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MEDICATION ADHERENCE

Detection of Non-Adherence to Antihypertensive Treatment by Measurements of Serum Drug Concentrations

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ABSTRACT: Non-adherence to drugs is a challenge in hypertension treatment. We aimed to assess the prevalence of non-adherence by serum drug concentrations compared with two indirect methods and relate to the prescribed drug regimens in a nationwide multicenter study. Five-hundred-and-fifty hypertensive patients using ≥2 antihypertensive agents participated. We measured concentrations of 23 antihypertensive drugs using ultra-high-performance liquid-chromatography tandem mass-spectrometry, and compared with patients' self-reports and investigators' assessment based on structured interview. We identified 40 non-adherent patients (7.3%) using serum drug concentrations. They had higher office diastolic blood pressure (BP) (90 vs. 83 mmHg, p<0.01) and daytime diastolic BP (85 vs. 80 mmHg, p<0.01) though systolic BPs did not differ significantly. They had more prescribed daily antihypertensive pills (2.5 vs. 2.1 pills, p<0.01) and total daily pills (5.5 vs. 4.4 pills, p=0.03). Prescription of fixed-dose combination pills were lower among the non-adherent patients identified by serum concentrations (45.0 vs. 67.1%, p<0.01). Fifty-three patients self-reported non-adherence, while the investigators suspected 69 non-adherent patients. These groups showed no or few differences in drug regimens, respectively. In summary, we detected 7.3% prevalence of non-adherence by serum drug measurements in patients using ≥2 antihypertensive agents in a nationwide study; they had higher office and ambulatory diastolic BPs, higher number of prescribed daily pills, more daily antihypertensive pills, and less frequent prescriptions of fixed-dose combination pills. Indirect methods showed poor overlap with serum drugs concentrations and no or minimal medication differences. Thus, serum measurements of drugs were useful in detection and characterization of non-adherence to antihypertensive treatment.

Key Words: antihypertensive drugs ▪ blood pressure ▪ hypertension ▪ non-adherence ▪ single-pill combination

INTRODUCTION

Non-adherence to antihypertensive medication is a challenge in the treatment of high blood pressure (BP) [1-4]. Despite emphasis on the importance of optimal BP control in recent guidelines, there is considerable variation in the rate of controlled hypertension in hypertensive populations [5-9]. Factors known to be associated with low adherence include younger age, female gender and higher number of prescribed pills, but characteristics of non-adherence need to be further explored [10]. Indirect methods such as patient questionnaires and physician opinions may provide information on non-adherence. Although readily available, these methods are insufficient, and existing adherence questionnaires suffer from lack of validation against objective, direct methods such as measurements of serum drug concentrations [11, 12]. Despite the increasing use of direct methods to measure antihypertensive drugs in body fluids, non-adherence remains an obstacle in hypertension treatment. There is considerable variation in the extent of non-adherence among hypertensive patients, largely depending on the particular method of assessment that has been applied [10, 13-15].

In this nationwide cross-sectional multicenter cohort study, we aimed to assess the prevalence of non-adherence among Norwegian hypertensive patients using ≥2 antihypertensive agents by using direct measurements of serum drug concentrations, characterize drug regimens in non-adherent patients, and investigate other associated factors of non-adherence to antihypertensive medication. In addition, we aimed to assess the accuracy of two indirect methods namely patient-reported non-adherence and physician-reported non-adherence, by comparing them to the direct method of serum drug concentrations.

METHODS

Patients were included at the four major university hospitals across Norway (Oslo University Hospital, Ullevål, Haukeland University Hospital, Bergen, University Hospital of North

Norway, Tromsø and Trondheim University Hospital). Referrals to the study came from primary physicians, secondary centers or tertiary specialist centers. In addition, patients could contact our research unit directly, in response to advertisement in local newspapers and social media. The Regional Ethical Committee had approved the advertisement (**Fig. S1**). Participation was free, and patients were not paid. The advertisement had similar wordings as letters to hospital outpatient clinics, practicing specialist and physicians in general practice, inviting them to refer patients to the study.

Patients were included if they were ≥18 years old, being prescribed ≥2 antihypertensive agents (or ≥1 fixed-dose combination pill) and on a stable treatment regimen for at least 4 weeks. Exclusion criteria were eGFR <30 ml/min/1.73m^2, urine albumin/creatinine ratio >300 mg/mmol, poor Norwegian language skills, pregnancy, admitted illicit drug abuse, or any conditions that could limit the ability to evaluate the efficacy or safety of the protocol (such as psychiatric disorders and impaired cognitive function). Patients did not receive any particular instructions prior to the consultation in order to minimize the Hawthorne effect [16].

Study Approvals and Data Capture

The study protocol was approved by the Regional Ethical Committee, conducted in accordance with the Helsinki declaration and overseen by an independent safety and monitoring board. All participants provided written informed consent. Electronic data capture was managed with VieDoc (PCG Solutions, Uppsala, Sweden), approved by the local Data Safety Officer. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Physician-Patient Interview

Fig. 1 shows a schematic illustration of patients' activities on the day of blood sampling for measurements of serum drug concentrations. After signing the informed consent, remaining uninformed about the intention to screen for non-adherence and collection of serum samples for drug analyses, patients underwent a structured physician-patient interview. The interview collected information about demographic and lifestyle data, socioeconomic factors, medical and family history, and previous antihypertensive treatment. We recorded the patients' weight, height and body mass index.

The patients reported all information on current antihypertensive and concomitant treatment. We recorded each separate antihypertensive agent, along with the total number of prescribed daily antihypertensive pills – both single-agent pills and fixed-dose combination pills. We also recorded the reported time of intake of last dose of antihypertensive pills and duration of treatment. We calculated the total number of prescribed daily antihypertensive and concomitant pills by summation of all prescribed pills per week divided by 7 days, in order to account for pills taken every other day or once a week. In addition, we counted each separate concomitant agent to reflect also medications that were not pills (such as inhaled medications, eye drops, ointments etc.). We measured attended office BP following this comprehensive interview.

