer fitted with a Jet Stream electrospray ionization source (Agilent, Santa Clara, CA, USA). Mobile phase A contained 0.1% acetic acid in water and mobile phase B had 0.1% acetic acid in methanol (Optima™ LC–MS grade, Fisher Scientific, Loughborough, UK). The initial conditions were set as follows: 5% B/95% A for 2 min and then raised to 60% B and 40% A at 6 min, followed by 100% B at 9 min. For 1 minute, the gradient was maintained for 1 minute at 100% B and subsequently equilibrated at 5% B for 11 min. The total run time for each sample was 12 minutes. Agilent Technologies Zorbax Eclipse Plus C18 2.1×50 mm column was used for the high-performance liquid chromatography (HPLC) separation. Multiple reaction monitoring modes were performed in the mass spectrometer process and the status of positive ion mode and negative ion mode was applied for each urine sample, which means that each sample was analyzed twice. The lower limits of detection (LODs) for this method range from 1 to 200 ng/mL, as previously described (11). The LODs acted as a decision point between those deemed adherent and non-adherent.

UCrt was measured by the kinetic Jaffé method using an automated spectrophotometric immunoassay on Olympus AU640 (Olympus America Inc., Centre Valley, PA, USA) using Beckman Coulter (Beckman Coulter, Inc., Kraemer Blvd. Brea, CA, USA) (1).

Statistical Analysis

Adherence results were categorized as follows: 1 for total non-adherence (none of the prescribed medications detected), 2 for partial adherence (at least one medication but not all medications detected) and 3 for total adherence (all prescribed medications detected). The adherence score for medication was calculated as the ratio of the total number of subjects in which the drug was detected to the total number of subjects to whom the drug was prescribed ($n = 463$). The median UCrt was calculated for each category and compared using Kruskal–Wallis chi-square analysis. All statistical analyses were performed using SSPS statistics 25.

Results and Discussion

The demographics and clinical characteristics of the cohort are as described in Table I. There was a total of 463 subjects, 52% of subjects were female, and mean age 57.6 ± 14.9 years, who were prescribed in total 1,709 antihypertensive medications of 22 different types. Each subject was prescribed a mean and standard deviation of 3.7 ± 1.5 medications with the mean number and standard deviation of detected medications being 2.5 ± 1.6. Nearly 42% of subjects were partially non-adherent to at least one of the prescribed medications. Fifty-eight percentage of subjects were adherent to all of the prescribed medications. These demographics and findings are comparable to previous adherence studies (4–7).

Table I. Demographic and Clinical Characteristics of Study Population

Characteristic	Value
Number	463
Age (years)	57.6 (14.9)
Female	245 (52)
Number of prescribed medications by class	
A	402 (23.5)
B	253 (14.8)
C	346 (20.2)
D	441 (25.8)
E	267 (15.6)
Total	1709 (100%)
Average number of prescribed medications	3.7 (1.5)
Average number of detected medications	2.5 (1.6)
Any non-adherence	193 (41.7)
Total non-adherence	61 (13.2)
Partial non-adherence	132 (28.5)
Total adherence	270 (58.3)
Urine creatinine (mmol/L)	9.4 (7.1)

Data are counts (percentages) (female and class of medications), means (standard deviations) (age and number of prescribed and detected medications) and medians (interquartile ranges) (creatinine). A, angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor antagonists and renin inhibitors; B, beta-blockers; C, calcium channel antagonists; D, diuretics and E, other antihypertensive medications.
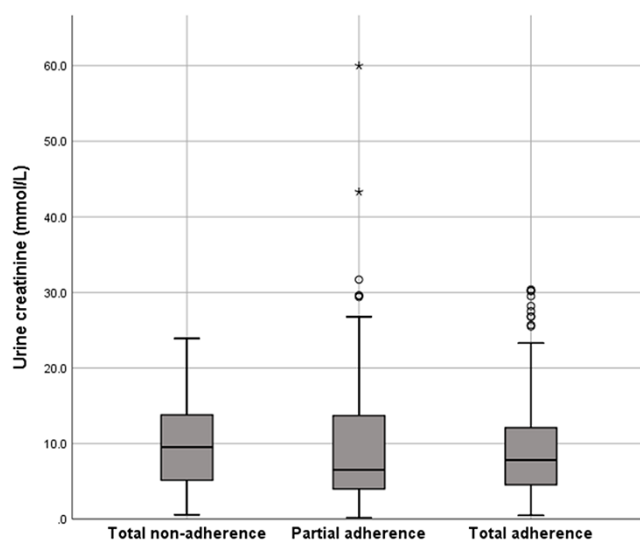

Figure 1. Boxplot of adherence groups and urine creatinine. Interquartile ranges (IQR) and medians are shown with within the box, and range is within 1.5*IQR. Outliers (circles) and extremes (stars) fall within 3*IQR and outside 3*IQR, respectively.

There were no significant differences between the median UCrt values between the detected and non-detected groups for 22 of the antihypertensive drugs (Table II). The range of UCrt values of detected medications was between 3.6 and 11.9 mmol/L. The median UCrt values between non-adherence (9.5 mmol/L, $n = 59$), partial adherence (6.5 mmol/L, $n = 129$) and complete adherence (7.6 mmol/L, $n = 263$) groups are shown in Figure 1. The adherence scores across the UCrt values were not statistically significant ($P = 0.229$) (Figure 2).

