Online Supplement

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

*Ola Undrum Bergland, *Lene V. Halvorsen, Camilla L. Søraas, Ulla Hjørnholm, Vibeke N. Kjær, Stine Rognstad, Karl Marius Brobak, Arleen Aune, Eirik Olsen, Ylva M. Fauchald, Sondre Heimark, Christian W. Thorstensen, Knut Liestøl, Marit D. Solbu, Eva Gerdts, Rune Mo, Morten Rostrup, Sverre E. Kjeldsen[ID], Aud Høieggen, Mimi S. Opdal, Anne Cecilie K. Larstorp, Fadl Elmula M. Fadl Elmula

*These authors are joint first author

From the Section for Cardiovascular and Renal Research (OUB, LVH, CLS, UH, VNK, SR, YMF, SH, MR, SEK, AH, ACKL, FEMFE), Oslo University Hospital Ullevål, Oslo; Institute of Clinical Medicine (OUB, LVH, SR, SH, SEK, AH, ACKL), University of Oslo, Oslo; Department of Nephrology (LVH, SH, AH), Oslo University Hospital Ullevål, Oslo; Section for Environmental and Occupational Medicine (CLS), Oslo University Hospital Ullevål, Oslo; Department of Pharmacology (SR, CWT, MSO), Oslo University Hospital Ullevål, Oslo; Metabolic and Renal Research Group (KMB, MDS), UiT The Arctic University of Norway, and Section of Nephrology (KMB, MDS), University Hospital of North Norway, Tromsø; Department of Clinical Science (AA, EG), University of Bergen, Bergen, and Department of Heart Disease (AA, EG), Haukeland University Hospital, Bergen; Department of Cardiology (EO, RM), Trondheim University Hospital, University of Trondheim, Trondheim; Department of Informatics (KL), University of Oslo, Oslo; Department of Behavioral Sciences (MR), Institute of Basic Medical Sciences, University of Oslo, Oslo; Department of Acute Medicine (MR, FEMFE), Oslo University Hospital Ullevål, Oslo; Department of Cardiology (SEK), Oslo University Hospital Ullevål, Oslo; Department of Medical Biochemistry (ACKL), Oslo University Hospital Ullevål, Oslo, Norway.

METHODS

Office Blood Pressure Measurement

Office BP was measured using Microlife WatchBP O3 (Microlife Health Management Ltd., Cambridge, UK), SunTech Oscar 2 (SunTech Medical, Morrisville, NC, USA) or Omron HEM-907 (Omron Corporation, Kyoto, Japan). Trained personnel measured the circumference of both upper arms to assure correct cuff size. BP was measured on both arms, and the non-dominant arm was chosen unless we detected a difference of ≥10 mmHg in systolic BP between the left- and right arms, in which case the arm with the highest BP was used. Patients who for some reason were unable to have their BP measured on both arms had their measurement performed on the available arm. The first measurement was omitted, and the mean of the second-and third measurements was used as office BP. If the last two measurements differed by ≥7 mmHg, an additional two measurements were performed, and the mean of the last four measurements was used to denote the office BP. Directly following office BP measurement, a standardized orthostatic test was performed with BP recordings

after one- and three minutes. Any orthostatism (defined as a systolic BP reduction ≥20 mmHg or diastolic BP reduction ≥10 mmHg) was recorded.

Ambulatory Blood Pressure Measurement
The device was programmed to inflate every 20 minutes during the day (06:00-22:00), and every 30 minutes at night (22:00-06:00). Patients were instructed to go about their day as they normally would, but particularly strenuous- and unusual activity was to be avoided. If such activities was unavoidable, patients were to record activities in a standardized ABPM journal where they also recorded when they fell asleep and when they woke up. Upon extraction of data from the device, we corrected the readings in the included software to accurately reflect the reported sleep cycle. For a reading to be valid, at least 70% of measurements had to be successful, and no more than two hours without recordings could be present during daytime. In cases where an insufficient amount of valid measurements were obtained, we repeated the ambulatory measurement. In a selected few cases, where a repeated measurement was not feasible, we permitted 65 % of valid readings.

Patients' Motivations and Possible Incentives to Participate in the Present Study
Soon after patients had signed the written informed consent, while seated by themselves, they responded in writing and filled in free-text on a form to the specific question "what would be your motivation to follow-up on your treatment for hypertension?" Patients explained, though using various wordings and expressions that they were motivated to follow-up on their hypertension treatments because they cared for their own health and they considered participation in this study being an opportunity to receive additional thorough work-up by medical experts in the field.

Follow-up and Safety
Patients who had ABPM ≥160/105 mmHg, would undergo thorough evaluation by a designated safety monitoring board, consisting of two specialists from the Dpt. of cardiology otherwise not involved in the study, in order to ensure rapid- and adequate treatment. Patients with controlled hypertension were referred back to their primary physician, while those with uncontrolled hypertension either had their antihypertensive medication optimized by the investigators following the end of the study protocol, or referred to a specialist center as needed.

Serum Drug Cut-off Limits Applied to Define Non-adherence
Table S1 shows the cut-off values applied for definition of non-adherence. These concentrations are shown at steady state at 24 hours after intake of the lowest recommended prescribed dose combined with the highest clearance for all but depot formulas. For depot formula drugs the average concentration at steady state was applied. Non-adherence of a drug was defined as a value less than the cut-off level.