Blood Pressure Measurements

We measured office BP with a validated device in a standardized manner according to the latest guidelines from ESC/ESH [5, 17] and ambulatory BP measurement (ABPM) using the same device. We labelled patients with a mean daytime systolic BP of ≥135 mmHg as uncontrolled for subsequent follow-up not reported here. Details on BP measurements are included in the Online Supplement.

Urine Sample and Blood Tests

Patients delivered morning urine samples for analysis. Following office BP measurement and before 24-hour ABPM, blood samples for hematological- and biochemical analyses were collected, as well as a 5 mL Vacutainer tube (BD, Franklin Lakes, NJ, USA) without additives for analyses of antihypertensive agents. If patients used nifedipine, we covered the tubes with aluminum foil to prevent photo-degradation. We centrifuged the tubes for 10 min at 3000 rpm, and pipetted serum into Sarstedt tubes (Sarstedt, Nümbrecht, Germany) before storing in the freezer at -20°C. We thawed the tubes before serum drug analyses.

Serum Drug Concentrations

The Department of Pharmacology, Oslo University Hospital, received all serum samples for analyses. Measurements of serum drug concentration were available for 23 of the most commonly prescribed antihypertensive agents in Norway using ultra-high-performance liquid chromatography coupled with tandem mass-spectrometry (UPLC-MS/MS). A cut-off value was decided for each antihypertensive agent (**Table S1**). This represents the serum concentration 24 hours after last drug intake at steady state for all but depot formulas. For depot formula drugs the average concentration at steady state was applied. Details on this methodology have been described previously [18] and are included in the Online Supplement. An experienced clinical pharmacologist, masked for all variables except for current medication (including dose, and reported time of last intake), gender and age, decided the adherence status of the patient. Taking into consideration the dosage and reported time of last intake, patients with serum drug concentrations below the cut-off were defined as non-adherent. Patients were defined as adherent if all prescribed antihypertensive agents were present in serum above the cut-off value, and non-adherent if at least one agent was

undetectable- or below the cut-off. If in doubt, two clinical pharmacologists reviewed the results and reached a consensus on the adherence status of the patient. The consideration of drug non-adherence was not applied to loop diuretics (furosemide, bumetanide), as these drugs could not be detected in serum after 12-24 hours [19].

Indirect Assessment of Non-Adherence

We also applied two indirect methods of non-adherence assessment (**Fig. 1**),

1) Physician-reported non-adherence. The investigating physician made a qualified prediction as to whether the patients were adherent or non-adherent to their antihypertensive medication. This assumption depended on the subjective opinion and clinical intuition of the individual investigating physician following the structured physician-patient interview and comprehensive registration of medications. The investigators made their prediction before performing BP measurements.

2) Patient-reported non-adherence. Self-reported non-adherence was based on a written question presented to the patients; "I take my blood pressure medication as agreed with my doctor". We asked the patients to tick one of four boxes to indicate their degree of adherence: "never", "rarely", "mostly", or "always". Interpretation of the results were assessed using two models. In model 1, we considered "never", "rarely" and "mostly" as indicative of non-adherence, and "always" as indicative of adherence. In model 2, we only considered "never" and "rarely" as indicative of non-adherence, and "mostly" and "always" as indicative of adherence.

Statistical Analyses

We used IBM SPSS Statistics 26 (SPSS, Chicago, IL, USA) for statistical analyses. Continuous variables are presented as mean (SD, Standard Deviation) and categorical

variables are presented as absolute numbers with percentages, unless otherwise specified. We tested variables for normality using the Kolmogorov-Smirnov test. We tested differences between groups using a Student t-test for normally distributed continuous variables or Mann-Whitney U-test for those non-normally distributed, while we used Fisher's exact test for categorical variables. A two-sided $p < 0.05$ was considered significant.

We used univariate logistic regression analyses to identify factors significantly associated with non-adherence. After testing for differences between the two groups, all variables with a p-value ≤ 0.10 were included in univariate logistic regression analyses with non-adherence as the dependent variable. We then included the strongest associated factors from the univariate analyses in multivariate logistic regression models for non-adherence. Significant univariate factors that remained significant in multivariate analyses using stepwise forward regression decided the final models. In addition, the final model could not include more than 10% of the number of endpoints, as the number of non-adherent patients was 40 [20].

In the evaluation of agreement between methods of assessing non-adherence, we used Cohen's Kappa (κ), reported with the 95% confidence intervals (CI).

RESULTS

Study Population and Patients Identified by Serum Drug Concentrations

Of the 550 patients investigated, three patients had incomplete measurements of serum concentration. Thus, we classified 547 (99.5 %) patients according to their adherence status; 40 patients (7.3 %) were non-adherent and 507 (92.7 %) were adherent. Non-adherent patients were younger (58.3 vs. 63.6 years, $p=0.01$), had shorter duration of hypertension (nine vs. 14 years, $p<0.01$), and included a larger proportion of non-Caucasian ethnicity (82.5 vs. 95.7 %, $p<0.01$). The groups had otherwise comparable characteristics (**Table 1**). They also carried a similar burden of comorbidities (**Table S2**). Socio-economic factors and lifestyle habits did

not differ between the non-adherent and adherent groups (**Table S3**). The prevalence of non-adherence did not differ significantly between the self-referred patients (8.6%), patients referred from hospital physicians (8.5%) and patients referred from primary physicians (5.0%).

Blood Pressures and Biochemical Analyses in Non-Adherent Patients by Serum Drugs

The non-adherent group had higher office diastolic BP (85 vs. 80 mmHg, $p<0.01$) and daytime diastolic ABPM (90 vs. 83 mmHg, $p<0.01$) compared to the adherent group. However, there were no significant differences in systolic BPs (office systolic BP 148 vs. 144 mmHg, $p=0.36$) and daytime systolic ABPM (142 vs. 137 mmHg, $p=0.08$) (**Table 1**). Additionally, mean ambulatory heart rate was higher in the non-adherent group compared to the adherent group (74 vs. 69 beats/min, $p=0.03$). We found orthostatism in 22 patients (4.0 %) none of whom was in the non-adherent group.

