Original Article

Resistant hypertension? Assessment of adherence by toxicological urine analysis

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Objective: Uncontrolled hypertension under antihypertensive multidrug regimen is not necessarily always true resistance. Incomplete adherence is one of several possible causes of uncontrolled hypertension. Nonadherence remains largely unrecognized and is falsely interpreted as treatment resistance, as it is difficult to confirm or exclude objectively. This is the first study assessing adherence in patients with apparent resistant hypertension systematically via toxicological urine screening.

Methods: All patients referred from primary care physicians because of uncontrolled hypertension between 2004 and 2011 were analysed. Adherence was assessed in all patients with uncontrolled hypertension despite the concurrent use of at least four antihypertensive agents by using liquid chromatography-mass spectrometry analysis for antihypertensive drugs or their corresponding metabolites in urine.

Results: A total of 375 patients with uncontrolled hypertension were referred. After optimization of drug therapy and exclusion of white coat hypertension, 108 patients met criteria for resistant hypertension. Of those, 15 patients had secondary causes of hypertension and 17 achieved goal blood pressure with quadruple antihypertensive therapy. Of the remaining 76 patients, 40 patients (53%) were found to be nonadherent. Among nonadherent patients, 30% had complete and 70% had incomplete adherence; 85% of the latter had taken less than 50% of drugs prescribed. Lack of adherence was almost evenly distributed between different classes of antihypertensive drugs.

Conclusion: Low adherence was the most common cause of poor blood pressure control in patients with apparent resistant hypertension, being twice as frequent as secondary causes of hypertension. Incomplete adherence was far more common than complete nonadherence; thus, assessment of adherence in patients on multiple drug regime is only reliable when all drugs are included in assessment. Assessing adherence by toxicological urine screening is a useful tool in detecting low adherence, especially in the setting of multidrug regimen as a cause of apparently resistant hypertension.

Keywords: adherence, liquid chromatography-mass spectrometry, resistant hypertension, toxicological screening, urine analysis

Abbreviations: ABPM, 24-h ambulatory blood pressure monitoring; ACEi, angiotensin-converting enzyme-inhibitor; ARB, angiotensin-II receptor blocker; B-blocker, beta-blocker; BP, blood pressure; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; EIC, extracted ion chromatogram; ESH, European Society of Hypertension; ESI+, positive electrospray ionization mode; GC-MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LDL, low-density lipoprotein; LVH, left ventricular hypertrophy; MEMS, Medication Events Monitoring Systems; RH, resistant hypertension; Sympathetic-B, sympathetic-blocker

INTRODUCTION

Hypertension is a common medical disorder affecting as many as 25% of the adult population. Some hypertensive patients appear resistant to combinations of antihypertensive drugs, prompting referral for specialist care, which includes expensive investigations for secondary causes of hypertension in conjunction with an increase in antihypertensive drug therapy as well as nondrug therapy. Resistant hypertension has been defined as blood pressure (BP) remaining above goal despite the concurrent use of antihypertensive medications from at least three drug classes, at full doses, preferably one of them being a diuretic [1,2]. Individuals with controlled BP using at least four drug classes are also considered to have resistant hypertension [3]. Patients with resistant hypertension are of great importance for public health, because they have a higher prevalence of secondary hypertension and target organ damage, higher risk of future cardiovascular and renal events, and require greater healthcare expenditures [2–4]. The true prevalence of resistant hypertension is unknown, but resistant hypertension is thought to be a

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common problem [3]. Moreover, a growing number of patients appear to have treatment resistance [5]. On the basis of data from clinical trials, it has been suggested that 20–35% of hypertensive patients can be considered as having resistant hypertension, but study participants are generally highly selected and may not be representative for the general hypertensive population [3]. Two recent cross-sectional studies from Europe and the USA have reported a similar prevalence of resistant hypertension of at least 12–15% [6–8]. However, uncontrolled hypertension under an antihypertensive multidrug regimen is not necessarily always 'truly' resistant. An estimated 30–50% may have pseudo-resistance, because of several confounders, including white-coat hypertension or lack of BP control secondary to poor medication adherence [3,5].

Incomplete adherence is thought to be one major cause of uncontrolled hypertension and clinicians are often not aware of issues concerning adherence [9,10]. Almost half of patients become nonadherent to antihypertensive therapy within 1 year of initiating therapy [11,12]. To what degree poor adherence is contributing to the apparent treatment resistance is to date unknown. Nonadherence remains largely unrecognized and is falsely interpreted as treatment resistance, because it is difficult to confirm or exclude objectively [8,13]. Not detecting nonadherence is likely to result in wrong measures being taken. In addition, nonadherence with taking antihypertensive medications results in high BP, poor clinical outcome and preventable healthcare costs [10]. Consequently, evaluation of patients with resistant hypertension should be directed towards confirming true treatment resistance, which includes evaluation of adherence [1,2].