Urine and blood samples adherence testing using MS has gained popularity among many clinicians investigating apparent resistant hypertension, but the limitations of such a screening have not been
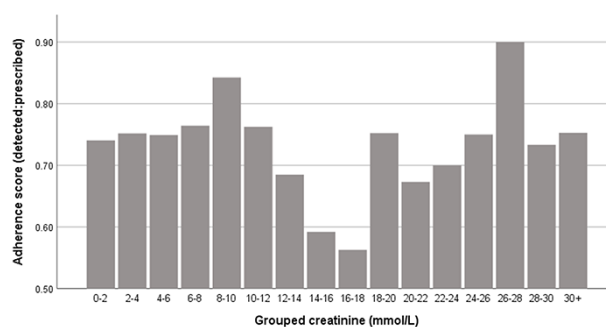

Figure 2. Average adherence scores (medications detected against prescribed) compared to grouped creatinine.

fully explored. Berra et al. (12) suggested that neither urine nor blood could prove conclusively if the drugs are taken regularly between the medical appointments due to the sensitivity of urine and blood samples, especially for those drugs that have higher clearance. The so-called white-coat adherence or the tooth-brush effect is an established phenomenon in which the patient starts taking medication prior to attending a clinical appointment and is a major limitation of biochemical adherence testing that can only assess biochemical adherence at the time of patient sampling. Moreover, pharmacokinetic variability between subjects could affect the concentration of drugs in blood or urine and therefore alter the classification of adherence. Half-life—a parameter commonly used to explain a drugs clearance rate—has been shown to have no effect on a qualitative adherence test (13). However, a difference would likely be seen with a quantitative adherence assessment.

Our group has set out to investigate the reliability of biochemical adherence testing with our previous study demonstrating that there is no correlation between the stability of medications in urine and biochemical adherence screening (11). The present study confirms the robustness of urinary screening for biochemical adherence, answering the question raised regarding urine concentration with no evidence to suggest that dilute urines result in false non-adherence results.

Our study has several strengths—it is a real-world study and consists of a large data set in terms of the total number of subjects being screened for adherence. It also has a varied spread of medications that are commonly used in treating hypertension, and the data set also consists of a wide range of UCrt values.

The major limitation of our study is the use of UCrt as a marker of urine concentration. We acknowledge that using UCrt to normalize urinary biomarkers can potentially lead to underestimation or overestimation of the biomarker excretion rate depending on the clinical context (14); however, the use of 24-h collections in this context is impractical and we believe that most patients being investigated have stable renal function and hence excretion of creatinine should be relatively constant. The use of LOD to determine adherence is a crude measure. Using a drug concentration as quantitative measure could have better discerned the adherent from those partially adherent, that is, detectable but below the therapeutic range that indicates regular dosing (15). A further limitation is noted in the lack of information on the dose that the subjects were prescribed, which may impact the findings; however, for our cohort, it is unlikely that the doses of each medication would be different for those who were adherent and non-adherent. We were also unable to question whether patients self-reported adherence or non-adherence—this information could further validate our results but was not available in this study.

Table II. List of Individual Medications and Differences in Non-adherence Rates

Drug class	Drug		Count	Median UCrt (mmol/L)	P value
A	Candesartan	Non-detected	9	11.8	0.106
		Detected	43	7.9	
	Irbesartan	Non-detected	13	6.8	0.644
		Detected	28	7.8	
	Lisinopril	Non-detected	15	11.5	0.125
		Detected	32	6.7	
	Losartan	Non-detected	18	7.8	0.682
		Detected	74	7.2	
	Perindopril	Non-detected	7	7.8	0.698
		Detected	15	8.3	
	Ramipril	Non-detected	35	7.1	0.251
		Detected	75	9.3	
B	Atenolol	Non-detected	19	7.2	0.217
		Detected	45	5.5	
	Bisoprolol	Non-detected	32	8.0	0.944
		Detected	128	7.2	
C	Amlodipine	Non-detected	66	8.4	0.535
		Detected	178	7.5	
	Diltiazem	Non-detected	8	8.2	1.000
		Detected	23	8.0	
	Felodipine	Non-detected	4	13.1	0.750
		Detected	15	9.7	
	Nifedipine	Non-detected	5	7.9	0.095
		Detected	19	11.9	
D	Amiloride	Non-detected	6	5.5	0.509
		Detected	17	8.1	
	Bendroflumethiazide	Non-detected	31	6.6	0.517
		Detected	45	7.1	
	Bumetanide	Non-detected	5	1.4	0.292
		Detected	12	3.6	
	Furosemide	Non-detected	28	6.8	0.366
		Detected	45	6.0	
	Hydrochlorothiazide	Non-detected	4	14.7	0.080
		Detected	11	5.7	
	Indapamide	Non-detected	44	7.9	0.204
		Detected	72	8.9	
	Spironolactone	Non-detected	53	9.4	0.052
		Detected	60	6.0	
E	Clonidine	Non-detected	6	6.2	0.831
		Detected	4	5.5	
	Doxazosin	Non-detected	66	9.2	0.470
		Detected	132	8.1	
	Moxonidine	Non-detected	12	10.6	0.135
		Detected	26	7.0	

A, angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor antagonists and renin inhibitors; B, beta-blockers; C, calcium channel antagonists; D, diuretics and E, other antihypertensive medications. UCrt (mmol/L) of spot urine sample that was analyzed for biochemical detection of adherence by LC–MS-MS. UCrt data are in medians. $P < 0.05$ was considered as significant.

Conclusion

To the best of our knowledge, this is the first study that demonstrates that UCrt does not affect the detection rates of antihypertensive medications. This provides more evidence of the reliability of using a random urine sample to test for adherence using LC–MS-MS for 22 of the most commonly prescribed antihypertensive medications.

Funding

Conflict of Interests

No conflict of interest.

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