Except for a lower level of serum triglycerides in the non-adherent group ($p=0.04$), the two groups did not differ in biochemical analyses, including biochemical markers of renal function (**Table S4**).

Current Antihypertensive and Concomitant Medication in Non-Adherent Patients by Serum Concentrations

Overall, the two groups of patients were similar in their prescription of antihypertensive drug-classes. The mean total number of antihypertensive pills in the non-adherent group was significantly higher than in the adherent group (2.5 vs. 2.1 pills, $p<0.01$). Non-adherent patients used fixed-dose combination pills less frequently than adherent patients (45.0 vs. 67.1 %, $p<0.01$). Non-adherent patients were prescribed fixed-dose double-agent combination pills less frequently than adherent patients (37.5 vs. 55.6 %, $p=0.04$). The two groups did not differ

significantly in their use of fixed-dose triple-agent combination pills (7.5 % of non-adherent patients vs. 11.4 % of adherent patients, p=0.61) (**Table 2**). The Online Supplement includes further details (**Fig. S2-6)**.

A larger fraction of non-adherent patients used at least one single-agent pill compared to adherent patients (90.0 vs. 74.6 %, p=0.03). Although most patients used at least one fixed-dose combination pill, the rate of patients being treated with only single-agent pills (i.e. multiple single-agent pills without addition of any fixed-dose combination pills) was borderline significantly different between the two groups (50.0 % in the non-adherent group vs. 33.1 % in the adherent group, p=0.054).

Use of concomitant agents did not differ between the two groups (82.5 % of the non-adherent group vs. 82.5 % of the adherent group, p=1.00). The total number of prescribed agents (i.e. the sum of antihypertensive agents and concomitant agents by all routes of administration), was significantly higher among the non-adherent patients than the adherent patients (5.6 vs. 4.8 agents, p=0.02). Additionally, the total number of prescribed daily pills was significantly higher in the non-adherent group (5.5 vs. 4.4 pills, p=0.03). The two groups did not differ in their prescription of lipid-lowering-, antidiabetic- or anticoagulant agents, reflecting the similarities in comorbidities between the groups.

An overview of non-adherence by drug class is included in **Table S5**.

Factors Associated with Non-Adherence in Non-Adherent Patients by Serum Concentrations

Univariate logistic regression analyses are reported in **Table S6**. The final multivariate model included ethnicity, age, use of only single-agent pills and office diastolic BP. We included office diastolic BP as it was the BP variable with the strongest association from univariate analyses (**Table 3, Upper Panel**). The factor strongest associated with non-adherence in the

final model was the prescription of only single-agent pills (Odds ratio (OR) 3.130 [95% CIs 1.571, 6.235], p<0.001). Since BP could be an effect of non-adherence rather than a predictor, we also computed a model without inclusion of any BP measure (**Table 3, Lower Panel**).

Age as a continuous variable was not significantly associated with non-adherence in a multivariate model. However, age was highly significant in univariate analyses when categorized into quartiles and computed as a dichotomous variable with quartile 1 (age 18-56) as the reference category vs. quartiles 2-4 (age 57-88). Age (≤ 56 vs. ≥ 57 years) was then included in the final model.

Assessment of Non-Adherence by Indirect Methods

Our results demonstrated poor overlap between the direct and indirect methods, and only two non-adherent patients emerged by all three methods (**Fig. 2**).

Four hundred and eighty four of 547 patients responded to the question "I take my blood pressure medication as agreed with my doctor" of which 27 were pharmacologically identified as non-adherent. Twenty-two of the non-adherent patients and 409 of the adherent patients replied "always". Five of the non-adherent patients and 48 of the adherent patients replied "mostly". No patients replied "rarely" or "never". The two groups did not differ significantly in their replies (p=0.20).

Using model 1, 53 patients reported non-adherence. Only seven patients (13.2%) reported non-adherence overlapping with the serum drug measurements. Thus, the model mislabeled 46 patients. By Cohen's κ test, a low agreement between patient-reported adherence and serum drug concentration was indicated (κ 0.06 [95% CI -0.04, 0.16], p=0.20) (**Fig. 2**). Using model 2, no patients reported non-adherence as all patients replied either "always" or "mostly" (**Fig. S7**). Neither of the models showed satisfactory overlap with the direct method.

Physician-reported non-adherence was available in 536 of 547 patients. The investigators assessed 69 patients as non-adherent. Only 17 of these patients (24.6%) were non-adherent when pharmacologically evaluated. The remaining 52 patients were labeled as non-adherent by the investigating physicians, when, in fact, they were adherent according to the pharmacological evaluation. Cohen's κ yielded a value of 0.21 [95% CI 0.09, 0.33], $p<0.05$, showing a weak agreement between the two methods.

There were some few findings of higher pill numbers in the patients assumed non-adherent by the investigators (**Table 4, Upper Panel**), while there were no statistically significant findings in the characteristics of pharmacological regimens in the patients admitting non-adherence (**Table 4, Lower Panel**).

DISCUSSION

We assessed non-adherence to antihypertensive medication in 547 patients using measurements of serum drug concentration and found a prevalence of 7.3 %. To our knowledge this is the first time such a large population of patients with hypertension was assessed for directly measured non-adherence to more than 20 antihypertensive agents in serum by UPLC-MS/MS. The non-adherent patients had more prescribed daily antihypertensive pills, and total daily pills and less fixed-dose combination pills. There was no significant difference in referral method for non-adherent and adherent patients. Seven patients (13.2%) self-reported non-adherence overlapping with the pharmacological analyses, while the same overlap was seen in 17 patients (24.6%) identified as non-adherent by physicians. The non-adherent group had higher office diastolic BP and higher daytime diastolic ABPM while systolic BPs did not differ significantly between groups.