Yet, detection and quantitative assessment of nonadherence is a particularly difficult task. Physicians generally tend to overestimate patient's adherence [9]. Studies have demonstrated that clinicians' estimates of nonadherence are very poor, with a positive predictive value of only approximately 30% [14]. In fact, detecting nonadherence in clinical practice is almost impossible [13–15]. There are several ways to monitor compliance, including self-reported compliance, counting pills, rates of refilling prescriptions, electronic monitoring systems as well as direct measurements of drugs or biological markers. All of these methods have their limitations [9]. International guideline committees have called for more aggressive approaches to implement strategies known to improve adherence in antihypertensive treatment, which indeed includes better methods to measure adherence [10]. This is the first study using a liquid chromatography-mass spectrometry (LC-MS) technique to determine the impact of adherence in patients with apparent resistant hypertension and to assess possible factors related to drug therapy adherence.

MATERIALS AND METHODS

Patients

The outpatient clinic of the department of nephrology at the Goethe-University hospital provides service for primary care physicians in evaluation for secondary hypertension and assistance in controlling BP. All patients referred receive a complete history and physical examination including evaluation of causes of secondary hypertension. If no recent 24-h ambulatory BP monitoring (ABPM) is provided by the primary care physicians, ABPM is performed after initial presentation, to exclude white-coat hypertension. Offices BP as well as 24-h ABPM are measured according to international guidelines [2]. In patients with poor BP control, antihypertensive therapy is escalated to achieve goal BP according to international guidelines [2]. All patients return to follow-up visits until BP goal is achieved. During follow-up visits, patients are routinely asked after office BP measurements, whether they have taken their current medication to lower BP as prescribed. In patients not achieving BP goal despite concurrent use of at least four antihypertensive agents after exclusion of secondary causes of hypertension, we routinely assess adherence by toxicological screening for antihypertensive drugs or corresponding metabolites in spot urine as described below. If the patient had confirmed regular drug intake during the last days prior to the clinical visit, a urine sample is obtained with the patient being unaware that adherence is assessed.

Study design

The charts of all new patients referred from their primary care physicians because of uncontrolled hypertension (as stated on referral document – based on the International Classification of Diseases, 10th revision diagnosis) seen after 1 January 2004 who completed work up on hypertension until 30 December 2011 were reviewed. Control of BP was defined as office BP less than 140/90 mmHg in two consecutive office visits and/or ABPM less than 130/80 mmHg. Patients were excluded from this observation if they had incident hypertension, were unable to live independently and had known psychiatric/mental disease or drug dependency.

All patients meeting international criteria for resistant hypertension [2], defined as BP remaining above goal despite the concurrent use of antihypertensive medications from at least three drug classes after exclusion of white-coat hypertension, were further investigated and stratified into three different groups: patients having secondary causes of hypertension, patients achieving goal BP under antihypertensive therapy with four different drugs and patients not achieving BP goal despite concurrent use of at least four antihypertensive agents without secondary causes of hypertension, in whom adherence was assessed because of unexplained resistant hypertension.

Variables were collected for each patient on the basis of the interviews and physical examination at the time of visits and on data drawn from clinical records. These included age, sex, weight, height, BMI (obesity defined as BMI $\geq$30 kg/m^2), duration of hypertension, known cardiovascular risk factors, such as smoking and diabetes mellitus (fasting glucose $\geq$7 mmol/l in at least two occasions or current treatment with antidiabetic agents), dyslipidemia [total serum cholesterol >6.5 mmol/l, low-density lipoprotein (LDL)-cholesterol >4.0 mmol/l, or current treatment with lipid-lowering agents], target organ damage including urinary albumin (microalbuminuria defined as

>20 mg/l) and total protein (proteinuria defined as >300 mg/l), excretion, reduced renal function [estimated glomerular filtration rate (eGFR) Modification of Diet in Renal Disease (MDRD) equation], echocardiography [left ventricular hypertrophy (LVH) defined as LVMI men M≥125 g/m^2, women ≥110 g/m^2] as well as known clinical cardiovascular disease (coronary heart disease, cerebrovascular disease, peripheral occlusive disease). Additional details about antihypertensive treatment (class and doses of drugs, dosing time, total count of antihypertensive drugs taken per day) were collected.

Analysis of urine samples for antihypertensive drugs using high-performance liquid chromatography-mass spectrometry

