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# Routine urinary detection of antihypertensive drugs for systematic evaluation of adherence to treatment in hypertensive patients

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**Background:** Nonadherence to antihypertensive therapy is an important cause of poor blood pressure control. However, to date, few effective and accurate tools exist to routinely evaluate drug nonadherence.

**Methods:** In this observational study, performed under conditions of routine clinical practice, we included 174 patients (aged  $67 \pm 11$  years) with treated essential hypertension who attended the outpatient hypertension clinic of a university hospital. Adherence to antihypertensive treatment was measured by using ultraperformance liquid chromatography–tandem mass spectrometry in spot urine at the time of clinical appointment and blood pressure measurement. Patients were also asked to report their adherence using a validated questionnaire (four-item Morisky Medication Adherence Scale).

**Results:** The prevalence of directly measured nonadherence by urine drug detection was approximately 10%. Compared with adherent patients, those who did not adhere to their treatment ( $n = 15$ ) had a higher number of antihypertensive pills and drugs ( $P = 0.02$ ), cotreatment with cardiovascular drugs ( $P < 0.05$ ), and total concurrent medications and pills ( $P < 0.01$ ). After adjustment for age, SBP and DBP were higher in nonadherent than adherent group (SBP:  $146 \pm 18$  vs.  $131 \pm 14$ , respectively,  $P < 0.01$ ; and DBP:  $77 \pm 15$  vs.  $73 \pm 9$ , respectively,  $P < 0.01$ ). There was no significant association between four-item Morisky Medication Adherence Scale score and directly measured nonadherence. A longitudinal analysis, performed in a subpopulation of 105 patients after a median follow-up of 11 months, showed that the adherence status remained unchanged in 88% of patients.

**Conclusion:** These results indicate a good adherence to antihypertensive drugs in patients attending the outpatient clinics of a university hospital. They suggest that urine detection of antihypertensive drugs by ultraperformance liquid chromatography–tandem mass spectrometry is an accurate and practical tool for directly monitoring adherence. This direct information is not overlapping with self-report questionnaire.

**Keywords:** adherence, hypertension, urinary drug detection

**Abbreviations:** ACEi, angiotensin-converting enzymes inhibitor; AHT, antihypertensive; ARB, AT1-R blocker; BP, blood pressure; CV, cardiovascular; DENERHTN, Renal Denervation for Hypertension study; IQR, interquartile range; LOQ, limit of quantification; MMAS-4, four-item Morisky Medication Adherence Scale; SE, standard error; UPLC–MS/MS, ultraperformance liquid chromatography–tandem mass spectrometry

## INTRODUCTION

Adherence to antihypertensive medication is a major challenge that clinicians often face in the management of hypertension [1]. Convergent evidences have confirmed that adherence is a crucial key determinant of adequate blood pressure (BP) control [2] and cardiovascular outcomes [3]. However, few effective and accurate tools exist to routinely evaluate drug adherence.

An ideal assessment method should be both accurate and practical in detecting drug nonadherence. In this context, several methods have been suggested for daily evaluation of adherence including patient self-report, pill count, electronic pillboxes, or direct measurement of drug in biological fluids [4]. Recently, measurement of antihypertensive drugs in urine, accurately determined by liquid chromatography–tandem mass spectrometry, has become available [5,6]. The presence of the unchanged drug or its appropriate metabolite in urine certifies that the patient has taken the prescribed drug in the days preceding the analysis. The delay between last drug intake and positive detection in urine depends on the dose and half-life of the drug. This noninvasive approach may give reliable

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information for the physician about his actual patient's adherence based on directly measured parameter.

We recently reported, by using such method as indicator of adherence, an alarming rate (51%) of nonadherence to treatment in a clinical trial including patients with resistant hypertension, the DENERHTN trial [7]. This high level of nonadherence has been observed previously using the same methodology in other observational studies [5,6,8–10] among patients with resistant hypertension. However, very few of the published studies have looked at the reasons for nonadherence and impact of directly measured nonadherence on the BP control. Furthermore, most of them underlined important methodological limitations including the unavailability of indirect methods and single-point time measurements. More importantly, all studies were conducted under a research setting conditions and notably restricted to patients with resistant hypertension. Whether such approach is effective under conditions of routine clinical practice is still debated.