The prevalence of non-adherence in our study was modest compared to other studies using drug monitoring [15, 21]. However, several of these studies investigated patients with

apparent treatment-resistant hypertension, using ≥3 antihypertensive agents. As non-adherence seems to increase with an increasing number of prescribed pills, lower prevalence of non-adherence in our study of patients on ≥2 antihypertensive agents is reasonable [10, 22, 23]. Additionally, patients who participate in a clinical study may perhaps display awareness about their condition and be more adherent to treatment. This, in turn, makes non-adherence particularly challenging to investigate in clinical studies. However, Hamdidouche et al. found a rate of non-adherence comparable to our findings [24].

Our study adds to previous reports, and finds that younger age, more daily antihypertensive pills, and a higher total number of prescribed agents and daily pills were associated with non-adherence [23, 25, 26]. In addition, we found that a higher rate of single-agent antihypertensive pill use was associated with non-adherence. Ethnicity was associated with non-adherence; however, these results should be interpreted with caution as the vast majority of patients were Caucasian. Some studies have found female gender to be associated with non-adherence; but our study did not confirm this [27, 28].

The rate of patients treated with fixed-dose combination pills was significantly lower in the non-adherent group, while the rate of patients treated with *only* single-agent antihypertensive pills was significantly higher. The prescription of fixed-dose combination pills is an important tool to improve adherence and prevent treatment discontinuation [24, 29]. The fact that the prescription of single-agent pills remained a strongly associated factor in a multivariate model even when adjusting for office diastolic BP, age and ethnicity, supports results from other studies, highlighting the importance of combination-pill therapy as opposed to multiple single-agent pills [10, 30]. By reducing the number of daily antihypertensive pills, the overall pill burden is reduced, thereby likely improving both treatment adherence and persistence [31]. This is particularly relevant in patients who receive medical treatment for other conditions and have a high number of total daily pills. Even though we found less fixed-dose

combination pills to be strongly associated with non-adherence, this was only the case for double-agent combination pills and not for triple-agent combination pills. This is possibly caused by the low number of patients using triple-agent combination pills, which might reflect the fact that only one triple-agent combination pill is available on the market in Norway.

Patients who participate in clinical studies are subjects to the Hawthorne effect [16]. This presents a challenge in the pharmacological evaluation based on UPLC-MS/MS of serum, as this method only gives information over a limited time span [29, 32]. This is particularly relevant as most drugs require several half-lives in order to reach a pharmacological "steady state" and thus full therapeutic effect [18]. The patients in our study were supposed to be on stable medication regimes; possibly such inclusion criteria have contributed to a stable treatment situation with less noise in the investigations and findings.

UPLC-MS/MS is a precise and widely used method for therapeutic drug monitoring. However, the establishment of serum reference ranges for antihypertensive agents is rather novel and in continuous development. The cut-off values for non-adherence were set to evaluate whether the patient had taken their prescribed antihypertensive agents within the last 24 hours. Our method gives reliable information about this time-period if patients are in a pharmacological steady state. This implies that patients using their drugs as prescribed would classify as adherent, regardless of drug intake on the particular study day or not. Using this method, the risk of overestimation of non-adherence remained low. On the other hand, patients influenced by the Hawthorne effect or who only use their drugs intermittently, could classify as adherent when they in fact were not. Such mechanism may in part explain the low prevalence of non-adherence in our study and explain why systolic BPs are rather modestly elevated in both groups [8, 9].

Other direct methods of assessing non-adherence to antihypertensive drugs exist, most notably urinalysis, and oral fluids analyses [14, 33]. Urine samples are less invasive than serum

samples. However, they provide a qualitative assessment and may be less sensitive for certain drugs as not all antihypertensive drugs are excreted with urine [34]. Furthermore, urinalyses may underestimate drug non-adherence because some drugs are detectable in urine several days after absorption, depending on dose and drug half-life [35]. Serum drug measurements are useful to detect drug non-adherence but also to quantify drug levels in serum to optimize drug dosage. This might allow a more accurate assessment of the true drug levels in the body and more information on the time since last intake, as several half-lives of a drug might be needed to reach a pharmacological steady state [18].

Even though direct methods are more accurate, they tend to be costly, labor-intensive and time-consuming. Thus, indirect methods are more widely used [32]. Patient-reported questionnaires and assessments by physicians tend to underestimate non-adherence when compared against direct measurements [36-38]. Our results support that patients do not accurately report their non-adherence, in accordance with other studies [11, 39]. Although our single question method has not been validated this limitation also applies to commonly used adherence questionnaires as all lack external verification against direct methods and are of limited accuracy [37]. The non-adherent and adherent patients did not differ significantly in their replies. One may speculate that some patients regard "mostly" as being almost synonymous with "always", which is why we chose to apply two different models for comparison of direct- and indirect methods. Hence, our findings illustrate the difficulties in collecting patient-reported data on medication non-adherence. Likewise, physicians correctly identified only 17 of 40 (42.5%) patients labelled as non-adherent by serum drug measurements which is consistent with previous data [36] and supports the use of more accurate, direct methods.

Interestingly, 46 patients who self-reported non-adherence were identified as adherent by pharmacological evaluation. These patients may take their medication irregularly (and thus self-report as non-adherent), but have taken tem prior to the visit, causing them to be

evaluated as adherent based on serum measurements. This may also be the case for some of the 52 patients who were reported as non-adherent by physicians; yet identified as adherent by pharmacological evaluation. Fifteen of these additionally self-reported non-adherence. The true prevalence of non-adherence over time is thus likely to be found within the range of those who self-report as non-adherent, those identified by physicians and those who are pharmacologically evaluated as non-adherent, as these methods elucidate non-adherence from different perspectives. The disagreements between these methods may potentially represent a limitation of adherence defined by serum drug concentrations though the differences in drug measurements were rather strongly related to numerous differences in patients' characteristics and drug regimens.