Urine (0.5 ml) was mixed with 100 μl of saturated aqueous sodium sulfate solution and 100 μl of internal standards mixture (1 ng/μl in acetonitrile of various deuterated analogues of medical drugs and drugs of abuse, c.f. [16]). For extraction of neutral and acidic compounds, 20 μl of formic acid was added to adjust the pH to 2.5. The extraction was performed by intensive shaking for 1 min with 1 ml of ethyl acetate, centrifugation for 10 min at 16 000*g* and transferring the organic phase to a clean vial. For extraction of basic compounds, the aqueous phase was alkalinized with 60 μl of ammonia (25%) and 90 μl of 1 mol/l diammonium hydrogen phosphate (pH 9.5), which resulted in a pH of 8.5. The mixture was again extracted for 1 min using 1-chlorobutane/diethylether (1 : 1, v/v), and after centrifugation, the organic phase was transferred to and combined with the first extract. After evaporation at 60°C, the dry residue was redissolved in 100 μl acetonitrile/water (80 : 20, v/v) containing 0.1% formic acid of which 2 μl was analysed. The analysis was performed using an Agilent (Waldbronn, Germany) 1100 series liquid chromatograph interfaced to an Agilent 1100 series oa-TOF system operated in electrospray ionization mode (ESI). ESI source and mass spectrometry parameters were set according to the recommendations of the supplier except for nebulizer pressure (45 psig), capillary voltage (3000 V) and drying gas flow (10 l/min at 350°C), which were set according to the flow rate of 0.4 ml/min. The fragmentor voltage was set to 125 V. Data acquisition was performed in a mass range from m/z 101 to 1100 with simultaneous internal mass calibration in each recorded spectrum (system reference mixture supplied by the Agilent dual-sprayer interface). Chromatographic separation was achieved on a 100 × 2.0 mm Polaris C18-Ether 3 μm column (Varian, Darmstadt, Germany) at 50°C. For hydrochlorothiazide, furosemide and torasemide, a gradient of acetonitrile/0.05% acetic acid (5% for 0.5 min, increasing to 75% during 6 min, cleaning at 100% for 2.1 min, re-equilibration to 5% for 3.7 min) over a total run time of 12 min in negative ESI was used, and all other compounds were analysed in positive ESI mode using a gradient of acetonitrile/0.1% formic acid (5% for 0.5 min, increase to 75% during 5.5 min, cleaning at 100% for 2.1 min, re-equilibration to 5% for 3.7 min) over a total run time of 12 min. Identification was achieved on the basis of retention time in comparison to a solution containing reference substance of all target compounds (1 ng/μl) and accurate masses of the protonated molecular ion with at least one isotope exhibiting deviations of less than 10 ppm from the theoretical mass.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR) or as proportions as appropriate. Continuous and categorical variables were compared for univariate analysis between groups using the *t*-test or Mann–Whitney *U*-test and Fisher exact test, respectively. All *P* values reported are two-sided. Statistical significance was assumed when the *P* value was less than 0.05.

RESULTS

Study cohort

Between 1 January 2004 and 30 December 2011, a total of 375 patients – meeting inclusion criteria – were referred from their primary care physicians because of uncontrolled hypertension to the outpatient clinic of the Department of Nephrology at the Goethe-University Hospital, Frankfurt. Of these, eight patients (2.1%) did not return for follow-up visits and were lost to follow-up. Therefore, 367 cases were investigated. Of these patients, uncontrolled BP was attributable to white-coat hypertension in nine patients (2.5%); another 250 patients (68.1%) had their BP controlled with three or less antihypertensive agents largely from regimen optimization and intensification, including proper use of diuretics. Consecutively, 108 patients (29.4%) met international guidelines criteria for resistant hypertension [2].

Among these patients with apparent resistant hypertension, 15 patients (13.9%) were found to have secondary causes of hypertension and received specific therapy of the underlying disease. Another 17 patients (15.7%) achieved excellent BP control under quadruple antihypertensive therapy as assessed by ABPM. Thus, 76 patients showed poor blood control despite prescription of at least four antihypertensive drugs. In these cases of unexplained

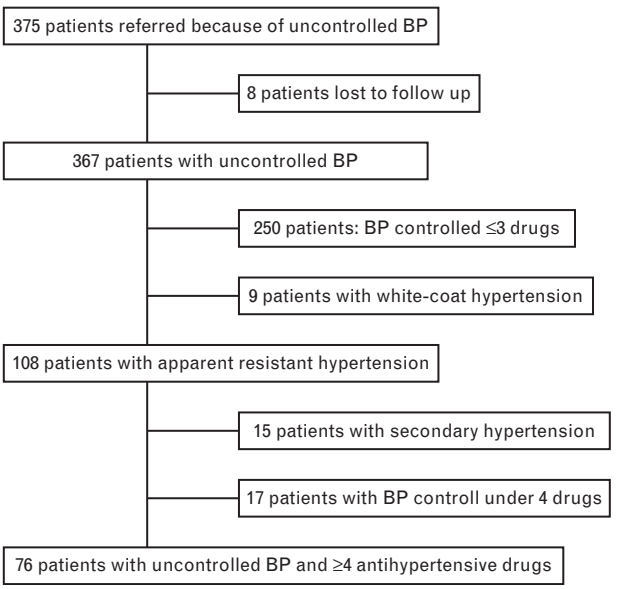

FIGURE 1 Referral and reasons for inclusion or exclusion.

apparent resistant hypertension, adherence was assessed (Fig. 1 Referral and reasons for study inclusion or exclusion).