Accordingly, the purpose of the current study was to assess, in a cohort of outpatients with essential hypertension referred to an academic medical center specialty hypertension clinic, the prevalence of drug nonadherence under routine clinical conditions using urine drug detection, the factors associated with nonadherence, and the impact of directly measured nonadherence on BP control.

## METHODS

### Patients

All consecutive outpatients attending the hypertension clinic of one physician (S.L.) at the hypertension department of the Pitié-Salpêtrière university hospital in Paris were asked to participate in the study, from January to April 2015. The main but not the exclusive reason why patients were referred to the clinic was to guide antihypertensive treatment. Eligible patients were those who were 18 years or older, prescribed at least one antihypertensive drug, and had essential hypertension at the baseline study visit and at their previous clinic visit. Patients were ineligible for participation if any of the following criteria were met: severe uncontrolled hypertension (SBP  $\geq$  200 mmHg and/or DBP  $\geq$  130 mmHg), severe reduced kidney function that may influence renal excretion of antihypertensive drugs, and serious physical or psychiatric impairment that limited ability to self-administer antihypertensive medications.

All patients underwent a clinical investigation, including a detailed patient history analysis and physical examination. Variables were collected for each patient on the basis of the interviews, physical examination, and on data drawn from clinical records at the time of visits. These included basic sociodemographic data (sex, age, and BMI); duration of hypertension; and known cardiovascular risk factors, such as smoking, diabetes mellitus (abnormal fasting plasma glucose levels or the current use of insulin or an oral hypoglycemic agent), and dyslipidemia (abnormal fasting plasma cholesterol levels or the current use of lipid-lowering agents). Current antihypertensive medication use (dosing, administration timing, total count of antihypertensive drugs, and pills taken each day), number of antihypertensive fixed-dose combination pills,

antidiabetics, antiplatelet and lipid-lowering drugs were registered, as well as total count of all drugs and pills taken each day.

Baseline visit was considered as the first visit during which antihypertensive medication adherence was evaluated. After this initial visit, a second visit was performed in a subset of 105 patients, during which antihypertensive medication adherence was also monitored. Thus, the 69 other patients should not be considered as lost for follow-up, but only as not having benefited from a second measurement of adherence.

### Office and home blood pressure measurements

During each clinic visit, office BP was measured supine and unattended by a trained nurse blinded to participant adherence status, using the validated electronic device Omron M5 (Omron Healthcare, Kyoto, Japan), according to a standardized and validated procedure used for more than 20 years in our hypertension center. After the patient had been resting quietly for 5 min, office BP was automatically measured three times separated by 1 min, and BP values were averaged. Office BP measurements were recorded prior to urine collection. Controlled office BP was defined as SBP less than 140 mmHg and DBP less than 90 mmHg.

Patients were asked to measure their BP under seated conditions at home during the week preceding the outpatient clinic to exclude a possible white-coat effect or masked hypertension. Three BP measurements were taken in the morning and in the evening by the patient. The average home BP measurement values, over the monitoring period, were used to determine an individual's home BP. Controlled home BP was defined as SBP less than 135 mmHg and DBP less than 85 mmHg, according to the guidelines [1].

### Urine analysis of antihypertensive drugs by ultraperformance liquid chromatography–tandem mass spectrometry

All the patients were unaware that urine detection of antihypertensive drugs could be assessed until they attended our clinic. The urine collection was performed by nurses during an ordinary office visit. Before the urine sampling, patients received clear information about the purpose and the procedures of the study and provided written informed consent. The written informed consent clearly stated that the urine sample was performed to assess antihypertensive drug adherence. No patient refused to participate in the study. Urine collection was performed before the visit with the physician.