Despite one of the exclusion criteria being poor Norwegian language skills, which may have led to low number of study participants from non-Caucasian ethnicities, a surprisingly large fraction of the non-Caucasian patients was non-adherent. Different cultural aspects and language skills may limit patient-physician relationship with subsequent lower adherence to prescribed medications. Other large cohort-studies in the USA have also found non-Caucasian ethnicity to be associated with non-adherence to medication therapy [40, 41]. The socioeconomic status of the patient plays a role in the capability to keep follow-up appointments, as well as to buy prescribed medications. However, in contrast to in the USA, patients in Norway receive public reimbursements for all regular costs in the treatment of chronic diseases – including hypertension. Thus, socioeconomic status should have less impact on our results. Furthermore, one cannot completely rule out that pharmacogenetic variations across different ethnic groups may exist, which warrants further investigation.

Our nationwide multicenter study provides novel insight into the incidence of directly measured non-adherence in a hypertensive cohort treated with ≥2 antihypertensive agents. An important strength is that we centralized all serum drug measurements at a single laboratory

using a sensitive and specific assay. Moreover, all patients received thorough work-up and assessment of a wide variety of factors known to be associated with non-adherence. Taking into account the unselected nature of this large cohort, our results may be generalizable to many patients with hypertension in clinical practice.

A limitation of our study is the inevitable selection bias of patients; probably the largest prevalence of non-adherence exists among patients who are unwilling to participate in clinical studies. Further, as serum drug measurement is only a momentary reflection of adherence, one would have to sample patients repeatedly on random days in order to reflect everyday non-adherence and persistence. In addition, one should consider the inherent limitation of questionnaires to collect reliable information regarding patient self-reporting of non-adherence to antihypertensive medication.

We based the cut-off values for serum drug concentrations on published literature as well as calculations with known pharmacokinetic variables and patient samples[18]. Yet, we cannot completely rule out misclassification of patients in some few cases where the serum drug concentration was immediately above or below the lower detection limit. Additionally, the limited number of non-adherent patients carries an increased risk of type II errors.

In conclusion, we found measurements of serum drug concentration useful in detecting non-adherence to antihypertensive medication. Our data showed that non-adherence to antihypertensive medication by this direct method was associated with higher BP, prescription of a higher number of daily antihypertensive pills-, prescribed agents-, and prescribed daily pills in total, along with less prescription of fixed-dose combination pills. Indirect methods of assessing adherence compared poorly with the direct method and insufficiently described the drug regimens of the non-adherent patients.

PERSPECTIVES

Measurements of serum drug concentrations are useful in detecting non-adherence to antihypertensive medication in patients using ≥2 antihypertensive agents. The large discrepancies among direct- and indirect methods of assessing non-adherence illustrate the challenge of detecting patients who are non-adherent to their antihypertensive medication. Non-adherent patients detected by serum drug concentrations had higher blood pressures, more pills and more agents prescribed, but they were also on less fixed-dose combination pills compared to adherent patients. We believe that the direct method of serum drug measurements using UPLC-MS/MS may improve detection of non-adherence to antihypertensive medication, and that this method of detection should be included in more extensive research and possibly routine clinical evaluations of adherence status in hypertensive patients. Our data also suggest that physicians may improve adherence by prescribing more fixed-dose combination pills in line with guidelines, as use of only single-agent pills were associated with non-adherence detected with measurement of serum drug concentrations.

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Novelty and Significance

What Is New?

- We investigated non-adherence to antihypertensive medication with serum measurements of 23 commonly used antihypertensive agents in patients unaware of drug testing.

- Non-adherence to treatment by serum drugs appeared in 7.3% of patients prescribed the most common antihypertensive agents.
- Investigator prediction and patients' admittance of non-adherence correlated poorly to serum drug measurements.
- Less use of fixed-dose combination pills and increased number of prescriptions characterized medication regimens of the non-adherent patients by drug measurements.

What Is Relevant?

- Non-adherence to drugs is one of the main problems in the treatment of patients with hypertension and contributes to poor blood pressure control, which is associated with increased morbidity and mortality.
- Increased knowledge of mechanisms explaining non-adherence to antihypertensive drugs is crucial to improve adherence to antihypertensive drugs in the many patients with hypertension.

Summary

Non-adherence to antihypertensive drugs is a major clinical problem. With measurements of serum concentrations of 23 commonly prescribed antihypertensive agents, we detected non-adherence in 7.3% of hypertensive patients prescribed ≥2 agents in a national study. Serum drug measurements correlated poorly with investigator prediction and patients' admittance of non-adherence. Higher blood pressure, less use of fixed-dose combination pills and more drug prescriptions characterized the non-adherent patients identified by serum drug measurements.

Legends Figure 1.

Figure comprised of two parts. *Part one* is a flowchart of the study visit in chronological order from left to right. There are three icons denoting participants; a) grey, with the letter "P" denoting the patient, b) black, with the letter "I" denoting investigating physician, and c) white, with the letters TP denoting trained personell. Each box in the flowchart represents an event in the study visit; the icons above each box represent the person/people participating in that event. The three groups of patients illustrate the ways of referral into the study. The line scoring vertically after the box labeled "structured interview" and the following dotted line, illustrates the time the investigating physician has left the room in order to assess the patient's adherence status. Following the box labeled "ABPM", the dotted line with "24h" above it, denotes the passing of 24 hours with ABPM measurement. *Part two* with three separate boxes in the lower half of the figure further describes the outline of the study.

Abbreviations: BP = Blood pressure, ABPM = Ambulatory BP measurement.

Legends Figure 2.

Venn-diagram illustrating model 1 of patient-reported non-adherence and its overlap with physician (investigator)-reported and pharmacological evaluation of non-adherence. Patient-reported non-adherence is based on a written question presented to the patient, and represented by the gray circle. Physician-reported non-adherence is the assessment of the investigating physician based only on physician-patient interview, an represented by the dark grey circle. Pharmacological evaluation is based on the serum drug concentration measurements, and represented by the white circle. The intersecting areas between two circles represent, along with the corresponding number, patients detected by two methods. The central area where the three circles intersect represent, along with the corresponding number, patients detected by all three methods.