Patients' characteristics

Clinical characteristics of patients with unexplained apparent resistant hypertension under at least four antihypertensive drugs are summarized in Table 1. Patients were all white and predominantly men (57.9%); median age was 58 years (IQR: 51–69 years). Diagnosis of hypertension had been established several years prior to referral to our centre (median 12 years, IQR: 5–22 years), but most patients had hypertension grade II or III at initial presentation. The majority of patients were obese (92.1% of patients had a BMI $\geq$30 kg/m^2) and comorbidities, such as cardiovascular disease or diabetes mellitus as well as target organ damage, including renal dysfunction, were frequently found.

Antihypertensive therapy of patients at time of adherence assessment is summarized in Table 2. A median of five drugs (IQR: 4–6) was prescribed per patient. Most patients had fixed-dose combination tablets prescribed (71.1%). Diuretics had been prescribed in all patients. Most patients were on angiotensin-converting enzyme inhibitor (ACEi) or angiotensin-II receptor blocker (ARB) therapy, with some patients receiving both for BP control. Beta-blockers and calcium channel blocker were prescribed in most patients, reflecting current therapeutic guidelines.

Results of toxicological urine screening for antihypertensive drugs or metabolites

In the 76 patients, cumulatively 388 antihypertensive drugs were prescribed. Out of these 388 drugs, LC-MS analysis was performed for 368 drugs, as to date, we are not able to detect lercanidipine and nitrates by the LC-MS method (17 patients had lercanidipine and three had nitrates prescribed for BP therapy). Estimations upon adherence versus nonadherence as well as degree of nonadherence were thus calculated on the basis of drugs in which assessment by LC-MS was possible. Thirty-six patients (47.4%) were adherent to therapy (all antihypertensive drugs prescribed were detected in urine), whereas 40 patients (52.6%) showed nonadherence as assessed by LC-MS. Examples of typical chromatographic mass spectrometric data in such cases are illustrated in Figure 2.

Among the 40 nonadherent patients, 12 (30%) had complete nonadherence, as none of the drugs prescribed was detected, and 28 (70%) had incomplete adherence: seveb patients (17.5%) had taken less than 25% of drugs prescribed, 16 patients (40%) had taken 26–50% of drugs prescribed, three patients (7.5%) had taken 51–75% of drugs prescribed and two patients (5.0%) had taken more than 75% of drugs prescribed (Fig. 3).

When comparing adherence with different classes of antihypertensive drugs, we found that rates of nonadherence were comparable among drug classes ranging from 51% (vasodilators) to 77.6% (beta-blocker) (Fig. 4).

LC-MS analysis was performed only when patients had previously confirmed regular drug intake during the last days prior to clinical visit. After being informed about the results of adherence analysis, 87.5% of patients with nonadherence assessed by LC-MS analysis attested – in contrast to their initial statement – not having taken their medication, at least not regularly.

TABLE 1. Patients' characteristics

	N = 76
Age (years)	58 (51–69)
Hypertension – *n* (%)	
Grade I	20 (26.3%)
Grade II	31 (40.8%)
Grade III	25 (32.9%)
Hypertension since (years)	12 (5–22)
Male – *n* (%)	44 (57.9%)
BMI >30 kg/m^2, *n* (%)	70 (92.1%)
Smoker, *n* (%)	32 (42.1%)
Family history of hypertension, *n* (%)	66 (86.8%)
Concomitant disease and/or target organ damage, *n* (%)	69 (90.8%)
Diabetes mellitus type II	24 (31.6%)
Dyslipidemia, *n* (%)	57 (75.0%)
Albuminuria (30–300 mg/day)	6 (7.9%)
Proteinuria (>300 mg/day)	21 (27.6)
eGFR (45–60 ml/min)	7 (9.2%)
Left ventricular hypertrophy	66 (86.8%)
Coronary artery disease	6 (7.9%)
Cerebrovascular disease	6 (7.9%)
Peripheral occlusive disease	3 (3.9%)

Variables are expressed as median and interquartile range (IQR) or as proportions as appropriate. eGFR, estimated glomerular filtration rate.

TABLE 2. Antihypertensive therapy at the time of adherence assessment

	N = 76
No. of antihypertensive drugs per patient	5 (4–6)
Antihypertensive tablets per day	6 (5–8)
Fixed-dose combination tablet prescribed, *n* (%)	54 (71.1%)
Antihypertensive drug prescribed, *n* (%)	
ACE inhibitor	39 (51.3%)
Angiotensin II receptor blocker	54 (71.1%)
Renin inhibitor	5 (6.6%)
Beta-blocker	67 (88.2%)
Calcium channel blocker	57 (75.0%)
Diuretic	76 (100%)
Sympathetic blockers	41 (53.9%)
Other vasodilators	30 (26.3%)

Variables are expressed as median and interquartile range (IQR) or as proportions as appropriate. ACE, angiotensin-converting enzyme.