The serum cut-off values for antihypertensive drugs were developed as explained by Rognstad et al (18). Three approaches were used; 1) retrieval of serum ranges from reported authoritative literature, 2) calculation of expected drug concentrations from pharmacokinetic variables and common doses retrieved from authoritative literature at 24 hours after drug intake at steady state and 3) serum drug measurements from hypertensive patients. Combining these three approaches, minimum serum concentrations at 24 hours after drug intake were established. However, the cut-off values in Table S1 deviate slightly from the reference ranges published by Rognstad et al because we at the onset time of this study (2017) had fewer measurements from patient samples available to adjust the calculated ranges.

The serum sample preparation consisted of a precipitation step removing proteins. An aliquot of the supernatant was injected onto the UPLC-MS/MS (Agilent Technologies 6490 Triple Quad LC/MS, Matrix). By applying controls at low, intermediate and high drug concentrations for each drug the mean (SD) total analytical uncertainty = $\sqrt{\text{bias}^2 + \text{variation coefficient}^2}$ for all drugs were 10.7 (2.8) %. The analytical calibration ranges are shown in Table S1. Lercanidipine, bendroflumethiazide and eplerenone were read off the calibration line below the lowest calibrators since the limit of quantification/limit of detection were (0.12/0.04 nmol/L), (6.92/2.08 nmol/L) and (3.37/0.55 nmol/L), respectively for these substances.

Table S1. Overview of Antihypertensive Agents and Serum Cut-off Values

Antihypertensive agents	Analytical calibration ranges * (nmol/L)	Established cut-off values (nmol/L)
Selective α-adrenoreceptor blockers		
Doxazosin †	10-500	10
Beta-blockers		
Atenolol	20-6000	75
Bisoprolol	1-1000	10
Carvedilol	1-1000	5
Labetolol	1-1000	50
Metoprolol †	10-3000	10
Calcium channel blockers		
Amlodipine	1-1000	5
Diltiazem †	10-1000	150
Lercanidipine	1-100	0.15
Nifedipine †	1-1000	10
Verapamil †	9-924	50
ACE inhibitors		
Enalaprilat ‡	1-1000	10
Lisinopril	10-500	10
Ramiprilat ‡	1-1000	4
Angiotensin II receptor blockers		
Candesartan	1-1000	15
Irbesartan	240-20000	400
Losartan carboxylic acid ‡	10-3000	30
Telmisartan	4-6000	10
Valsartan	240-20000	100
Thiazide diuretics		
Bendroflumethiazide	10-500	2.5
Hydrochlorothiazide	10-3000	10
Aldosterone antagonists		
Canrenone ‡	1-1000	30
Eplerenone	20-6000	3.5

ACE=Angiotensin Converting Enzyme
* For each drug six calibrators, quadratic or linear models of fit for the calibration curves were applied
† Depot drugs, calculated using average concentration
‡ Metabolites of the antihypertensive agents (enalaprilat; enalapril, ramiprilat; ramipril, losartan carboxylic acid; losartan, canrenone; spironolactone)

Table S2. Patient Referral, Comorbidities and Hereditary Risk Factors (Non-Adherent by Serum Drug Concentrations)

Variable	Adherent (n = 507)	Non-Adherent (n=40)	p-value
Source of patient referral, n (%)			
Self-referred	222 (44.0)	21 (52.5)	0.32
Primary physician	208 (41.2)	11 (27.5)	0.18
Hospital physician	75 (14.9)	7 (17.5)	0.65
Comorbidities, n (%)			
Hypercholesterolemia	221 (43.6)	15 (37.5)	0.51
Stroke	29 (5.7)	2 (5.1)	1.00
Transient ischemic attack	18 (4.0)	2 (6.7)	0.36
Atrial fibrillation	64 (12.6)	3 (7.7)	0.46
Heart failure	16 (3.2)	1 (2.6)	1.00
Myocardial infarction/angina	52 (11.6)	4 (13.3)	0.77
Undergone CABG surgery/PCI	50 (11.1)	5 (16.7)	0.37
Diabetes mellitus type 1	6 (1.2)	1 (2.5)	0.41
Diabetes mellitus type 2	76 (15.0)	5 (12.8)	0.89
Kidney disease	45 (8.9)	5 (12.8)	0.39
Peripheral artery disease	20 (3.9)	3 (7.7)	0.33
Autoimmune disease *	46 (10.2)	2 (6.7)	0.76
Obstructive sleep apnea	92 (18.2)	8 (20.5)	0.67
Malignant disease	57 (11.2)	4 (10.0)	1.00
Hereditary risk factors, n (%)			
Parental hypertension	309 (60.9)	20 (50.0)	0.18
Parental stroke	161 (31.8)	11 (27.5)	0.72
Parental myocardial infarction	197 (38.9)	11 (27.5)	0.18

Results are reported as n (%), p-value denotes differences between the adherent group and the non-adherent group
CABG=Coronary Artery Bypass Graft, PCI = Percutaneous Coronary Intervention
* Excluding Diabetes mellitus type 1

Table S3. Socio-Economic Factors and Lifestyle Habits (Non-Adherent by Serum Drug Concentrations)

