Hypertension

REVIEW

Nonadherence in Hypertension: How to Develop and Implement Chemical Adherence Testing

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ABSTRACT: Nonadherence to antihypertensive medication is common, especially in those with apparent treatment-resistant hypertension (true treatment-resistant hypertension requires exclusion of nonadherence), and its routine detection is supported by clinical guidelines. Chemical adherence testing is a reliable and valid method to detect adherence, yet methods are unstandardized and are not ubiquitous. This article describes the principles of chemical adherence testing for hypertensive patients and provides a set of recommendations for centers wishing to develop the test. We recommend testing should be done in either of two instances: (1) in those who have resistant hypertension or (2) in those on 2 antihypertensives who have a less than 10 mm Hg drop in systolic blood pressure on addition of the second antihypertensive medication. Furthermore, we recommend that verbal consent is secured before undertaking the test, and the results should be discussed with the patient. Based on medications prescribed in United Kingdom, European Union, and United States, we list top 20 to 24 drugs that cover >95% of hypertension prescriptions which may be included in the testing panel. Information required to identify these medications on mass spectrometry platforms is likewise provided. We discuss issues related to ethics, sample collection, transport, stability, urine versus blood samples, qualitative versus quantitative testing, pharmacokinetics, instrumentation, validation, quality assurance, and gaps in knowledge. We consider how to best present, interpret, and discuss chemical adherence test results with the patient. In summary, this guidance should help clinicians and their laboratories in the development of chemical adherence testing of prescribed antihypertensive drugs.

Key Words: adherence ■ guidelines ■ hypertension ■ mass spectrometry ■ compliance ■ urine

Hypertension is the most important modifiable risk factor of cardiovascular disease, accounting for 1.16 million deaths and 21.5 million disability-adjusted life-years globally in 2019.[1] Nonadherence to antihypertensive medications is common (rates between 30%–50%)[2–5] and associated with uncontrolled blood pressure (BP), poor clinical outcomes,[6] and an increased cost to the healthcare system.[7] Nonadherence has been known since the time of Hippocrates,[8] but an important impediment in improving it has been that, until recently, there was the lack of an objective and feasible method to assess nonadherence.[9]

In this guidance article, we summarize the current state of chemical adherence testing (Figure 1), which is a robust technique used to objectively confirm medication ingestion. We aim to provide an understanding and framework for hypertension specialists aiming to develop chemical adherence testing for antihypertensive medications in their centers. We provide pragmatic recommendations that could be applied to suit local health systems with the aim to standardize testing in the future and summarize the gaps in knowledge in the field.

MATERIALS AND METHODS

Up to date, literature were sought from MEDLINE via PubMed, as well as relevant international guidelines, to inform the

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Nonstandard Abbreviations and Acronyms

BP	blood pressure
HR	high resolution
LC-MS/MS	liquid chromatography–tandem mass spectrometry

recommendations made in this article. Subsequent iterations of the article were reviewed in regular meetings of the authors to reach the consensus on the usefulness and weight of the recommendations. Details of how data were extracted from the medication dispensary databases are outlined in the Supplemental Material (Methods section). Prescription rate data is available on request from the authors.

MEASURES OF NONADHERENCE

Physicians' perception of whether a patient is adherent to their medication or not has been shown to be no better than flipping a coin.[10] Self-reported measures such as questionnaires suffer from an over-reporting of adherence by patients.[11] Prescription records are used to assess nonadherence in some clinical research studies, although they also tend to over-report adherence, require seamless integration of all electronic pharmacy records, suffer from methodological issues in calculating nonadherence, and do not reflect the current status of nonadherence of a patient.[11,12] Pill counting is objective but is time-consuming and is dependent on patients remembering to bring their pillboxes with them at their clinic visit. An obvious limitation of this method is that pills may also be taken out but not necessarily ingested. One of the objective methods to assess nonadherence is the use of electronic monitoring systems, such as pill dispensers, which record every time a pill container is opened.[13,14] This method is generally able to monitor only a single medication per bottle but can provide a dosing history over months or years. Today, it is primarily used for research proposes. The advantages of all above methods may be circumvented in case of intentional nonadherence. Directly observed therapy, or witnessed intake of medications, avoids this potential bias but requires supervision by a senior nurse or a doctor during a day-ward admission and may unintentionally cause severe hypotension.[15] It is also complex to organize for a large population of patients with resistant hypertension and expensive limiting its use in routine care. These methods are summarized in the Supplemental Material (Table S1 in the Supplemental Material).