The timing of urine collection occurred on average 5 h (2–8 h) after the presumed antihypertensive drug intake. Urine samples were stored immediately at  $-80^{\circ}\text{C}$  until further analysis. The urine analysis of antihypertensive drugs was simple and rapid. Briefly, after 1:100 urine sample dilution in water:acetonitrile (90:10 v/v) and vortex-mixing, the mixture was transferred to vials, and 5  $\mu\text{l}$  was injected into the ultraperformance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) system. The UPLC–MS/MS system was performed using an Acquity UPLC hyphenated to a Xevo TS-Q triple quadrupole Mass Spectrometer (Waters Corp., Milford,

**TABLE 1. Antihypertensive medications and/or their metabolites analyzed in urine by ultra-performance liquid chromatography–tandem mass spectrometry**

No	Molecule (metabolite)	LOQ <sup>a</sup> (pg/ml)	Dilution factor
1	Irbesartan	50	100
2	Valsartan	100	1000
3	Bisoprolol	50	100
4	Atenolol	25	100
5	Nebivolol	25	10
6	Celiprolol	100	100
7	Amlodipine	50	100
8	Manidipine	25	10
9	Spironolactone (canrenone)	100	100
10	Furosemide	500	100
11	Hydrochlorothiazide	250	100
12	Indapamide	50	100
13	Prazosine	10	100
14	Rilmenidine	50	100

<sup>a</sup>LOQ: the limit of quantification was defined as the lowest calibrator with a signal-to-noise ratio of at least 10 for which precision and accuracy were within  $\pm 20\%$ , for each molecule.

Massachusetts, USA). A Waters BEH Acquity UPLC C18 (1.7  $\mu\text{m}$ ,  $2.1 \times 50 \text{ mm}^2$ ) column was used for the chromatographic separation at 40 °C. The mobile phase was operated at a flow rate of 0.4 ml/min in gradient mode using 0.1% acetic acid in water for mobile phase (A) and acetonitrile for mobile phase (B). The initial conditions (B: 10%) were held for 1 min followed by a linear ramp to 90% (B) during 3 min and finally 1 min for period of re-equilibration. Total run time was 5 min. The detection of the compounds was performed via heated electrospray ionization and targeted multiple reactions monitoring mode. Capillary voltage and cone voltage were set at 2 kV and 35 V, respectively. The source temperature was 150 °C. The desolvation gas flow was set to 650 l/h, and the cone gas flow was 50 l/h. Detection of indapamide, furosemide, hydrochlorothiazide, and irbesartan was performed in the negative ESI mode, whereas detection of all other compounds was performed in the positive ESI mode. A total of 14 different antihypertensive drugs or their metabolites were screened simultaneously (Table 1).

Full nonadherence was defined as complete absence of any prescribed antihypertensive medications (or their metabolites when appropriate) in a spot urine sample on UPLC/MS–MS method. Partial nonadherence was defined as the absence of at least one, but not all, prescribed antihypertensive drugs. Full adherence was defined by the presence of all prescribed antihypertensive drugs.

Adherence to angiotensin-converting enzyme inhibitors (ACEis) was determined from spot urine Ac SDKP/creatinine ratio; a threshold of 4 nmol/mmol was used to discriminate between adherent and nonadherent patients, as reported previously [11]. All samples were analyzed blind to the prescribed antihypertensive treatment.

### Self-reported antihypertensive medication adherence (four-item Morisky Medication Adherence Scale)

In addition to the evaluation of adherence by urine drug detection, patients were asked to report their adherence by

a previously validated four-item Morisky Medication Adherence Scale (MMAS-4) [12]. The MMAS-4 questionnaire was administered by the nurse immediately after the urine collection to query adherence to all of antihypertensive medications used since the last visit. Each ‘yes’ reply was scored as 1 and each ‘no’ reply was scored as 0. The questionnaire was thus scored from 0 to 4. Using previously published cut points [12], high, medium, and low MMAS-4 adherence were defined as scores of 0, 1–2, and more than 2, respectively. For the analyses, only patients with high adherence (MMAS score = 0) were considered adherent.