Variable	Adherent (n = 507)	Non-Adherent (n=40)	p-value
Marital status, n (%)			
Married/cohabitation	347 (69.3)	29 (74.4)	0.59
Single	106 (21.2)	10 (25.6)	0.54
Widowed	31 (6.2)	0 (0)	0.16
In a relationship, not living together	13 (2.6)	0 (0)	0.61
Other	4 (0.8)	0 (0)	1.00
Highest completed education, n (%)			
Elementary school (9 yrs)	41 (8.1)	2 (5.0)	0.76
High school (vocational)	62 (12.3)	3 (7.5)	0.61
High school (academic)	59 (11.7)	4 (10.0)	1.00
Higher education (≤ 4 yrs)	194 (38.3)	20 (50.0)	0.18
Higher education (> 4 yrs)	127 (25.1)	10 (25.0)	1.00
Other	23 (4.5)	1 (2.5)	1.00
Employment status, n (%)			
Employed, full time	178 (38.1)	16 (50.0)	0.61
Employed, part time	42 (9.0)	2 (6.3)	0.76
Retired	238 (51.0)	14 (43.7)	0.19
Other public benefit	9 (1.9)	0 (0)	0.46
Average hrs. of sleep/night, n (%)			
Low amount (<6 hours)	129 (25.5)	15 (39.5)	0.08
Moderate amount (6-8 hours)	335 (66.2)	20 (52.6)	0.11
High amount (8-10 hours)*	42 (8.3)	3 (7.9)	1.00
Low-intensity physical activity/week, n (%) †			
None	16 (3.2)	1 (2.6)	1.00
Low amount(<2 hours)	116 (22.9)	8 (20.5)	0.84
Moderate amount (3-6 hours)	199 (39.3)	11 (28.2)	0.23
High amount (>6 hours)	176 (34.7)	19 (48.7)	0.09
High-intensity physical activity/week, n (%) ‡			
None	234 (46.5)	19 (48.7)	0.87
Low amount (<1 hour)	54 (10.7)	2 (5.1)	0.41
Moderate amount (1-2 hours)	110 (21.9)	7 (17.9)	0.69
High amount (>2 hours) §	105 (20.9)	11 (28.2)	0.31
Reporting a healthy diet, n (%)			
Yes	337 (74.5)	24 (61.5)	0.09
Occasionally	100 (19.8)	11 (28.2)	0.22
No	29 (5.7)	4 (10.3)	0.28

Reporting a limited salt intake, n (%)			
Yes	282 (55.7)	25 (64.1)	0.40
Sometimes	102 (20.2)	7 (17.9)	0.84
No	122 (24.1)	7 (17.9)	0.44
Standard units of alcohol/week, n (%)			
Low consumption (<1 unit/week)	127 (27.5)	9 (36.0)	0.36
Moderate consumption (1-10 units/week)	273 (59.1)	10 (40.0)	0.06
High consumption (11-20 units/week)	46 (10.0)	5 (20.0)	0.17
Very high consumption (>20 units/week)	16 (3.2)	1 (4.0)	0.60
Never smoker, n (%)	242 (47.7)	18 (45.0)	0.60
Previous regular smoker, n (%)	265 (52.3)	16 (40.0)	0.58
Current regular smoker, n (%)	43 (8.5)	6 (15.0)	0.21

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

* No patients reported >10 hours of sleep, † activity without excessive shortness of breath and perspiration, ‡ activity with shortness of breath and excessive perspiration, § no patients reported >20 hours of high-intensity physical activity

Table S4. Results of Analysis of Blood and Urine Samples (Non-Adherent by Serum Drug Concentrations)

Variable	Adherent (n = 507)	Non-Adherent (n=40)	p-value
Serum Total Cholesterol (mmol/L)	5.0 (1.2)	4.7 (1.0)	0.09
Serum HDL (mmol/L)	1.5 (0.5)	1.4 (0.5)	0.26
Serum LDL (mmol/L)	3.2 (1.1)	3.0 (0.9)	0.31
Serum Triglycerides (mmol/L)	1.8 (1.0)	1.5 (1.1)	0.04
Serum Potassium (mmol/L)	4.2 (0.4)	4.2 (0.4)	0.88
Serum Creatinine (µmol/L)	81.2 (23.8)	83.3 (31.3)	0.67
eGFR (mL/min/1.73m^2)	80.5 (17.2)	83.8 (22.3)	0.10
Urine A/C Ratio (mg/mmol)	5.8 (24.0)	2.8 (7.0)	0.84

Results are reported as mean (Standard Deviation), p-value denotes differences between the adherent group and the non-adherent group
SD=Standard Deviation, HDL=High-Density Lipoprotein, LDL=Low-Density Lipoprotein, GFR= Glomerular Filtration Rate, eGFR calculated by CKD-EPI=Chronic Kidney Disease Epidemiology Collaboration, A/C=Albumine/Creatinine

Table S5. Non-Adherence to Antihypertensive Medication (Non-Adherent by Serum Drug Concentrations)

Variable	Samples Below Cut-Off*
ACE inhibitors	5 (50.0)
Angiotensin II receptor blockers	12 (40.0)
Calcium channel blockers	13 (41.9)
Diuretics	14 (63.6)
Aldosterone antagonists	1 (33.3)
Beta blockers	9 (56.3)
Non-selective α/β blockers	n.a.
Selective α-adrenoreceptor blockers	1 (33.3)
Centrally acting sympatholytics	n.a.