Comparison between adherent and nonadherent patients

Patients being not adherent to antihypertensive therapy had significantly higher SBP and DBP levels as well as higher heart rate. All other clinical characteristics, such as age, sex, therapeutic parameters, lifestyle factors or comorbid disease state, did not differ between adherent and nonadherent patients (Table 3).

DISCUSSION

Poor adherence with drug taking is thought to be one of the major factors contributing to apparent treatment-resistant hypertension, but is difficult to assess in daily clinical practice [8,9,17]. Estimated prevalence of adherence is

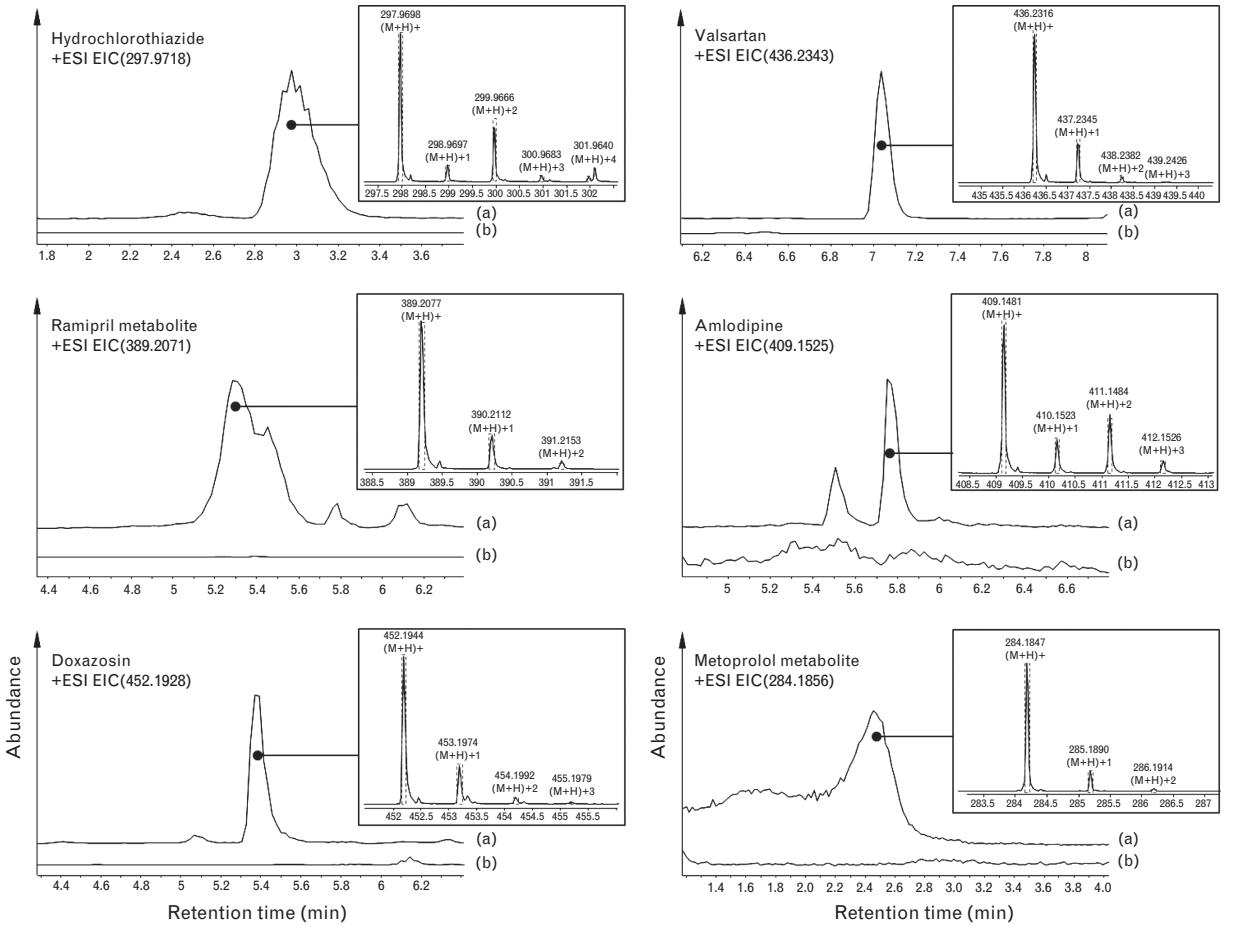

FIGURE 2 Analytical data of six antihypertensive drugs in urine samples of compliant patients (a) and of patients without the respective medication (b, blank urine). Shown are the extracted ion chromatograms (EIC) of the protonated molecular ions of the drugs in the positive electrospray ionization mode (ESI+). The inserts are the mass spectra with the theoretical abundances of the isotopes indicated by dashed boxes.

varying largely between 40 and 90% depending on the population and the method used [9,12,15,17–20]. A recent study [7] found that 70% of patients with apparent resistant hypertension on at least three medications had uncontrolled BP. This frequently encountered resistance to a combination of antihypertensive agents in a given hypertensive patient always raises the difficult question of inadequate pharmacological regimen versus inadequate adherence.