### Enrolled and final cohort

At baseline, out of the 213 patients screened, 10 (4.7%) were ineligible. After exclusion, 203 patients enrolled in the study. Among the 203 enrolled patients, 179 (88%) had data available from the urinary drug detection. Indeed, we did not include 19 (9%) patients receiving only antihypertensive drugs that we could not detect with our UPLC/MS–MS method, two (2%) patients with difficulty to urinate at the clinical appointment, and three (1%) patients with missed urine samples. Among those 179 patients, 174 completed the MMAS-4 questionnaire. Among those 174 patients, 105 completed a second study visit with a mean follow-up of 11 months [interquartile range (IQR): 4–13].

### Statistical analysis

The chi-square or Fisher test was used to compare categorical variables. Continuous variables were examined by using the *t* test or the Mann–Whitney test. We assessed the impact of nonadherence on BP by using multiple linear regression models with BP as dependent quantitative variable; also age and antihypertensive medication adherence (categorized into binary phenotype) as independent parameters. Statistical analysis was performed using NCSS 2007 [Hintze J. (2009), Kaysville, Utah, USA]. Data are presented as mean  $\pm$  SD, that is, mean (SD). Two-sided *P* values less than 0.05 were considered to be significant.

## RESULTS

The baseline clinical characteristics – mean (SD) – of patients are shown in Table 2. Age of the population was 67 (11) years; BMI was 26 (4); women were predominantly included (57%). Diagnosis of hypertension had been established 14 years (10) prior to this analysis. At time of adherence assessment, patients were prescribed 2.6 (1.2) antihypertensive medications (Table 3). Urine drug detection was possible for 2.2 (1.0) of the prescribed antihypertensive drugs. Fifty-two percent of patients had fixed-dose combination tablets. SBP and DBP were most often well controlled at the baseline clinical visit: 133 (16) and 73 (10) mmHg, respectively. A majority (64%) of participants have controlled home BP (SBP < 135 mmHg and DBP < 85 mmHg) at the baseline clinical visit.

According to urine drug detection, 159 patients out of 174 (91%) were deemed to be adherent. The 12 patients (7%) who were partially nonadherent and the three (2%) who were fully nonadherent were gathered in one group of nonadherent patients. Characteristics of patients are given in Tables 2 and 3. Patients not adherent to antihypertensive

**TABLE 2. Adherence and clinical characteristics of study patients at baseline**

	Overall	Directly measured adherence		P value
		Adherent	Nonadherent	
Total number	174	159	15	
Age (years)	67 ± 11	67 ± 11	70 ± 12	0.1567
Female, n (%)	100 (57)	92 (58)	8 (53)	0.7345
Hypertension duration (years)	14 ± 10	14 ± 10	11 ± 9	0.3641
BMI (kg/m <sup>2</sup> )	26 ± 4	25 ± 4	27 ± 4	0.2452
Smoker, n (%)	22 (13)	20 (13)	2 (13)	0.9329
Type II diabetes, n (%)	27 (15)	4 (27)	23 (15)	0.8069
Office SBP (mmHg)	133 ± 16	131 ± 14	146 ± 18	0.0026
Office DBP (mmHg)	73 ± 10	73 ± 9	77 ± 15	0.1309
Not controlled on office BP, n (%)	43 (25)	35 (22)	8 (53)	0.0071
Home SBP (mmHg)	130 ± 12	128 ± 11	142 ± 15	0.0008
Home DBP (mmHg)	76 ± 9	76 ± 8	79 ± 12	0.5205
Not controlled on home BP, n (%) <sup>a</sup>	46 (36)	37 (32)	9 (64)	0.0191
MMAS-4 score at baseline [mean (SD)]	0.23 ± 0.62	0.22 ± 0.60	0.26 ± 0.79	0.8546

MMAS-4, four-item Morisky Medication Adherence Scale.

<sup>a</sup>Home BP: information available for 127 patients.

therapy received a significantly higher number of prescribed antihypertensive drugs as well as a higher number of antihypertensive pills. A significantly higher number of cardiovascular drugs, total number of prescribed drugs, and total number of prescribed pills was observed in patients in the nonadherent group ( $P < 0.05$ ). When comparing different classes of antihypertensive drugs according to the adherence status, a significantly higher number of patients receiving beta-blockers (73 vs. 37%;  $P = 0.006$ ) were observed in the nonadherent group (Table 3).