*n of samples below cut-off was 55 among the 40 non-adherent patients.
Results are reported as n (% of non-adherent users of each drug within group).
n.a. = not applicable

Table S6. Univariate Logistic Analyses with Non-Adherence as Dependent Variable (Non-Adherent by Serum Drug Concentrations)

Variable group	Variable name	Beta (SE)	Odds Ratio (95% CI)	p-value
AGE	Age (dichotomized)*	1.168 (0.333)	3.215 (1.674, 6.174)	<0.001
	Age (continuous)	-0.046 (0.014)	0.959 (0.932, 0.986)	0.003
BACKGROUND	Ethnicity	-1.549 (0.470)	0.213 (0.085, 0.534)	0.001
	Years since diagnosis of hypertension	-0.051 (0.019)	0.950 (0.916, 0.986)	0.007
	At least one parent with a cardiovascular event†	-0.627 (0.332)	0.534 (0.279, 1.025)	0.059
BLOOD PRESSURE	Office diastolic BP	0.047 (0.013)	1.048 (1.021, 1.075)	<0.001
	Ambulatory daytime diastolic BP	0.048 (0.016)	1.049 (1.016, 1.082)	0.003
	Ambulatory daytime heart rate	0.033 (0.015)	1.033 (1.002, 1.065)	0.035
	Ambulatory daytime systolic BP	0.020 (0.010)	1.020 (1.000, 1.040)	0.045
ANTI-HYPERTENSIVE MEDICATION	Patients prescribed only single-agent pills	0.900 (0.331)	2.459 (1.284, 4.709)	0.007
	Number of prescribed daily antihypertensive pills	0.381 (0.156)	1.463 (1.078, 1.987)	0.015
	Patients prescribed ≥1 fixed-dose combination pill	0.736 (0.339)	2.087 (1.075, 4.052)	0.030
	Patients prescribed ≥1 single-agent pills	-1.127 (0.573)	0.324 (0.113, 0.928)	0.036
	Patients prescribed only one antihypertensive pill	-1.117 (0.537)	0.327 (0.114, 0.937)	0.037
	Total number of prescribed daily pills	0.092 (0.045)	1.096 (1.003, 1.198)	0.043

SE=Standard Error, CI=Confidence Interval, BP=Blood Pressure
* Dichotomization; youngest quartile (Q1) vs. three oldest quartiles (Q2-Q4)
† Cardiovascular event includes stroke, transitory ischemic attack or myocardial infarction.
Non-significant variables: Urine albumin/creatinine ratio, serum triglycerides, office heart rate, ambulatory daytime pulse pressure, body mass index, current smoking status (yes/no), glomerular filtration rate, number of prescribed concomitant agents, total number of prescribed agents, patients reporting 11-20 units of alcohol/week, patients reporting 6-8 hours of sleep, previous cardiovascular event and higher education (>9 yrs.)

Figure S1

Figure S1. Advertisement for patients to participate in the study.

The figure contains the main elements in newspaper advertisements and letters to practicing physicians and hospital outpatient clinics seeking patients who would volunteer to participate in the study.