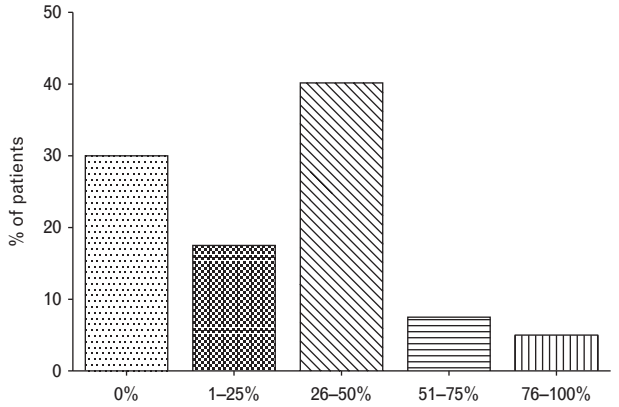

FIGURE 3 Percentage of prescribed drugs taken by nonadherent patients.

After excluding common confounders in the office diagnosis of resistant hypertension, for example therapeutic inertia and white coat hypertension, we found 108 (29.4%) out of 367 patients to have apparent resistant hypertension according to international guidelines [2]. This finding is in accordance with previous observations, indicating that underuse of adequate drug therapy is far more often than true resistance [7,19,21,22].

The majority of patients with apparent resistant hypertension were obese, and patients exhibited a high burden of comorbidities, such as cardiovascular disease, diabetes mellitus and renal dysfunction, which is concordant with previous observations [4,5,7,19,23,24]. Resistant hypertension was attributable to secondary causes in 13.8% of patients and another 15.7% achieved goal BP under quadruple antihypertensive therapy. The present study attempted to assess the adherence in all the remaining patients who had uncontrolled BP despite the concurrent use of at least four antihypertensive drugs from different classes after exclusion of secondary forms of hypertension.

Toxicological urine screenings were used as an objective technique to assess drug intake in these cases of apparent resistant hypertension. Systematic development

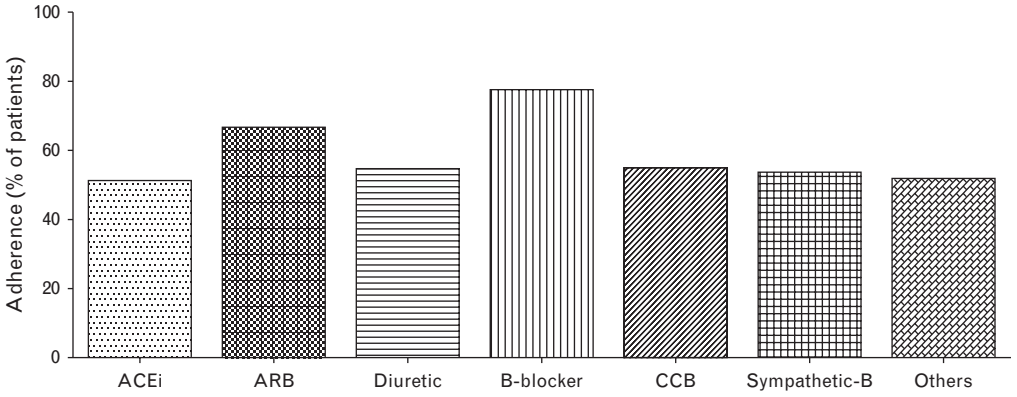

FIGURE 4 Adherence to therapy in different drug classes. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; B-blocker, beta-blocker; CCB, calcium channel blocker; sympathetic-B, sympathetic blockers.

of analytical procedures for a general toxicological screening in urine, first using gas chromatography-mass spectrometry (GC-MS), has evolved since the 1980s (e.g. [25,26]). The recent improvement of LC-MS instrumentation has led to the development of analytical procedures for the detection of various drug classes [27,28] including antihypertensive drugs (e.g. [29,30]). To our knowledge, this is the first study using toxicological urine screening with LC-MS in a clinical setting to assess adherence systematically. As all patients visited the outpatient clinic in the morning, all urine samples were obtained within a maximum of 5 h after the expected time of taking the regular morning doses of their medication. Therefore, sufficient amounts of all antihypertensive drugs should have been excreted in urine, rendering them detectable using a screening procedure that has been used routinely in clinical and forensic toxicology [16].

Toxicological urine screening revealed nonadherence to the prescribed drug regimen in more than 50% of patients. Of those patients being nonadherent, 30% were completely nonadherent and 70% had incomplete adherence to antihypertensive therapy. Of note, 85% of nonadherent patients had taken less than 50% of antihypertensive drugs prescribed. After being confronted with the results of the toxicological urine analysis indicating nonadherence, 87.5% of these patients admitted not having taken their medication, at least not regularly. Taken together with those patients found to be adherent, adherence assessment using toxicological urine screening demonstrated a rather high accuracy of 93.4% (adherence status correctly diagnosed in 71 out of 76 patients). Apart from higher BP levels and elevated heart rate, adherent and nonadherent patients were merely indistinguishable with regard to clinical characteristics, supporting the notion that clinical approaches such as physician's impression, patient's interviews or pill counts overestimate adherence and are thus unreliable, as they cannot finally confirm that medication has been taken [14,15,20,31].