After adjustment for age, office SBP and DBP, as well as home SBP and DBP were higher in the nonadherent than the adherent group (Table 4). The proportions of patients with uncontrolled office or home BP were significantly greater in the nonadherent than adherent group (Table 2). All other clinical characteristics, such as age, sex, hypertension duration, BMI, smoking, and type II

diabetes, did not differ between adherent and nonadherent patients (Table 2).

Self-reported nonadherence (MMAS score  $> 0$ ) was observed in 27 (15%) patients. Age was significantly lower in the self-reported nonadherent group (Table S1, <http://links.lww.com/HJH/A775>). After adjustment for age, no significant differences in office SBP and DBP as well as in home SBP and DBP were observed between the nonadherent and adherent groups (Table S3, <http://links.lww.com/HJH/A775>). However, the proportion of patients with uncontrolled home BP (home SBP  $> 135$  mmHg or DBP  $> 85$  mmHg) was significantly greater in the nonadherent than adherent group (Table S1, <http://links.lww.com/HJH/A775>). All other clinical characteristics, such as sex, hypertension duration, BMI, smoking, and type II diabetes, did not differ between self-reported adherent and nonadherent patients (Tables S1–S4, <http://links.lww.com/HJH/>

**TABLE 3. Adherence and prescribed therapies of study patients at baseline**

	Overall	Directly measured adherence		P value
		Adherent	Nonadherent	
Total number	174	159	15	
Average no. AHT drugs per patient	2.6 ± 1.2 (1–7)	2.5 ± 1.1	3.6 ± 1.5	0.0060
Average no. of screened AHT per patient <sup>a</sup>	2.2 ± 1.0 (1–5)	2.1 ± 1.0	3.0 ± 1.1	0.0044
Average no AHT fixed-dose combination pills	0.5 ± 0.5 (0–2)	0.5 ± 0.5	0.6 ± 0.6	0.4604
Average no. AHT pills per day	2.3 ± 1.4 (1–9)	2.2 ± 1.3	3.4 ± 2.0	0.0065
Average no. of other CV drugs per patient <sup>b</sup>	0.9 ± 0.9 (0–3)	0.8 ± 0.9	1.4 ± 1.2	0.0446
Average no. other prescribed drugs	1.0 ± 1.4 (0–9)	1.2 ± 1.0	0.9 ± 1.4	0.1039
Average no. of total drugs prescribed	4.6 ± 2.7 (1–15)	4.4 ± 2.6	6.5 ± 3.4	0.0089
Average no. of total pills prescribed	4.7 ± 3.7 (1–24)	4.4 ± 3.5	7.0 ± 4.7	0.0303
ACEi, n (%)	18 (10)	16 (10)	2 (13)	0.6909
ARBs, n (%)	124 (71)	112 (70)	12 (80)	0.4341
Calcium antagonists, n (%)	102 (59)	92 (58)	10 (67)	0.5080
Diuretics, n (%)	104 (60)	92 (58)	12 (80)	0.0946
β-blockers, n (%)	70 (40)	59 (37)	11 (73)	0.0062
Central, n (%)	10 (6)	9 (6)	1 (7)	0.8728
Lipid-lowering agents, n (%)	73 (42)	64 (40)	9 (60)	0.1384
Antiplatelets, n (%)	54 (31)	46 (29)	8 (53)	0.0508
Antidiabetics, n (%)	26 (15)	22 (14)	4 (27)	0.1827

ACEi, angiotensin-converting enzyme inhibitors; AHT, antihypertensive drug; ARB, angiotensin II receptor antagonist; CV, cardiovascular.

<sup>a</sup>Average number of antihypertensive drugs screened out of the total antihypertensive drugs prescribed (Table 1).<sup>b</sup>Cardiovascular drugs included lipid-lowering and/or antiplatelet and/or antidiabetic drugs.