CHEMICAL ADHERENCE TESTING

New methods of chemical adherence testing have been developed for use as objective measures of

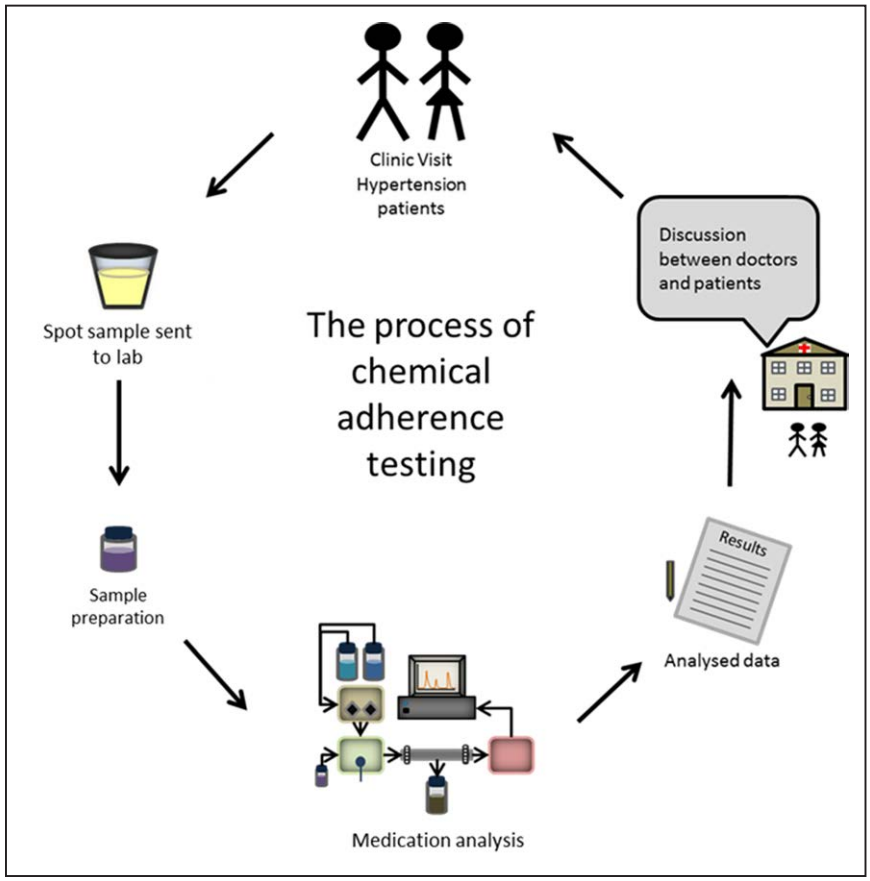

Figure 1. The schematic of chemical adherence testing.
Adapted from Gupta et al[81] with permission. Copyright ©2018, Springer Nature.

nonadherence to antihypertensive medications.[16] The technique typically uses liquid chromatography–tandem mass spectrometry (LC-MS/MS) to detect medications in patient samples (blood, urine, etc). Such methods represent a considerable improvement in the detection of nonadherence as their implementation has started to fulfill an unmet clinical need.[17] The latest European Society of Cardiology and European Society of Hypertension guidelines state that chemical testing for nonadherence shows "considerable promise but are not widely available."[17] The most recent International Society of Hypertension guidelines put a strong emphasis on screening for nonadherence in management of hypertension and recommend using objective (such as the chemical analysis of blood or urine) rather than subjective methods of detecting nonadherence in clinical practice.[18]

NOMENCLATURE OF ADHERENCE

The World Health Organization recommends using "adherence" to describe the extent to which a person's behavior (ie, taking prescribed medications) corresponds with agreed-upon healthcare provider recommendations.[16] In the past, adherence was typically described in a pejorative manner that associated the behavior with being difficult or disobedient. For example, "recalcitrant" and "noncompliant" were often used throughout the 20th century to indicate nonadherence.[19] "Concordance" was coined later to deviate from terminology that implicated patient blame. However, "adherence" was considered to be the least paternalistic term (ie, suggests that the patient has a say in their own healthcare and is not just following orders). For the analytical testing of adherence using blood and urine, we recommend using the phrase "chemical adherence testing."

CURRENT STATUS OF CHEMICAL ADHERENCE TESTING

Number of Centers

Chemical adherence testing is used in a limited number of secondary and tertiary healthcare settings. Although it is not used in primary care, there are data to suggest it is feasible to do so both in the United Kingdom,[20] and internationally.[21] There are also data to show that the test is cost-effective (at £40 or €46/$55 as of 2021, per sample, but this will vary largely between laboratories and countries).[22] The reasons for this lack of uptake could be the lack of awareness and the lack of availability of the test, which we hope will be rectified by this article. Currently, in the United Kingdom, there are several centers with access to this methodology; at least three of these centers receive samples from 60 NHS hospitals. Elsewhere, among the members of the European Society of Hypertension working group on adherence, 14 out of 50 centers regularly perform chemical adherence testing (unpublished result).

POPULATION, SCOPE OF SCREEN, AND ETHICAL CONSIDERATIONS

Who to Screen?

The European Society of Cardiology/European Society of Hypertension recommends that nonadherence be assessed especially in those who have suspected resistant hypertension,[23] which is defined as an uncontrolled hypertension despite optimal or best-tolerated doses of three or more antihypertensive drugs, including a diuretic. In this context, we stipulate suspected resistant hypertension should be denoted as apparent treatment resistance. Nonadherence increases with the number of prescribed antihypertensive medications in a near-linear fashion from 10% when a patient is on a single medication to around 70-80% in those prescribed six medications.[24] Considering this alongside previous European Society of Cardiology/European Society of Hypertension suggestions, we recommend that adherence should be assessed in patients treated for hypertension in the following circumstances:

1. In those with suspected resistant hypertension
2. In those on 2 antihypertensives who have a <10 mm Hg drop in systolic BP on addition of the second antihypertensive medication

The latter threshold is based on data by Wu et al,[25] who reviewed BP-lowering efficacy of monotherapy with main classes of antihypertensive medications and noted (on average) >10 mm Hg drop for each class. Furthermore, after addition of the second antihypertensive, one should wait until the next scheduled clinic visit to consider adherence.