Figure S2

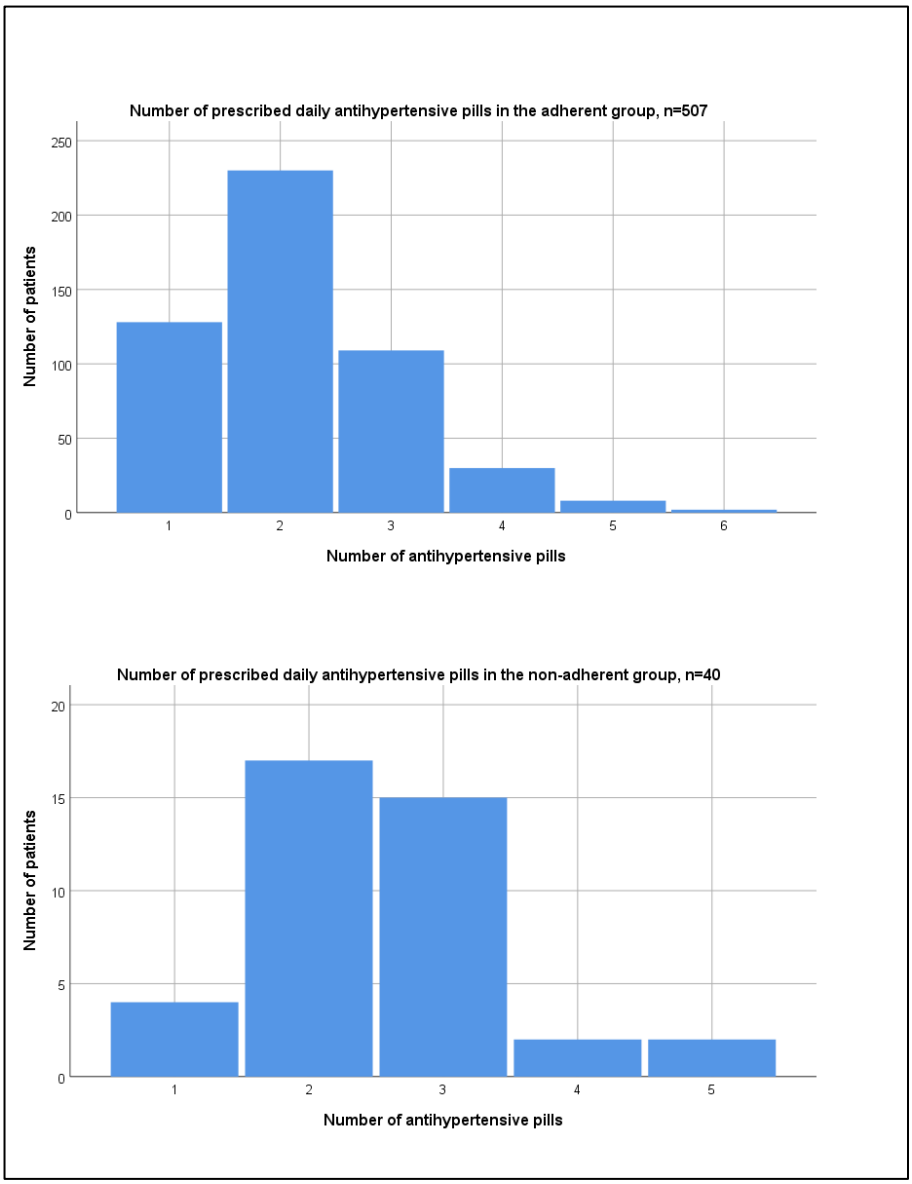

Figure S2. Number of prescribed daily antihypertensive pills across groups

The figure contains two histograms; one describing the total number of antihypertensive pills in the adherent group (n=507) and one describing the same in the non-adherent group (n=40). The X-axes denote the number of antihypertensive pills. Patients without a pharmacological evaluation of adherence status are not described separately (n=3). The Y-axes denote the number of patients, and are scaled differently in each histogram. Each column denotes the number of patients with the corresponding number of antihypertensive pills.

Figure S3

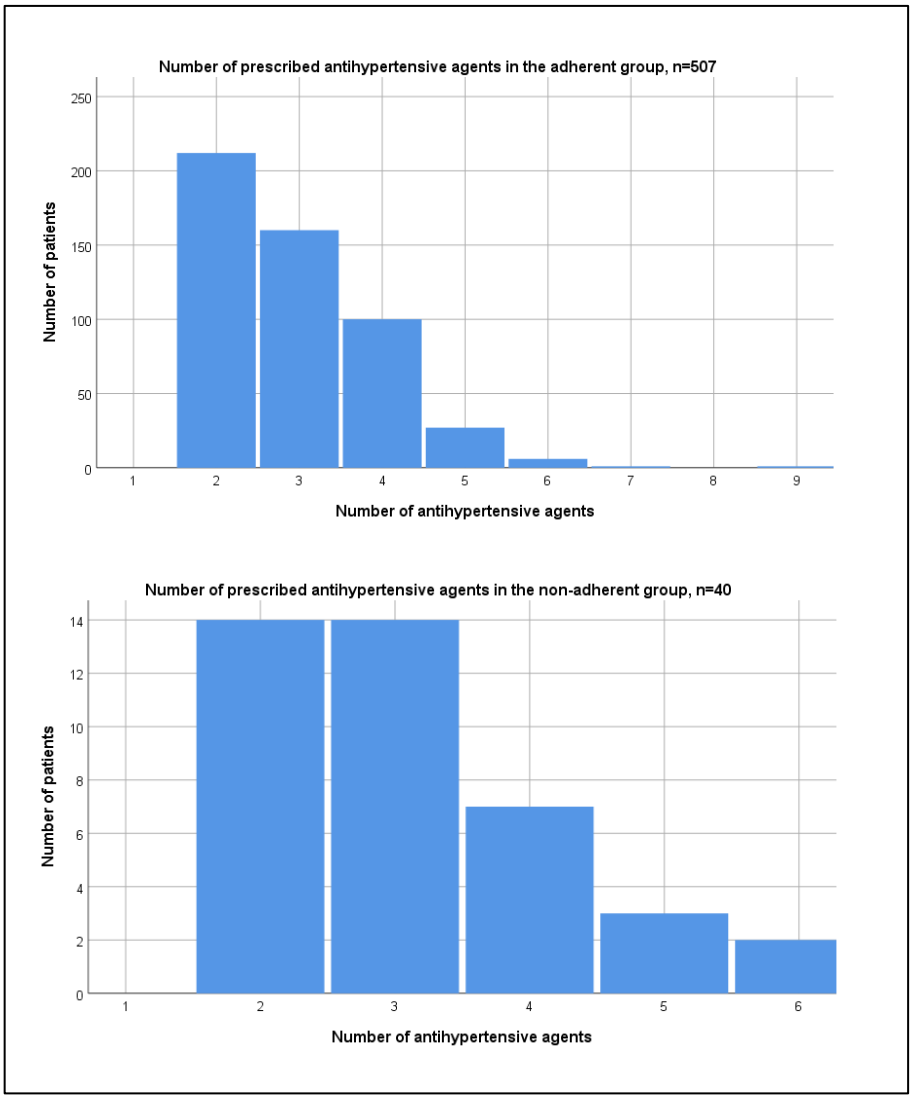

Figure S3. Number of prescribed antihypertensive agents across groups

The figure contains two histograms; one describing the total number of antihypertensive agents in the adherent group (n=507) and one describing the same in the non-adherent group (n=40). The X-axes denote the number of antihypertensive agents. Patients without a pharmacological evaluation of adherence status are not described separately (n=3). The Y-axes denote the number of patients, and are scaled differently in each histogram. Each column denotes the number of patients with the corresponding number of antihypertensive agents.