**TABLE 4. Association between blood pressure and adherence to antihypertensive treatment at baseline**

	Overall	Directly measured adherence		Beta (SE) <sup>a</sup>	P value*
		Adherent	Nonadherent		
Total number	174	159	15		
Office SBP (mmHg)	133 ± 16	131 ± 14	146 ± 18	-14 (4.1)	0.0008
Office DBP (mmHg)	73 ± 10	73 ± 9	77 ± 15	-6 (2.5)	0.0289
Home SBP (mmHg)	130 ± 12	128 ± 11	142 ± 15	-13 (3.2)	0.0001
Home DBP (mmHg)	76 ± 9	76 ± 8	79 ± 12	-3 (1.9)	0.0488

Home SBP – information was available for 113 adherents, 14 nonadherent; Home DBP – information available for 111 adherent, 14 nonadherent. BP, blood pressure.

<sup>a</sup>Beta –  $\beta$ -coefficient.

\*P value are all from adjusted linear regression models with BP as dependent quantitative variable and age as well as nonadherence to antihypertensive treatment (categorized into binary phenotype) as independent parameters included in the model.

A775). A significantly higher number of noncardiovascular drugs and lower total drugs were observed in the self-reported nonadherence group ( $P < 0.05$ ) (Table S2, <http://links.lww.com/HJH/A775>).

The MMAS-4 score was not significantly different between adherent and nonadherent patients according to urine drug detection (Table 2). There was no or weak agreement between urine drug detection and MMAS-4 questionnaire [Kappa test = -0.01 (95% confidence interval: -0.15 to 0.12);  $P = 0.80$ ], with 38 of 174 nonconcordant tests. Indeed, as shown in Table S5, <http://links.lww.com/HJH/A775>, 134 (77%) patients were labeled as adherent to treatment by both methods, whereas only two patients (1%) were deemed to be nonadherent by UPLC-MS/MS method and reported actually to have problems in adherence by MMAS-4 questionnaire (MMAS score > 0).

The analysis of the temporal stability of adherence was performed in a subgroup of 105 patients (96 adherent and nine nonadherent) after a median follow-up of 11 months (IQR: 4–13). It revealed that adherence and nonadherence, assessed by urinary detection, remained the same in 92 patients (88%): 90 patients and two patients, respectively, whereas seven nonadherent patients (6.6%) became adherent, and six adherent patients (5.7%) became nonadherent (Tables S5 and S6, <http://links.lww.com/HJH/A775>). The overall nonadherence did not significantly change from baseline to 11 months (8.6% at baseline vs. 7.6% at 11 months, McNemar test,  $P = 0.78$ ).

## DISCUSSION

Nonadherence to antihypertensive medication is now well recognized as a potential cause of noncontrol of BP in treated hypertensive patients [13]. However, few effective and accurate tools exist to routinely evaluate drug nonadherence. Until the development of direct measurement, clinicians have used indirect measures such as self-reports that are known to be less accurate and poorly informative [10]. The main results of the current study are the following: it is possible to directly monitor nonadherence under conditions of routine clinical practice using urinary drug detection by UPLC-MS/MS; the prevalence of drug nonadherence in patients attending the outpatient clinics of a university hospital is low, approximately 10%; compared with adherent patients those who did not adhere to their treatment had higher SBP/DBP, number of antihypertensive pills and drugs, cotreatment with cardiovascular drugs,

and total concurrent medications and pills; information on drug nonadherence obtained with urine detection did not overlap with self-report questionnaire; and the status of adherence or nonadherence, assessed by urinary detection, remained the same in 88% of patients during follow-up.

The low prevalence of drug nonadherence in patients attending the outpatient clinic of a university hospital is not surprising. Indeed, most of these patients regularly attended this outpatient clinic and had controlled BP, either under office or home conditions. This population differs from other populations most often included in clinical studies, such as patients having uncontrolled BP or resistant hypertension, in whom the same methodology reported a high degree of nonadherence often higher than 50% of patients [14]. Taken together, these data suggest that drug intake is associated with BP lowering and underline the necessity for monitoring drug adherence with urine-based detection in patients with apparent resistant hypertension. Of note, even though the majority of our patients ( $\approx 90\%$ ) were deemed to be adherent to their prescribed therapy at baseline, office and home BP were still not controlled in 22 and 32% of adherent patients. These results indicate either that patients considered as fully adherent may not have taken all their medications (i.e. urine detection overestimates the true adherence) or that a full adherence to treatment is not sufficient enough for controlling BP (i.e. the appropriate pharmacological class has not been prescribed and/or other factors contribute to the lack of BP control) [15].