It should be kept in mind that individual adherence profile may vary over time for various reasons, including depression,[26] and that repeated testing may be necessary. However, one should also consider that patients are more likely to take their medications before their outpatient appointment (and as such before chemical testing). This is often termed as white-coat adherence or toothbrush adherence.[27] No adherence measure, including chemical adherence testing, is immune from the problem of measurement reactivity; however, the impact is likely to be small, and this is outweighed by the advantages of such a measure.[28]

When to Screen?

We recommend testing is undertaken early in patients with suspected resistant hypertension before expensive investigations, interventions (ie, renal denervation) as well as treatment escalations/dose alterations.[29] This approach has been found to be cost-effective.[22]

Ethical Implications

Chemical adherence tests should only include medications if they directly affect patient care for the condition being investigated, that is, antihypertensive medications should be tested for if the objective is to improve BP control. Caution should be taken with off-target screening of compounds (eg, drugs of abuse) which could have ethical and legal implications.

The European Workplace Drug Testing Society indicates that sufficient information, meaning, and context of the test should be outlined to the recipient to allow for consent to be given.[30] We recommend that the standard medical practice of verbal informed consent used before any analytical test is sufficient in this situation. However, we recommend that to avoid influencing patient behavior; consent is best taken on the day of sampling. Consent should be recorded in the medical notes, in line with the local governance.

Given that the LC-MS/MS based techniques have the ability to detect a whole plethora of compounds, an argument can be made for the detection and reporting of substances other than those requested by the patient's doctor, thats is, off-target screening of nonantihypertensive substances (over the counter or nonprescribed) that decrease the efficacy of prescriptions. For example, St John's Wort may increase clearances of medications by enhancing drug metabolism via cytochrome P450 enzymes or excretion via p-glycoprotein.[31,32] Rifampicin and antiepileptics are also inducers of these proteins. The benefit of off-target screening alongside adherence testing is unknown, although a systematic review found that preventable drug-drug interactions were the cause of 1.1% of all hospital admissions.[33] Another study found unexpected (unprescribed) use of β-adrenoreceptor and renin-angiotensin-aldosterone system blockers in 25% of hypertensive patients.[34] Those patients may be receiving BP-lowering agents such as beta-blockers or RAS-blockers prescribed for cardiovascular disease protection (or postmyocardial infarction) rather than for BP-lowering per se.

We recommend approaching local ethical committees if off-target screening were to be undertaken. Also, hypertension specialists should have the list of medications tested available to show to patients before samples are collected. To mitigate unintended, off-target detection, we propose the implementation of reporting filters for systems that can screen a large library of drugs and metabolites.

Is Chemical Adherence Testing Acceptable to Patients?

A recent study assessing patients' attitudes and beliefs towards chemical adherence testing found that 91% of patients believed the test was a good idea and one that may be used frequently for the understanding of suboptimal disease control.[35] However, 63% of the patients stated concerns about the impact chemical adherence testing may have on the patient-physician relationship. Testing is, therefore, acceptable from the patients' perspective, although it is paramount to approach the topic without accusation (see How to Present, Interpret, and Discuss the Results to the Patients).

Composition of the Screening Panel

The medication panel for screening in a clinical setting should be based on local prescription habits, which differ between countries and regions. In the United Kingdom and European Union, 20 drugs describe 95% of dispensed treatments for hypertension.[36] In the United Kingdom, 4 medications span over half of the prescriptions (UK column, rank 1–4, Table 1). In Europe, the pattern is more diverse; 5 drugs represent over 50% of prescribed antihypertensives (European Union column, rank 1–5, Table 1). A US assay that covers 95% of hypertension therapies would likely include medications listed in Table 1. Five medications would also form half of all prescriptions (US column, rank 1–5, Table 1).[37] Information on how this data were selected is outlined in the Supplemental Material.

Therefore, we recommend that the top 95% of locally prescribed medications would be an acceptable screening panel for most clinics. At a minimum, the screen should include at least five of the most prescribed medications. This repertoire could be extended over time.

Evidently, panels for research that are used as part of international trials could include a variety of drugs that are different from drugs prescribed in a country or region. It is also worth noting the complexity of data interpretation increases with larger panels.

The potential of chemical adherence testing in combination pills has been shown in a recent pilot study.[38] Detection of only one of the pill constituents would likely indicate sporadic adherence. For example, in an amlodipine/hydrochlorothiazide combination, where amlodipine is eliminated from the body slowly and hydrochlorothiazide is eliminated quickly, the sole detection of amlodipine in chemical adherence testing would suggest intermittent dosing. We recommend hydrochlorothiazide should be included in the screening panel as it is a commonly encountered as a combination pill.