Figure S4

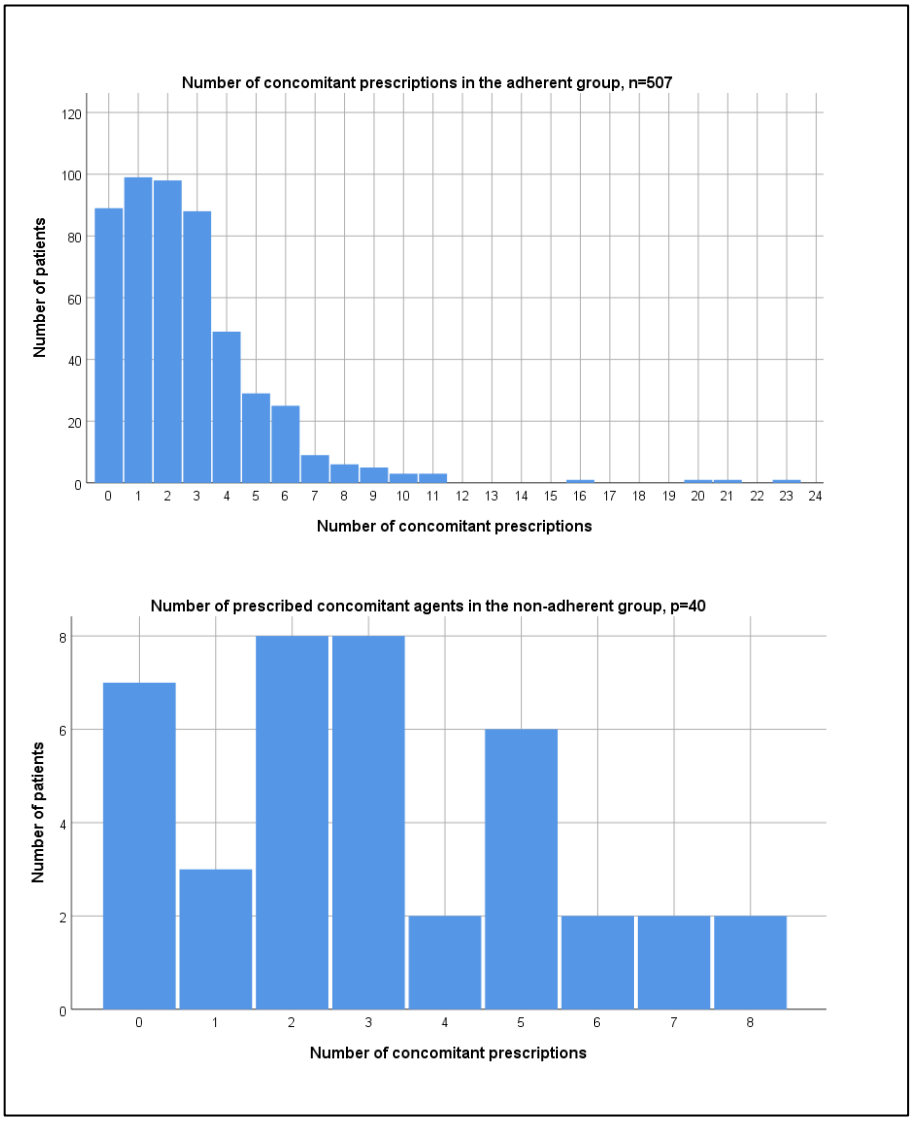

Figure S4. Number of prescribed concomitant agents across groups

The figure contains two histograms; one describing the total number of concomitant agents in the adherent group (n=507) and one describing the same in the non-adherent group (n=40). The X-axes denote the number of concomitant agents. Patients without a pharmacological evaluation of adherence status are not described separately (n=3). The Y-axes denote the number of patients, and are scaled differently in each histogram. Each column denotes the number of patients with the corresponding number of concomitant agents.