Similar proportions of nonadherence were found in a recent study [17] in patients with apparent resistant hypertension using supervised drug administration followed by BP monitoring in a clinical environment with ABPM. Moreover, studies using Medication Events Monitoring Systems (MEMS) found that almost half of patients become nonadherent to antihypertensive therapy within 1 year after initiation of therapy [11,12]. In contrast to these observations, other studies using MEMS found abundantly higher rates of adherence [20,22,32]. This discrepancy might be attributable to several reasons. First, overt monitoring of adherence itself has the – beneficial – side effect to improve

TABLE 3. Comparison between adherent and nonadherent patients

	Adherent ($n = 36$)	Nonadherent ($n = 40$)	*P*
Age (years)	60 (53–69)	58 (49–67)	0.147
Male, *n* (%)	24 (66.7%)	20 (50%)	0.168
Hypertension since (years)	15 (7–23)	10 (5–21)	0.113
SBP (mmHg)	166 (151–177)	175 (163–201)	0.011
DBP (mmHg)	95 (84–100)	101 (90–101)	0.023
Heart rate	67 (61–76)	77 (65–87)	0.019
Antihypertensive tablets per day	6 (5–8)	7 (5–9)	0.102
BMI	30 (28–35)	31 (28–36)	0.847
Smoker, *n* (%)	17 (47.2%)	15 (37.5%)	0.487
Family history of hypertension, *n* (%)	32 (80.0%)	34 (94.4%)	0.740
Concomitant disease or target organ damage, *n* (%)	31 (86.1%)	38 (95.0%)	0.246
Antihypertensive tablets per day	6 (5–8)	7 (5–9)	0.102
Fixed-dose combination, *n* (%)	26 (72.2%)	28 (70.0%)	1.000

Variables are expressed as median and inter quartile range (IQR) or as proportions as appropriate.

BP [20,32]. Second, patient's consent is necessary when using MEMS, and patients participating in clinical trials are generally more motivated or willing to achieve target BP [3,32]. Third, assessment of adherence by MEMS is impractical in patients on multiple drug regimens and therefore frequently not performed for all pills of the regimen [32]. We found that 70% of nonadherent patients had incomplete adherence, taking at least some of the drugs prescribed. Hence, the commonly practiced approach to measure drug intake of one specific drug within a multiple regimen as an indicator for adherence may substantially underestimate adherence to therapy, irrespective of the technique used [18,32,33].

In our patients, nonadherence was almost evenly distributed when comparing different classes of antihypertensive drugs. This finding stands in contrast to numerous literature reports both from clinical trials and observational studies describing an impact of antihypertensive drug class on adherence. For instance, a recent meta-analysis found that adherence was lower in diuretics and beta-blockers than in ARBs and ACEIs [34]. However, findings from that analysis cannot be generalized to patients who are already on at least two antihypertensive drugs. Each of the drug classes is associated with distinct side effects that may lead to discontinuation of drug intake, for example higher urinary frequency and erectile dysfunction when using diuretics [35]. But for patients on multiple drug regimens, it is merely impossible to discriminate side effects between specific drugs. In addition, fixed-dose combinations were prescribed in more than 70% of patients. Therefore, the discontinuation of one drug because of experienced side effects inevitably leads to the discontinuation of one or more additional drugs. This is underlined by our observation, that beta-blockers, which are rarely used in fixed-dose combinations, had the best level of adherence. Moreover, several reasons apart from distinct side effects influence adherence, such as copayments, variations in physicians' perceptions or patients' belief in benefit of medication [36–38]. Alternatively, as 30% of nonadherent patients in our study did not take any of the drugs prescribed, this might have diminished differences in adherence regarding different drug classes. The opposite way around: no difference in adherence between drug classes was also observed in a study in which proportions of adherence exceeded 90% [20].

Apart from the already discussed, this study has limitations. Although measuring blood or urinary concentrations of drugs prescribed is the only way to ascertain that a drug has been taken, it can be applied only at appointments. As drug intake in our study was measured qualitatively, but not quantitatively, we were unable to affirm that adequate drug doses were taken at the right time, especially in drugs with long half-life [39]. Patients commonly improve their medication-taking behaviour in the 5 days before and after an appointment with the healthcare provider, whereas it declines between clinical visits ('white-coat adherence') [9,40]. Thus, by toxicological urine screening, prevalence of adherence might have been overestimated in our study, as it fails to detect poor long-term adherence. However, in most patients, adherence and persistence are intrinsically linked [10,12]. At least from a more clinical perspective, it appears unlikely that patients not taking antihypertensive drugs on a regular basis can start a multiple drug regimen of at least four drugs and still present with abundantly elevated BP at clinical visit.