Multicentre studies, or laboratories that partake in external quality assessment, should consider including those drugs (N=8; amlodipine, enalapril, furosemide, lisinopril, losartan, propranolol, ramipril, and spironolactone) that are repeated in United Kingdom, European Union, and US lists to allow the sharing techniques, experience, and samples across centers. Furthermore, prescribing patterns are liable to change and, to future-proof the

Table 1. Annual Prescription Rates for Hypertension Medications in the United States (US; 2017), United Kingdom (UK; 2018), and European Union (EU; 2020)

US prescriptions 2017				UK prescriptions 2018			EU prescriptions 2020		
Rank	Chemical name	Items*	Cumulative %	Chemical name	Items	Cumulative %	Chemical name	Items	Cumulative %
1	Lisinopril	104 779 319	19.9	Amlodipine	29 052 338	17.0	Bisoprolol	7 907 237 153	15.9
2	Amlodipine	72 508 879	33.7	Ramipril	28 605 025	33.8	Ramipril	5 460 622 545	27.0
3	Metoprolol	68 243 168	46.7	Bisoprolol	23 625 562	47.7	Amlodipine	5 095 414 027	37.2
4	Losartan	51 989 444	56.6	Bendroflumethiazide†	9 968 237	53.5	Metoprolol	4 534 102 285	46.4
5	Hydrochlorothiazide	42 037 081	64.6	Losartan	9 842 443	59.3	Candesartan	2 787 880 664	52.0
6	Carvedilol	22 974 069	68.9	Lisinopril	8 866 133	64.5	Nebivolol	2 200 634 673	56.4
7	Atenolol	20 208 476	72.8	Atenolol	7 097 426	68.7	Enalapril	1 953 629 115	60.4
8	Propranolol	17 790 917	76.1	Candesartan	6 897 553	72.7	Lercanidipine	1 883 278 123	64.2
9	Spironolactone	11 641 507	78.4	Doxazosin	6 600 406	76.6	Perindopril	1 395 952 158	67.0
10	Diltiazem	10 504 285	80.4	Propranolol	5 287 584	79.7	Indapamide	1 346 020 255	69.7
11	Clonidine‡	10 239 431	82.3	Indapamide	4 618 639	82.4	Losartan	1 287 651 118	72.3
12	Valsartan	9 231 280	84.1	Perindopril	4 541 353	85.0	Carvedilol	1 263 623 967	74.9
13	Benazepril‡	7 163 351	85.4	Felodipine†	3 680 683	87.2	Spironolactone	1 213 840 475	77.3
14	Hydralazine‡	7 117 426	86.8	Diltiazem	2 736 225	88.8	Valsartan	1 023 788 822	79.4
15	Nifedipine	6 213 121	88.0	Spironolactone	2 702 441	90.4	Telmisartan§	947 591 995	81.3
16	Enalapril	6 121 954	89.1	Lercanidipine	2 491 599	91.9	Irbesartan	927 910 758	83.2
17	Guanfacine‡	5 451 594	90.2	Nifedipine	1 845 850	92.9	Lisinopril	908 833 973	85.0
18	Ramipril	5 318 556	91.2	Irbesartan	1 841 482	94.0	Propranolol	882 513 822	86.8
19	Verapamil	4 662 812	92.0	Enalapril	1 662 282	95.0	Hydrochlorothiazide	875 562 021	88.5
20	Nebivolol	4 473 865	92.9				Doxazosin	788 105 735	90.1
21	Doxazosin	4 370 838	93.7				Atenolol	782 946 672	91.7
22	Chlorthalidone‡	3 601 956	94.4				Olmesartan§	656 225 749	93.0
23	Terazosin‡	2 936 434	95.0				Verapamil	544 212 462	94.1
24							Moxonidine§	522 969 802	95.2

Medications cover 95% of all dispensed drugs with hypertension as one of the indications. US data sourced from the Medical Expenditure Panel Survey (MEPS) HC-197A: 2017 Prescribed Medicines File.[37] UK data sourced from National Health Service (NHS) Digital, Prescription Cost Analysis–England, 2018.[36] All dose amounts were included, and only the most prevalent formulations were used. Polypill combinations were excluded.

*Weighting factor defined by US demographic data (PERWT71F) has been used to represent 321 529 965 people.

‡Medications independent in United States.

†Medications independent in United Kingdom.

§Medications independent in European Union.

assay, it is advisable to regularly consult local healthcare providers and pharmacy teams.