Figure S5

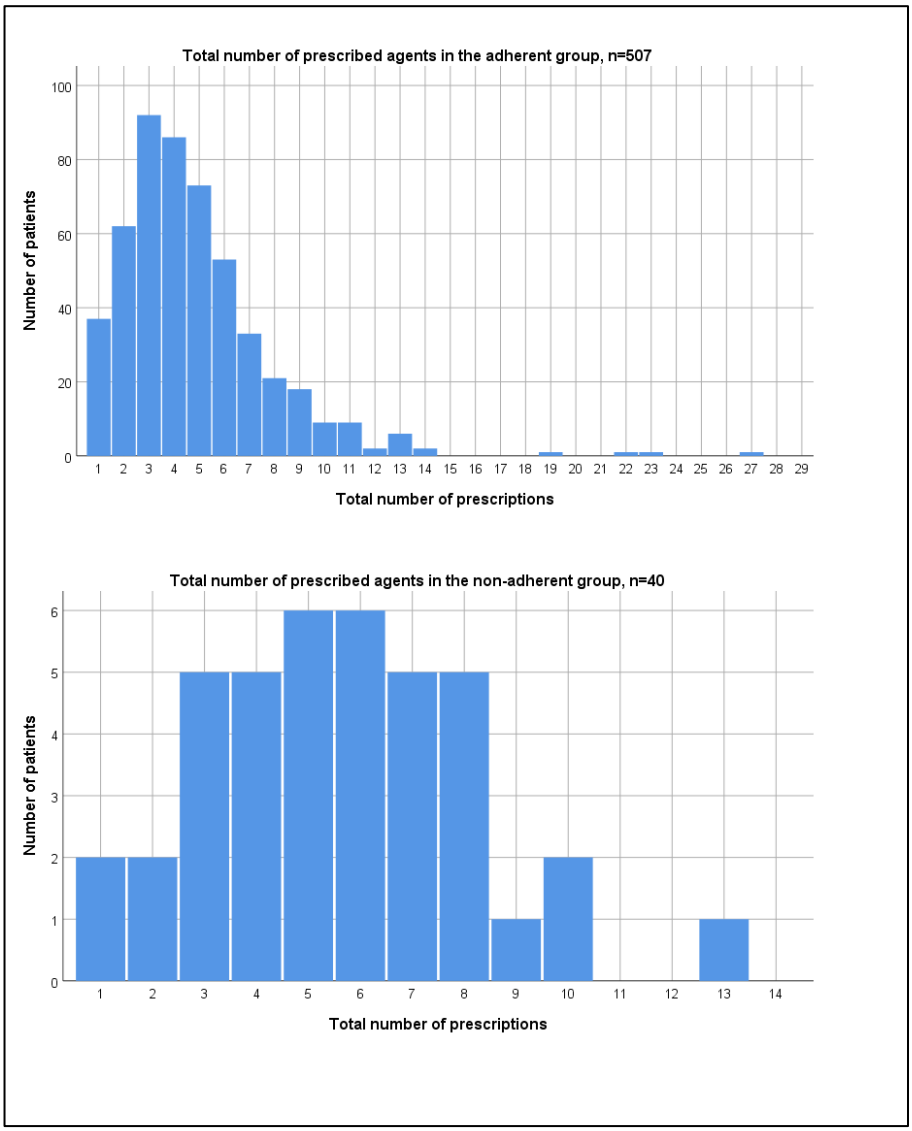

Figure S5. Total number of prescribed agents across groups

The figure contains two histograms; one describing the total number of prescribed agents in the adherent group (n=507) and one describing the same in the non-adherent group (n=40). The X-axes denote the number of prescribed agents. Patients without a pharmacological evaluation of adherence status are not described separately (n=3). The Y-axes denote the number of patients, and are scaled differently in each histogram. Each column denotes the number of patients with the corresponding number of prescribed agents.