Even though our study population was representative and the number of patients receiving at least four antihypertensive agents was comparable with other observations, even in large population studies, it is a single-centre study [7,19]. There are various reasons associated with nonadherence beyond patient and provider levels, for example access to care, copayments, healthcare system [15,41,42]. Thus, our results cannot be broadly generalized to other populations.

These issues notwithstanding, there are potential cost implications of our findings. Within the German healthcare system, the price invoiced for LC-MS measurement is approximately €60 (independent of the number of drugs assessed), which – based on our calculations – roughly covers all costs of the technique (including staff and so on). Although measuring drug intake by LC-MS appears costly, several considerations should be taken into account.

Without any objective measurement of drug adherence, the most common clinical response to apparent resistant hypertension is enhancing therapy, which apart from further evaluations and intensification of drug therapy nowadays may include new invasive measures for BP control. These options are also associated with additional costs.

First, the prescription of one or two additional drugs in a given patient with apparent resistant hypertension for a period of not more than 6–12 weeks will result in a similar increase in healthcare cost compared with those of LC-MS (analysis based on data on current annual average drug costs) [43]. In the situation of low adherence, escalating the prescribed regimen will be unlikely to solve the problem – with leastwise comparable costs – as increased complexity of the regimen and increased number of daily doses have been shown to further reduce drug adherence and persistence with the treatment [15]. Second, when physicians decide for new invasive measures to control BP in these patients, associated costs are tremendously higher, with direct interventional costs of at least €4000 for catheter-based renal denervation and more than €20 000 for implantable carotid body stimulator [44,45]. Assessment of adherence by LC-MS might be highly cost-effective in this setting. For example, if 100 patients would undergo catheter-based renal denervation because of apparent resistant hypertension, this would result in total healthcare expenditures exceeding €400 000. Given that urine toxicological analysis by LC-MS (additional costs of €60 per patient, cumulative costs €6000) is done prior to intervention and, based on our data, approximately 50% of patients were found to be nonadherent – thus having no indication for renal denervation according to the current European Society of Hypertension position article [46] – this would result in savings of €200 000 for the healthcare system.

Third, in patients with apparent resistant hypertension, clinical investigations looking for a secondary form of hypertension will be conducted at an absolute minimum

cost of €250. As nonadherence is far more common than true resistance due to secondary causes, LC-MS technique can be cost-effective, especially prior to more expensive investigations such as renal angiography.

Lastly, nonadherence *per se* has a cost, as it decreases cost-effectiveness of interventions, results in poor clinical outcomes and preventable healthcare costs [10,47,48]. But nonadherence is potentially remediable by enhanced patient involvement, support and education [32,49]. Detecting nonadherence is thus providing an opportunity for intervention, which is in this case not escalation of therapy, in an otherwise possibly fatal disease. In addition, overt monitoring of adherence itself improves adherence [20,32]. When comparing costs of LC-MS with other methods used to improve adherence, it is within the lower cost range [50].

Taken together, we found low adherence as the most common cause of poor BP control in patients with apparent resistant hypertension. Lack of adherence was observed twice as frequently as secondary causes of hypertension. Incomplete adherence was far more common than complete nonadherence; thus, assessment of adherence in patients on multiple drug regime is only reliable when all drugs are included in assessment.

These findings remind us that it is important for clinicians to pay more attention to the issue of adherence. Assessing adherence by toxicological urine analysis is a useful tool to identify nonadherence from patients who are truly resistant to the prescribed regimen. This distinction is clinically important, as not detecting nonadherence is likely to result in wrong measures being taken. Even treatment-resistant hypertension, despite proven adherence to treatment, is an important finding for the treating physician, as it requires a change in treatment strategy. In any case, assessment of adherence provides crucial information for the physician, allowing rational therapeutic decisions based on a measured parameter.

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Conflicts of interest

All authors have had no involvements that might raise the question of bias in the work reported or in the conclusions, implications or opinions stated. No funding was obtained for this study.

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Reviewers' Summary Evaluations

Referee 1

The paper is very important for the current understanding of the real-life prevalence of resistant hypertension and the impact of poor adherence. The direct measurement of drugs/metabolites documented that poor treatment adherence is relevant in about 30–60% of so-called resistant hypertensive patients. Especially in the era of new invasive technologies for treatment of this condition, the data reported can be highly interesting for the physicians. The study cannot be directly reproduced in general practice but provides important information about the necessity of considering poor adherence in all cases of refractory hypertension.

Referee 2

This paper gives useful information about poor compliance in hypertensive patients on multiple drug treatment. The results of the study encourage an evaluation with a cheap and simple urine drug analysis of all patients supposed to be drug resistant. Furthermore, the urine test would help to identify patients with poor drug compliance before undertaking complex radiological and hormonal evaluation for secondary hypertension.