Table 1. Characteristics of Study Groups When Non-Adherent Patients Are Identified by Serum Drug Concentrations

Variable	Adherent (n = 507)	Non-Adherent (n = 40)	p-value
Female gender, n (%)	218 (43.0)	15 (37.5)	0.62
Age, yrs.	63.6 (10.4)	58.3 (13.8)	0.01
Ethnicity, n (%)			
Caucasian	485 (95.7)	33 (82.5)	<0.01
Body mass index, kg/m^2	29.6 (5.2)	28.4 (5.2)	0.13
Time since diagnosis of hypertension, yrs.	15.5 (11.4)	10.3 (7.9)	<0.01
Blood pressures and heart rate			
Office systolic BP, mmHg	144 (18.9)	148 (22.0)	0.36
Office diastolic BP, mmHg	83 (11.3)	90 (10.6)	<0.01
Office heart rate, beats/min	68 (12.3)	70 (11.6)	0.16
Ambulatory daytime systolic BP, mmHg	137 (15.6)	142 (16.5)	0.08
Ambulatory daytime diastolic BP, mmHg	80 (9.6)	85 (10.8)	<0.01
Ambulatory daytime heart rate, beats/min	70 (10.7)	74 (10.4)	0.03
Uncontrolled hypertension, n (%)	254 (50.8)	24 (63.2)	0.18
Orthostatism, n (%)	22 (4.0)	0 (0)	0.39

Results are reported as n (%) or mean (SD), p-value denotes differences between the adherent and the non-adherent group, SD = Standard Deviation, BP=Blood Pressure

Table 2. Current Medications in the Study Groups (Non-Adherent Patients Identified by Serum Drug Concentrations)

Variable	Adherent (n = 507)	Non-Adherent (n = 40)	p-value
Medication overview, mean (SD)			
Number of prescribed daily antihypertensive pills	2.1 (0.9)	2.5 (0.9)	<0.01
Number of prescribed antihypertensive agents	2.9 (1.0)	3.1 (1.1)	0.35
Number of prescribed concomitant agents	2.7 (2.7)	3.1 (2.3)	0.14
Total number of prescribed agents *	4.8 (3.1)	5.6 (2.6)	0.02
Total number of prescribed daily pills †	4.4 (3.0)	5.5 (3.4)	0.03
Antihypertensive medications, n (%)			
ACE inhibitors	72 (14.2)	10 (25.0)	0.07
Angiotensin II receptor blockers	419 (82.6)	30 (75.0)	0.29
Calcium channel blockers	341 (67.3)	31 (77.5)	0.22
Diuretics ‡	343 (67.7)	22 (55.0)	0.12
Aldosterone antagonists	29 (5.7)	3 (7.5)	0.50
Beta-blockers	220 (43.4)	17 (42.5)	1.00
α-adrenoreceptor blockers	15 (3.0)	3 (7.5)	0.14
Centrally acting sympatholytics	25 (4.9)	2 (5.0)	1.00
Antihypertensive agents in combination pills, n (%)			
Patients prescribed ≥1 single-agent pill	378 (74.6)	36 (90.0)	0.03
Patients prescribed only single-agent pills	168 (33.1)	20 (50.0)	<0.01
Patients prescribed ≥1 fixed-dose combination pill	340 (67.1)	18 (45.0)	<0.01
Patients prescribed ≥1 fixed-dose double-agent combination pill	282 (55.6)	15 (37.5)	0.03
Patients prescribed ≥1 fixed-dose triple-agent combination pill	58 (11.4)	3 (7.5)	0.61
Patients prescribed only one antihypertensive pill	128 (25.2)	4 (10)	0.03
Selected concomitant medications, n (%)			
Lipid-lowering drugs §	232 (45.8)	17 (42.5)	0.74
Antidiabetic drugs ‖	70 (13.8)	5 (12.5)	1.00
Anticoagulants ¶	188 (37.1)	15 (37.5)	1.00

Table 2 (cont.)

Results are reported as n (%) or mean (SD), p-value denotes differences between the adherent and the non-adherent group

ACE = Angiotensin Converting Enzyme, SD = Standard Deviation

* All antihypertensive agents + all concomitant agents, † All antihypertensive pills + all concomitant pills, ‡ Diuretics include loop diuretics and thiazides , § Lipid-lowering drugs include all cholesterol-lowering drugs, ‖ Antidiabetic drugs include oral antidiabetics and insulin, ¶ Anticoagulants include antiplatelet drugs and direct oral anticoagulants

Table 3. Logistic Regression Model with Non-Adherence as Dependent Variable. With BP Included in Model (Upper Panel) and Without BP Included in Model (Lower Panel)

Variable	Beta (SE)	Odds ratio (95% CIs)	p-value
Age (dichotomized) *	0.725 (0.372)	2.064 (0.996, 4.279)	0.051
Ethnicity (Caucasian)	-1.346 (0.511)	0.260 (0.096, 0.708)	0.008
Patient prescribed only single-agent pills (yes vs. no)	1.141 (0.352)	3.130 (1.571, 6.235)	0.001
Office diastolic BP (mmHg)	0.033 (0.015)	1.034 (1.004, 1.064)	0.025

Variable	Beta (SE)	Odds ratio (95% CIs)	p-value
Age (dichotomized)*	1.017 (0.346)	2.766 (1.403, 5.454)	0.003
Ethnicity (Caucasian)	-1.456 (0.503)	0.233 (0.087, 0.625)	0.004
Patient prescribed only single-agent pills (yes vs. no)	1.122 (0.349)	3.071 (1.550, 6.087)	0.001

BP= blood pressure, SE=Standard Error, CI=Confidence Interval
*Dichotomization; Youngest quartile (Q1) vs. three oldest quartiles (Q2-Q4)

Table 4. Current Medications Classified by Physician Reported Non-Adherence (Upper Panel) and by Patient-Reported Non-Adherence (Lower Panel)