Figure S6

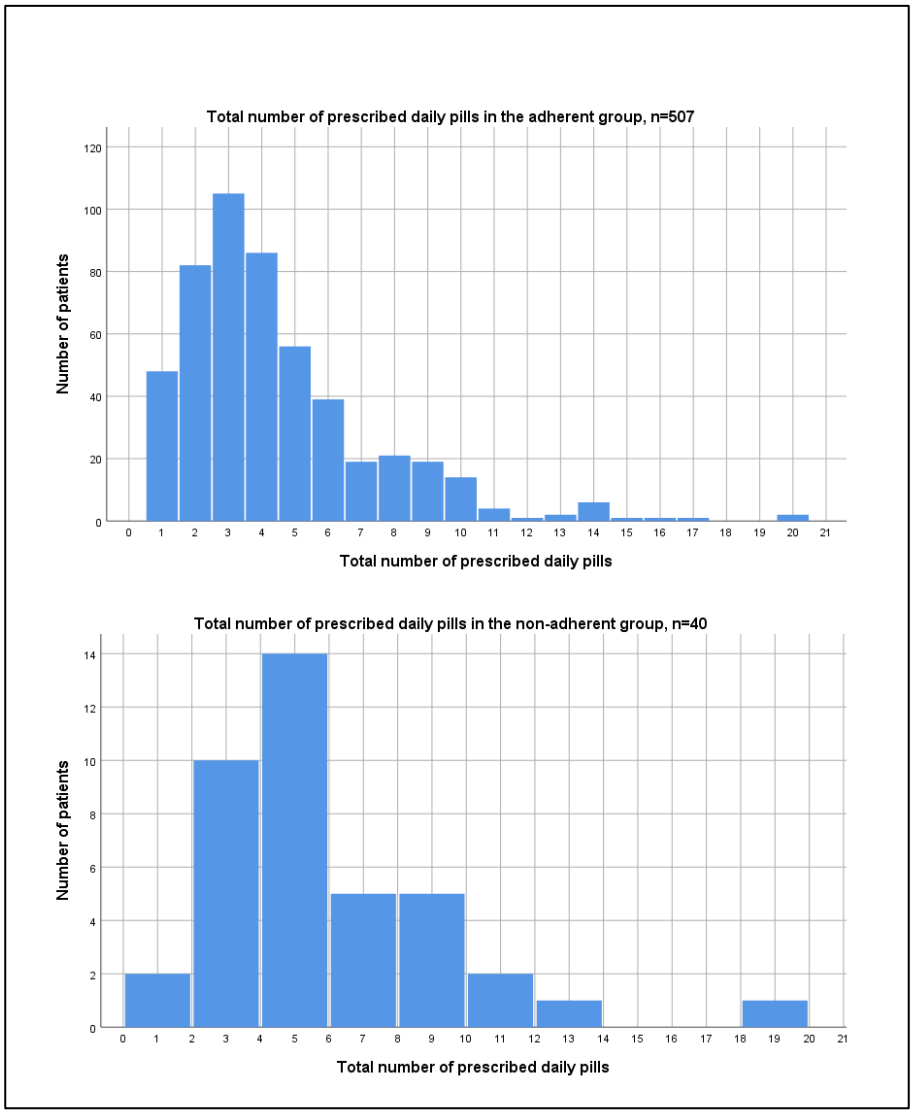

Figure S6. Total number of prescribed daily pills across groups

The figure contains two histograms; one describing the total number of prescribed daily pills in the adherent group (n=507) and one describing the same in the non-adherent group (n=40). The X-axes denote the number of prescribed daily pills. Patients without a pharmacological evaluation of adherence status are not described separately (n=3). The Y-axes denote the number of patients, and are scaled differently in each histogram. Each column denotes the number of patients with the corresponding number of prescribed daily pills.

Figure S7

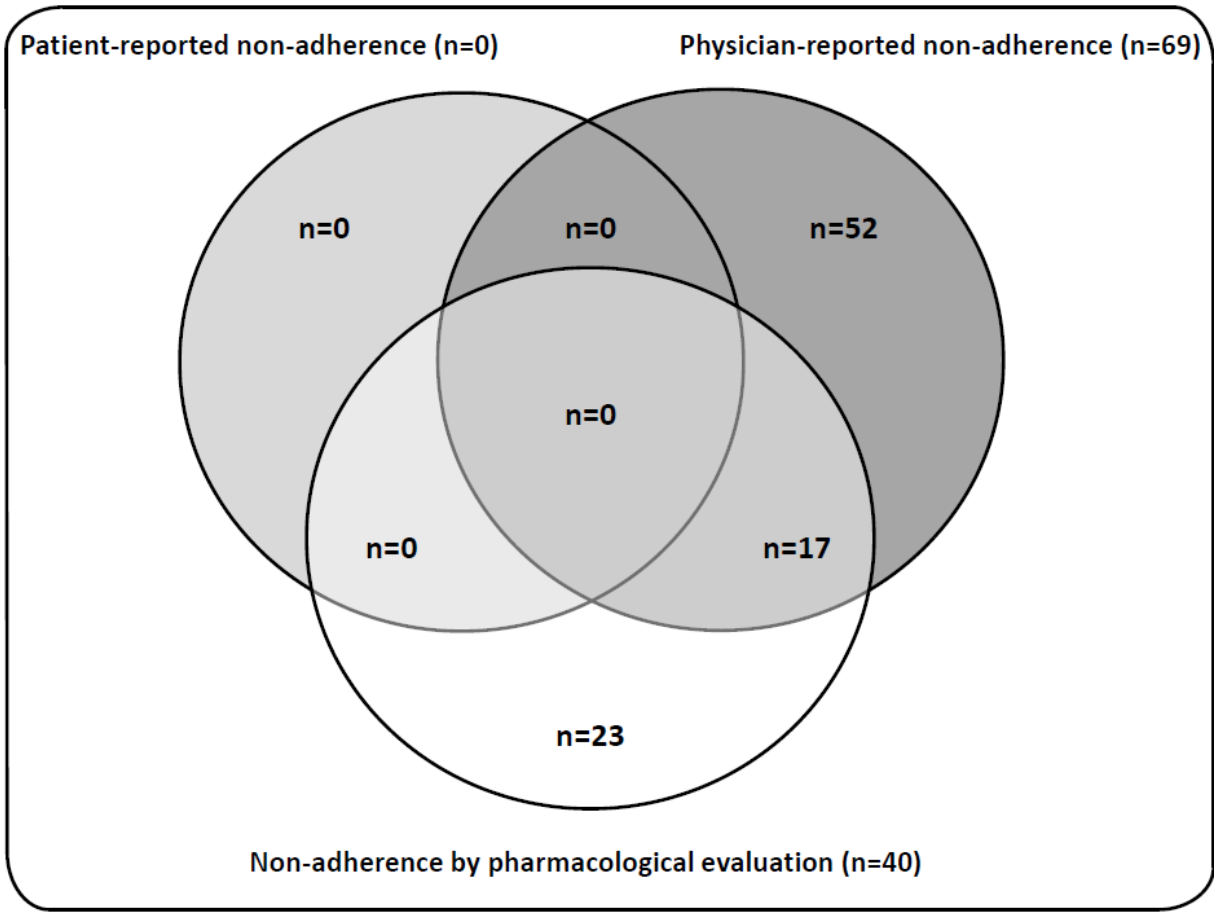

Figure S7. Model 2 of patient-reported non-adherence

Venn-diagram illustrating model 2 of patient-reported non-adherence and its overlap with physician-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 light black circle. Pharmacological evaluation is based on the serum drug concentration measurement, 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.