Understanding and accurately capturing factors associated with nonadherence can be useful in targeting subgroups for adherence analysis. The accuracy of urine drug detection allows a more finely grained analysis of adherence behavior. Table 3 shows that compared with adherent patients (using urine drug detection), those who did not adhere to their treatment had higher SBP/DBP, number of antihypertensive pills and drugs, cotreatment with cardiovascular drugs, total concurrent medications and pills, and were significantly more often prescribed beta-blockers. These results are consistent with previous studies [16–20]. Another point of interest is the higher number of cotreatment with cardiovascular drugs (i.e. not antihypertensive drugs), including antiplatelet agents, in nonadherent patients. These data may suggest that nonadherent patients may have more comorbidities. This means that nonadherence to antihypertensive drugs is more common and often more marked if hypertension coexists with serious diseases of cardiovascular

(diabetes, hyperlipidemia, etc.) or noncardiovascular nature. This analysis is also consistent with the high proportion of nonadherence observed in patients with apparent resistant hypertension [5,6,8–10].

The current study shows that information on drug non-adherence obtained with urine detection did not overlap with self-report questionnaire. Indeed MMAS-4 score was not associated with urine drug detection; in addition, 38 out of the 174 tests were nonconcordant (Supplemental data). A smaller number of parameters significantly differed between adherent and nonadherent patients when drug adherence was determined with MMAS-4 score, compared with data observed with urine detection. When drug adherence was determined with MMAS-4 score, patients who did not adhere to their treatment received lower number of total medications than adherent patients. But their office and home SBP/DBP did not differ, as well as the number of antihypertensive medications and pills, and beta-blockers. Thus, the MMAS-4 score has less accuracy than urine drug detection for determining the parameters associated with nonadherence. These data confirm the limited accuracy of self-report questionnaires to predict drug nonadherence that has been previously reported [10,12,21].

Drug nonadherence is a dynamic process, and patients may experience some changes in their behavior in parallel with the progress of treatment. As mentioned previously, recent studies using a similar methodology reported single-time point measurements of antihypertensive drugs in urine samples for adherence evaluation [5–7,10]. In the current study, we attempted to assess adherence both at baseline and after a mean follow-up of 11 months. Interestingly, the status of adherence or nonadherence, assessed by urinary detection, remained the same in 88% of patients during follow-up, and adherence behavior changed only in 13 out of 105 patients at the second study visit. These data indicate that adherence was mostly stable in our population. Whether these results apply to other populations remains to be determined. Indeed, most of our patients (82%) did not change their antihypertensive treatment between the two visits, and most of them had been treated with antihypertensive drugs for several years.

Our study has several limitations. First, the characteristics of the study population may not reflect the general population of a university hospital. Our patients had been followed in a university hospital for several years and thus had benefited from a therapeutic education regarding the need for a long-lasting, well observed antihypertensive treatment and acceptance of its side effects. This may explain the high rate of adherence ( $\approx 90\%$ ) reported in this study. Our population differs from other populations most often included in referral clinics, such as patients having uncontrolled BP or resistant hypertension, in whom the same methodology reported a high degree of nonadherence, often higher than 50%. However, our findings are reassuring and encouraging for hypertension specialists working in European Society of Hypertension (ESH) Excellence centers or hypertension centers and following large cohorts of patients with controlled BP. Second, because of the small number of nonadherent patients, we cannot exclude a chance finding concerning the differences between the nonadherent and adherent groups. Our results