Therapies that control hypertension describe only a portion of medications that could be targeted for adherence testing in patients at risk of cardiovascular disease, as people with hypertension are likely to have multi-morbidities including heart diseases, diabetes, and dyslipidemia. The usage of chemical adherence testing in these conditions is currently being established.[39,40]

ANALYTICAL METHOD

Several different techniques can be used to detect, or quantify, drugs from extracted matrices but for many clinical laboratories, LC-MS/MS has replaced other conventional chromatographic systems. This technique is available in most university laboratories. The instrument costs ≈£250 000 (€290 302 or $346 349 as of 2021) and is generally purchased on lease/rental model. LC-MS/MS screens can detect all classes of antihypertensive drugs or their metabolites in urine or plasma. Published methods have shown the detection of 9,[41] 13,[42] 14,[43] 23,[44] 40,[4] 46,[45] or >54[26] antihypertensives. The panel size can be readily increased, where running costs are largely unaffected, though additional chemical standards (≈ £150/€177/$209 each) will be required. Lower limits of detection using LC-MS/MS are typically less than 1 ng/mL, although lower ranges can be obtained with more advanced MS technologies. When reporting results around extremely low lower limits of detections, one must be mindful that basal levels of antihypertensives may indicate sporadic adherence—this idea is explored in the Pharmacokinetics section.

The next level of instrumentation is high resolution (HR)–MS—an MS platform with higher resolving power (<0.001 Da) like a quadrupole time of flight or an Orbitrap—offers similar advantages to MS/MS in excellent sensitivity and sample preparation, but offers

larger screen sizes (eg, >1000 medications) without compromising analytical performance. HR-MS has been used in the field for the simultaneous detection of 49 drugs (plus metabolites) with limits of detection similar to MS/MS methods.[46] However, HR-MS technique is more expensive than LC-MS/MS (≈ £400 000, €464 540, or $554 158) and requires personnel with a considerably higher degree of experience with mass spectrometry, which has to be considered if implementation and development of such procedures are planned.

Immunoassay

Immunoassay is commonly used for high throughput screening of urine and oral fluid samples for drugs of abuse. They use antibodies, which can cross-react with certain drug classes. The advantage of immunoassay screening is their ease of use, throughput, and sensitivity. The main disadvantage is their lack of specificity, with positive results requiring confirmation by MS platforms. For antihypertensive screening, some groups have developed immunoassays for a particular antihypertensive medication,[47–49] but none have developed a panel suitable for adherence testing. Commercial support would make development, validation, and automation of immunoassays easier. However, the present market for adherence testing is not large enough to gain significant interest of industry.

In summary, we recommend that due to the sensitive analyses required for clinically useful and comprehensive testing, currently only LC-MS/MS or HR-MS offer specifications that can meet adherence testing criteria. HR-MS offers several advantages over LC-MS/MS but currently is less ubiquitous than LC-MS/MS.

ANALYTICAL TARGETS

Adherence assays in both urine and blood should aim to be as sensitive as possible, aiming for a lower limits of detection of <10 ng/mL for all medications. For some drugs, targeting parent drug, metabolite, or a combination of the two will help to extend the detection window for the medications. The ratio of parent drug to metabolite may also give insight into white-coat adherence. A list of useful mass spectral characteristics (transitions) for the use by any laboratory that intends to set up an MS assay for adherence screening to antihypertensive medications, is available in the Supplemental Material (Table S2).

REQUESTING THE TEST

When requesting an adherence screen, information on the request form should ideally include demographic factors that can affect pharmacokinetics of the medications, such as age, sex, ethnicity, body mass index along with other useful information such as time of sample collection, timing and dose of medications, and a list of all prescribed medications. If feasible, estimated glomerular filtration rate and liver function may be added. Where possible, the request should be linked with electronic patient records.

Errors in prescriptions recorded by the requesting doctor may result in apparent positive off-target screening; hence, it is important that the list of medications that a patient is prescribed is detailed accurately when requesting the test.

SAMPLE TYPE

The type of biomatrix collected for medication screening will impact the detection limits, sample preparation, and reporting. Previous work has indicated that urine and blood assays are the most useful in adherence testing.[50] A comparison of these matrices is noted in the Supplemental Material (Table S3).

Blood Assays

The results of chemical adherence testing by blood or urine can be reported as a qualitative (present/not present) or a quantitative measure. Blood is less often used for qualitative analysis in adherence testing[51–53] but is more frequently used for quantification.[34,45,54–58] Certain groups have started quantifying antihypertensive drugs and comparing the results against the established peak (C_{max}) and minimal/trough (C_{min}) plasma drug concentrations.[45,54,57,59] These may provide a putative range indicative of regular intake (below steady-state range in Figure 2). A recent meta-analysis has shown great heterogeneity in plasma trough concentrations, and hence. caution is advised in using quantitative methods to ascertain adherence status.[60] From a practical point of view, quantitative analyses require considerably more use of laboratory resources.

Elsewhere, groups have introduced pin-prick sampling associated with dried matrix spotting techniques for antihypertensive detection to avoid venepuncture methods.[55,61] The approach is less invasive and is promising for those interested in developing a blood-based method.