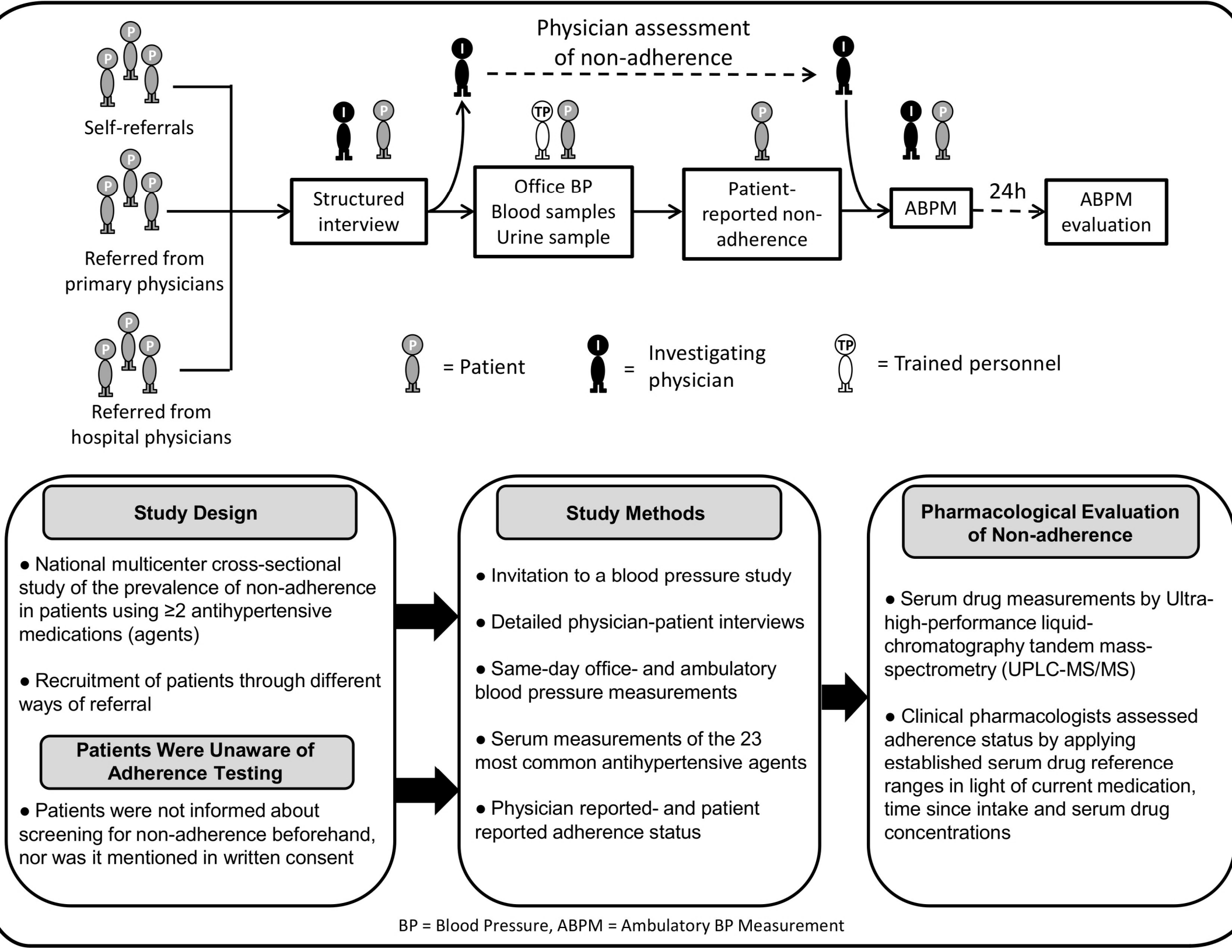
Variable	Physician Reported Adherence (n = 467)	Physician Reported Non-Adherence (n=69)	p-value
Medication overview, mean (SD)			
Number of prescribed daily antihypertensive pills	2.1 (0.9)	2.5 (1.0)	<0.01
Number of prescribed antihypertensive agents	2.9 (1.0)	3.2 (1.1)	0.02
Number of prescribed concomitant agents	2.7 (2.7)	2.6 (1.9)	0.40
Total number of prescribed agents *	4.8 (3.1)	5.2 (2.5)	0.06
Total number of prescribed daily pills †	4.4 (3.0)	5.1 (3.3)	0.10
Antihypertensive agents in combination pills, n (%)			
Patients prescribed ≥1 single-agent pill	343 (73.4)	62 (89.9)	<0.01
Patients prescribed only single-agent pills	124 (26.6)	7 (10.1)	0.18
Patients prescribed ≥1 fixed-dose combination pill	310 (66.4)	40 (58.0)	0.18
Patients prescribed ≥1 fixed-dose double-agent combination pill	259 (55.5)	34 (49.3)	0.37
Patients prescribed ≥1 fixed-dose triple-agent combination pill	52 (11.1)	7 (10.1)	1.00
Patients prescribed only one antihypertensive pill	123 (26.3)	7 (10.1)	<0.01

Variable	Patient Reported Adherence (n = 432)	Patient Reported Non-Adherence (n=53)	p-value
Medication overview, mean (SD)			
Number of prescribed daily antihypertensive pills	2.2 (1.0)	2.2 (0.9)	0.82
Number of prescribed antihypertensive agents	3.0 (1.0)	2.9 (1.1)	0.60
Number of prescribed concomitant agents	2.7 (2.6)	2.3 (2.1)	0.42
Total number of prescribed agents *	4.8 (3.0)	4.5 (2.5)	0.59
Total number of prescribed daily pills †	4.4 (2.9)	4.2 (2.9)	0.50
Antihypertensive agents in combination pills, n (%)			
Patients prescribed ≥1 single-agent pill	322 (74.5)	41 (77.4)	0.74
Patients prescribed only single-agent pills	110 (25.5)	12 (22.6)	0.45

Patients prescribed ≥1 fixed-dose combination pill	285 (66.0)	32 (60.4)	0.45
Patients prescribed ≥1 fixed-dose double-agent combination pill	236 (54.6)	27 (50.9)	0.66
Patients prescribed ≥1 fixed-dose triple-agent combination pill	50 (11.6)	5 (9.4)	0.82
Patients prescribed only one antihypertensive pill	109 (25.2)	11 (20.8)	0.61

Results are reported as n (%) or mean (SD), p-value denotes differences between the adherent group and the non-adherent group. SD = Standard Deviation.