thus need replication and confirmation in larger study sample including patients treated in general practice. Third, we acknowledge that socioeconomic, behavioral, and psychological parameters have not been measured. However, their recording is out of the scope of the current study that should rather be considered as a first-step proof of concept to determine whether it is possible to determine drug adherence in clinical practice by using urinary detection. Fourth, BP was measured under supine and unattended conditions. This method differs from the sitting and attended conditions recommended by the 2013 ESH and of the European Society of Cardiology (ESH-ESC) Guidelines [1]. Fifth, although urine drug detection can serve as an objective and direct measure of adherence, we acknowledge that the UPLC–MS/MS method may not be able to detect all antihypertensive drugs taken by the patients. However, 86% of prescribed antihypertensive drugs were available for analysis, which indicates a good average. Sixth, urine drug detection is sensitive to the ‘tooth-brush effect’, especially if the patient is informed in advance, which may cause ‘false positive’ results. This limitation has been partly overcome by performing an unplanned urine sampling. In addition, it is very unlikely that the patients who had urine sampling could have told to the other patients about the coming test. Indeed, patients waiting for being registered and taken in charge by the nurse and patients who already had the urine sample did not wait in the same waiting room. Finally, like many other methods of screening, the UPLC–MS/MS analysis provides a qualitative ‘yes/no’ answer to a question on presence/absence of antihypertensive medications. Indeed, a given drug of long half-life can be detected in the urine even if the pill has not been taken on the day of the urine sample. Thus, our analysis may overestimate the true rate of adherence.

The current study has several strengths including its prospective design under conditions of routine clinical practice, a relatively large population sample size drawn from a cohort of consecutive patients, and a broad range of data collected (sociodemographic, therapeutic, and clinical data). The assessment of the MMAS-4 score, an indirect method of evaluation of adherence, proved to be an advantage for comparison with a direct method. Furthermore, in addition to its noninvasive nature, the recent improvement in the UPLC–MS/MS systems allowed us to the development of a simple screening method. Indeed, samples were simply diluted and then directly injected onto the analytical system. This offers a rapid sample preparation, short analytical run time (5 min) with minimal staff time for a simultaneous analysis of broadest range of antihypertensive drugs. Finally, by contrast to other methods of measuring drug adherence, urine drug detection has an attractive accuracy/practicality ratio. Therefore, such approach could be easily implemented into a number of clinical laboratories, in which the UPLC–MS/MS technology is available.

The results of urine drug detection are available within few days in our center. They offer an accurate follow-up of drug intake and enable physicians to prevent incorrect interpretation of poor adherence as a lack of response and then make more rational therapeutic decisions in their

daily practice. Upcoming studies investigating whether discussing the results of urine drug detection with patients has a potential to improve adherence and BP control are warranted. The cost-effectiveness analysis of urine drug detection will likely be established in the near future in different settings of hypertensive patients, once the frequency of urine drug testing, the rate of nonresponders to urinary detection, and the magnitude of effect of urine drug detection on BP will be established [22]. Although urine-based detection has a cost, it is obviously much less expensive than escalation of treatment, repeated follow-up, investigations of a secondary cause of hypertension, and therapeutic strategies like renal denervation. Further cost-effectiveness analyses will also clarify which target group of hypertensive patients will benefit from systematic drug adherence detection.

In conclusion, in the current study, we demonstrated that it was possible to directly monitor drug adherence under conditions of routine clinical practice using urinary drug detection by UPLC–MS/MS. The prevalence of drug nonadherence in patients attending the outpatient clinics of a university hospital was low. Higher SBP/DBP, number of antihypertensive pills and drugs, cotreatment with cardiovascular drugs, and total concurrent medications and pills were observed in nonadherent patients, compared with adherent patients. Urinary drug detection by UPLC–MS/MS better detected the above parameters associated with nonadherence than MMAS–4. Finally, the status of adherence or nonadherence, assessed by urinary detection, remained the same in most patients during follow-up.

## ACKNOWLEDGEMENTS

### Conflicts of interest

There are no conflicts of interest.

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## **Reviewer's Summary Evaluation**

### **Reviewer 1**

The authors suggest urine detection of antihypertensive drugs by using ultra performance liquid chromatography-tandem mass spectrometry in spot urine as a simple marker of antihypertensive drug compliance. The compliance was unexpectedly good in their patient material in the university hospital. They did not find a significant association between

mass spectrometry score and directly measured nonadherence.

The measurement of urine concentration of antihypertensive drug is really a simple method for the measurement of compliance but the risk is that the nonadherent patients learn that the measurement is performed during the visits and take antihypertensive medication only during some preceding days before the visit.