Urine Assays

Urine chemical adherence tests are mostly qualitative.[4,17,51,52,62–65] Although, some groups have published quantitative methods.[44,58] Concentrations do not necessarily reflect when the drug was administered due to variances in hydration, pH, and frequency of urination. However, the collection of urine is noninvasive and easy to obtain in larger quantities. The matrix generally requires less preparation before being suitable for analysis compared with blood derivatives as phospholipids

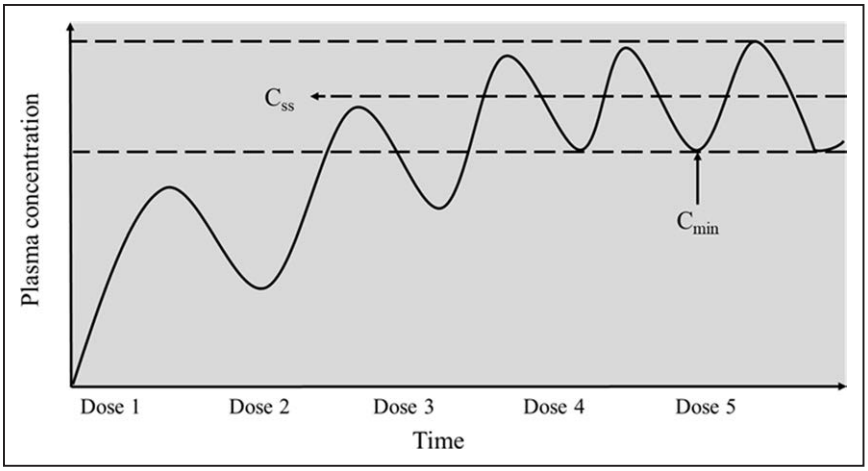

Figure 2. Pharmacokinetic curve monitoring changes in drug plasma concentration over time after sequential maintenance doses. The average (C_{ss}) and minimum (C_{min}) steady-state concentrations are labeled.

and other interfering compounds are absent. Simple dilute and shoot methods for urinary detection of 23 antihypertensives before liquid chromatography LC-MS/MS analysis have been published.[44,64] The relative ease of preparation allows timely processing in the lab, thus, has a high ceiling for throughput at minimal costs. Theoretically, factors such as the amount of excretion of a drug in urine may affect the results, although an observational study with limited demographic data failed to confirm such effect.[66] Furthermore, prospective studies are needed to confirm this finding.

In brief, we recommend the use of qualitative methods using either urine or blood samples to detect drug presence. Urine is often easier to acquire, although blood may allow more information to be sought on ingestion time and dosing regularity, given further research validates such techniques. Dried blood spots and other equivalent matrices may be considered if the laboratory has the required expertise.

PHARMACOKINETICS

In chemical adherence testing, some researchers have suggested drugs with extremely short or long elimination half-lives may incur false negative/positives.[64] For example, amlodipine is cleared slowly (half-life 34–50 hours[67]), thus dosing could be sporadic but still be analytically positive a week after the last ingestion. Conversely, hydrochlorothiazide is cleared rapidly (half-life 6.4 hours[68]), and sporadic dosing was suggested to appear analytically negative. However, recent report demonstrated no correlation between half-life and adherence rates for common antihypertensive medications in urine (Figure 3).[66] This may seem counterintuitive, although medications with short half-lives are often indicated in higher concentrations or in multiple daily doses, so adherent patients would likely be detected by the test. The fraction of renal elimination is also important for those drugs that are excreted in urine. Of note, people with impaired renal function may have altered clearances.

In blood assays, C_{min} can be used as a threshold for sporadic/regular dosing determination. C_{min} for each drug has been established using literature, although recent evidence has highlighted the large heterogeneity of C_{min} in different studies.[60] To avoid misclassification, some groups have used C_{min} merely as a basis to optimize cutoffs for their local population.[45] Other groups have optimized cutoffs on an individual basis (rather than population), using each patients' dose alongside scoped pharmacokinetics to estimate a dose-dependent concentration.[57] Further work, including pharmacokinetic modeling using data from adherent patients with hypertension, would help provide more information on these aspects.

Therefore, we recommend that reports mention the half-lives of medications (from published literature) and further research on the impact of pharmacokinetics on adherence status is undertaken.

SAMPLE REQUIREMENTS

Currently, there is no data on the optimal time of sample collection and further research is needed in this area. Therefore, for pragmatic reasons, we recommend collecting samples when the patient attends a clinic.

The minimum amount needed for urine analysis is small (<1.5 mL), although collection should be done in <20 mL standard universal containers. For blood samples, at least 1 mL should be collected in lithium heparin or, alternatively, an EDTA tube may be used for the collection of plasma. Serum may also be collected, as well as dried blood spot sampling (where the facilities for analysis of such samples are available).

SAMPLE STABILITY AND TRANSPORT

In a spoke-hub distribution laboratory model, samples are sent to a main site for testing. Delivery conditions are variable, so medication stability should be considered. Research results on stability are inconsistent.