* All antihypertensive agents + all concomitant agents, † All antihypertensive pills + all concomitant pill



Study Design

- National multicenter cross-sectional study of the prevalence of non-adherence in patients using ≥2 antihypertensive medications (agents)
- Recruitment of patients through different ways of referral

Patients Were Unaware of Adherence Testing

- Patients were not informed about screening for non-adherence beforehand, nor was it mentioned in written consent

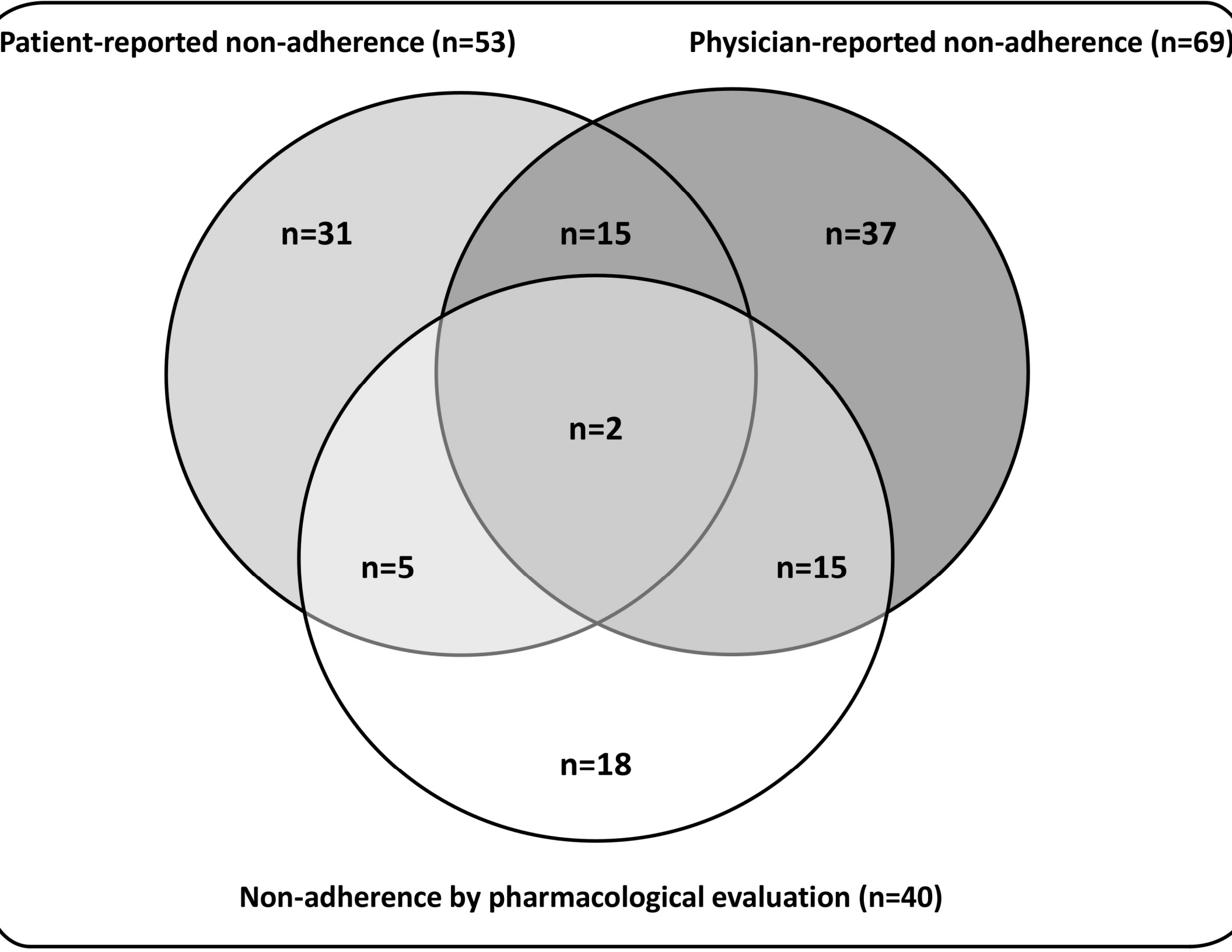
Study Methods

- Invitation to a blood pressure study
- Detailed physician-patient interviews
- Same-day office- and ambulatory blood pressure measurements
- Serum measurements of the 23 most common antihypertensive agents
- Physician reported- and patient reported adherence status

Pharmacological Evaluation of Non-adherence

- Serum drug measurements by Ultra-high-performance liquid-chromatography tandem mass-spectrometry (UPLC-MS/MS)
- Clinical pharmacologists assessed adherence status by applying established serum drug reference ranges in light of current medication, time since intake and serum drug concentrations

BP = Blood Pressure, ABPM = Ambulatory BP Measurement


Patient-reported non-adherence (n=53)
Physician-reported non-adherence (n=69)
n=31
n=15
n=37
n=2
n=5
n=15
n=18
Non-adherence by pharmacological evaluation (n=40)

Detection of Non-Adherence to Antihypertensive Treatment by Measurements of Serum Drug Concentrations

n=550
Hypertensive patients

42.7 %

≥ 2
Antihypertensive agents

4
University hospitals

7.3 % of patients non-adherent by serum-drug analyses

More daily antihypertensive pills and concomitant pills

Less fixed-dose combination antihypertensive pills

12.9 % of patients non-adherent in investigators' opinion

11.0 % of patients non-adherent by self-reporting

23 antihypertensive agents

Ultra-high-performance liquid-chromatography tandem mass-spectrometry

Patients were unaware about screening for non-adherence

Summary and Conclusion

Non-adherence associated with:

- ↑number antihypertensive pills
- ↓ combination pills
- ↓ age and ↑ blood pressure

Serum drug measurement is a useful tool to identify non-adherent patients

Key Words:

antihypertensive drugs ▪ blood pressure ▪ hypertension ▪ non-adherence ▪ single-pill combination