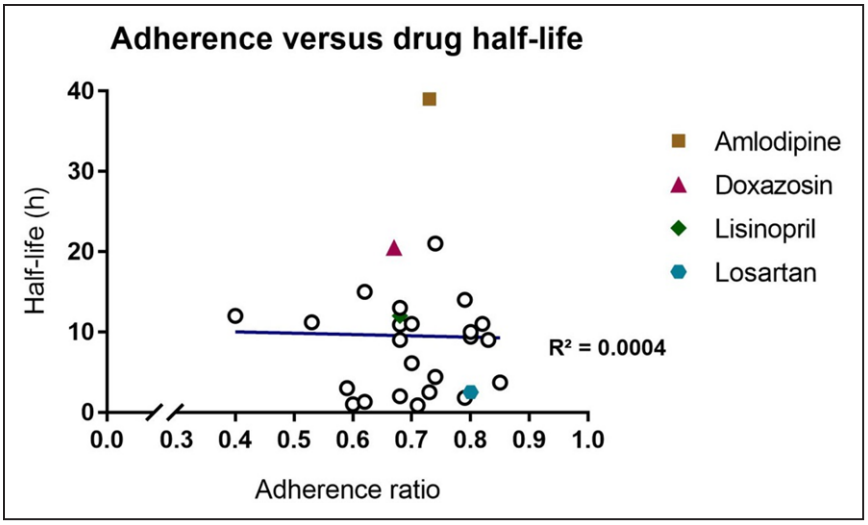

Figure 3. Adherence ratio (number of medications detected against the number that were prescribed) against half-life of 27 common antihypertensives.
[66] Four commonly prescribed medications are highlighted.

For example, antihypertensive drugs (N=29) in urine are stable at room temperature for three days.[69] In another study, the stability of 23 antihypertensive medications was investigated.[44] This study found the majority of drugs were stable in urine under a variety of conditions with significant losses seen for nifedipine (unstable when stored at 25 °C for 1 day) and bendroflumethiazide (unstable when stored at 25 °C for 1 day and when stored at 4 °C for 7 days). However, both drugs were stable when stored at −20 °C for 28 days. Another study of 6 antihypertensives found they were unstable at high pH but stable at a variety of temperatures.[70]

We recommend that urine samples be stored at 4 °C after collection—but can be transported by ordinary post within three days of sampling for most antihypertensives (nota bene consider transporting on ice for nifedipine and bendroflumethiazide).[69] Further research should investigate the stability of medications not previously reported. Once in the laboratory, samples can be stored at −20 °C for at least 4 weeks before analysis.

The stability of antihypertensive drugs in blood has not been investigated as extensively. One group demonstrated long-term stability at −80 °C from a panel of 20 analytes in plasma.[45] Three-hour room temperature stabilities were poor for some drugs, including captopril, hydralazine, and nifedipine. Catalytic ability of blood can be retained as free proteins can preserve their esterase function.[71] Storing these biomatrices cold (≤4 °C) will help to lessen these effects.

To decrease the effect of these enzymes, we recommend that blood should be centrifuged and the supernatant decanted, if possible. Serum or plasma should be stored frozen if possible; whole blood should not be frozen (to avoid hemolysis) but stored refrigerated. Shipping should take as short as possible and samples should be kept cool. Amber vials or other light exclusion techniques may also be used, especially in view of a possible degradation of dihydropyridine calcium channel antagonists,[72] although their efficacy is not well-understood for all antihypertensives.

Dried matrices can be sent by ordinary post and can be stored at room temperature.

LABORATORY CONSIDERATIONS

Important aspects for the laboratory including sample preparation, validation,[73–76] and external quality assurance have been outlined within the Supplemental Material.

FORMAT OF REPORTS

Nonadherence reports should be designed with the user in mind, with results being clear and concise for ease of interpretation. The technique used in the assay should be stated. Ideally, the list of hypertension medications prescribed should be detailed next to whether the drug was detectable or not detectable for the clinician to equate the result to adherence.

We recommend that for qualitative assays, the report should state the medication as “detected” or “not detected,” and for quantitative reports, the level of drug detected alongside the putative range for each drug should be noted. Cases around and below the putative range should be noted in the report as certain factors (eg, renal disease) may influence the concentration of excreted drug.[77] For a comprehensive report, the drugs that were not detected should be given as “negative (< lower limits of detection ng/mL)” and the prescribed drugs that were not covered by the analytical procedure should be mentioned.

The report may also contain an explanation that it can only conclude on the presence or absence of a drug over the half-live of medication and that period should be stated for the medication. It is not currently possible to predict ingestion of the dose given a concentration. Printing the half-life alongside the result provides context

if not detected *ie,* > 4 half-lives typically provides enough time to clear a single dose. The report should also state that malabsorption, among other reasons (see Fallacies of Chemical Adherence Testing and Gaps in Knowledge), maybe a reason for nondetection of medications.

HOW TO PRESENT, INTERPRET, AND DISCUSS THE RESULTS TO THE PATIENTS

It is imperative to give feedback on the test, regardless of result. This is designed to reinforce positive behavior where appropriate. In our experience, the results allow an open, nonjudgemental discussion about adherence. Most patients accept that they are nonadherent once the discussion is initiated. Although nonadherence is common, this aspect is not covered well in medical school training, therefore, clinicians may not feel comfortable about discussing the report of nonadherence with patients. Patients should be approached in an open-minded manner with the view to understand the reasoning for their nonadherence.

We recommend covering the common causes of nonadherence, such as forgetfulness, side-effects, costs, and patients' knowledge and beliefs about their illness and need to take medications. Also, it is useful to record nondetection (rather than nonadherence) by chemical testing in the patients' medical notes or correspondence. This information, if available to other clinicians, will avoid unnecessary investigations or potential complications if an admitted patient is given all their antihypertensives that he/she was not previously adherent to.

Patients on multiple medications who have tested positive for all but one medication (ie, 5/6) should be carefully reviewed. These patients may be considered adherent, as nondetection may be due to the medications pharmacokinetics. If there is persistent disagreement about the adherence status, a direct observed therapy could be undertaken.

Chemical adherence testing may only be the initial step of the adherence monitoring process since repeated measures on the same patient may be required.

FALLACIES OF CHEMICAL ADHERENCE TESTING AND GAPS IN KNOWLEDGE

Known contributors to low medication levels, other than nonadherence, have been previously outlined by Berra et al.[78] Since this publication, several contributors have been addressed (primarily the preanalytical artifacts). However, research is still required on issues with pharmacokinetics and effectors thereof (eg, gastrointestinal tract alterations on adsorption). Although there are data from observational studies, there is a need for well-designed prospective studies to answer the questions about the relationships between various pharmacokinetic parameters such as volume of distribution, half-life, urine excretion rate, and nondetection of drugs in blood or urine. Similarly, research is needed to study the effects of genetic polymorphisms in cytochromes P450, P-glycoprotein, and other drug transporters that effect pharmacokinetics on chemical adherence screening.

Observational studies have shown that chemical testing followed by discussion of results with a nonadherent patient, improves adherence status and BP.[5] Further evidence in the form of randomized control trials is needed as long-term outcomes after chemical adherence testing are poorly researched. The OUTREACH trial (https://www.clinicaltrials.gov; Unique identifier: NCT03293147) is aimed to answer this question.

Studies have shown that there are not only reasonable congruities but also differences between detection of medication in urine versus blood.[79,80] Larger studies are needed to study correlation between nonadherence results based on urine and blood samples, but if there is a good correlation between blood and urine chemical nonadherence results, this would not necessarily mean that these can be used interchangeably. Further studies are needed on the usefulness of quantitative measurement of drugs in adherence screening. Also, different matrices (eg, dried blood and urine, saliva, hair) should be explored to overcome the frailties in collection and stability. Long-term monitoring is also poorly researched. Dried matrices may allow for at-home sampling which could enable conduct of such a study.

Having multiple methods to assess adherence is always desirable. Self-reporting alongside chemical adherence testing can often provide context to the bioassay data. For example, adherent, older patients with limited mobility may forego their diuretics on the day of their clinic visit to circumvent needing to urinate outside their home. We suggest chemical adherence testing be used to triangulate with other assessments, especially those that gather patient perspective.

OTHER CONSIDERATIONS INCLUDE

- Does the ratio of parent drug to metabolite indicate long-term adherence?
- What is the optimal time of sample collection? For example, is an early morning urine sample better than collecting samples when patient comes to clinic? Similarly, should the blood sample be collected at the expected trough concentration (ie, before the next dose)?

SUMMARY OF RECOMMENDATIONS

We have outlined some considerations for groups interested in developing a chemical adherence test for clinical use. The drug panel and the aim of the test (qualitative or

Table 2. Summary of Recommendations for Developing and Implementing a Chemical Adherence Test

Summary of recommendations
Chemical adherence testing should be used as the terminology for assessing adherence by means of analysis of biofluids (eg, urine or plasma/serum)
Verbal consent should be obtained, and the scope of the test must be outlined. Ideally, this should be done on the same day as sampling
Chemical adherence testing should be done:
In those with suspected resistant hypertension (true treatment resistance requires exclusion)
In those on two antihypertensives who have a less than 10 mm Hg drop in systolic blood pressure on addition of the second antihypertensive medication[25]
The most common locally prescribed antihypertensive medications should be included in the assays scope
Liquid chromatography-mass spectrometry platforms should be used for analysis
Request forms should include detailed medication plan and time of last dose before sampling; other patient characteristics can be collected too
Qualitative screening using urine or blood is preferred over quantitative analysis as cutoffs (eg, for C_{min}) cannot be sourced from literature
Samples should be sent and stored on ice where pragmatic; room temperature delivery of urine and dried blood is possible
Reports should detail in simple terms which drugs have been detected and their putative ranges where applicable along with the drugs not detected and those that were not covered by the assay
Feedback of the result must be given, regardless of the result
Discussion of the results with patients should take place in nonconfrontational manner after excluding medical reasons for nondetection of medications.
Other measures, especially those that gain the patients' perspective (eg, self-report) should be considered alongside chemical adherence testing to give the full context to the results.
Further research is needed on impact of pharmacokinetic parameters, number of tests required, impact of the tests on change in adherence behavior, and correlation with long-term outcomes

quantitative) should be decided first. This will dictate the choice matrix for sample collection, which informs the sample preparation. LC-MS/MS and HR-MS are suitable platforms for analysis. The validation and implementation of the assay will help meet unmet clinical needs, identify true treatment resistance cases, reduce unwarranted treatment escalation, and prevent avoidable cardiovascular events. Table 2 summarizes recommendations for developing and implementing adherence testing.

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