Novel approach to adherence assessment based on parent drug and metabolite pharmacokinetics: pilot study with spironolactone

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Aim. The aim of this study was to evaluate adherence to spironolactone in a group of unselected patients with arterial hypertension by analysis of measured serum spironolactone and canrenone concentrations according to a proposed two-step decision scheme based on pharmacokinetic considerations.

Materials and Methods. Simulation of serum concentration-time profiles of spironolactone and canrenone based on population pharmacokinetic parameters described in literature and a body weight-normalized spironolactone dose / canrenone level nomogram derived from a group of adherent patients with conservatively treated primary hyperaldosteronism, were used to create a two-step decision scheme. 71 outpatients treated with spironolactone for resistant hypertension with spironolactone and canrenone serum concentrations measured between 2018 and 2021 were analyzed according to the proposed scheme. We compared our proposed methodology to the standard approach for adherence testing.

Results. With the most sensitive traditional approach to adherence assessment through detectable serum concentrations of spironolactone and/or canrenone, 9 (12.7%) non-adherent patients were identified. With our two-step assessment of adherence, we were able to identify 18 (25.4%) non-adherent patients.

Conclusion. Consideration of the pharmacokinetic properties of parental drug and its metabolite led to improved sensitivity in non-adherence detection in patients with arterial hypertension. This approach enables better interpretation of measured spironolactone and canrenone serum concentrations and should be used in clinical practice.

Key words: drug monitoring, pharmacokinetics, hypertension, laboratories, hospital

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INTRODUCTION

Adherence to medication is the extent to which a person's behaviour taking medication corresponds with the agreed recommendations of a health care provider. Non-adherence, the opposite, can be described as failure to achieve this goal and can be caused by many factors on the patient's or health care provider's part. Non-adherence may be intentional (caused, for example, by insufficient trust in the necessity of a medication, by negative experience with side effects or associated with economic barriers) or unintentional (for example, as a result of forgetfulness, lack of clear communication and understanding or inability to follow complicated drug regimens) (ref.[1]). A high prevalence of suboptimal adherence to antihypertensive treatment has been described in patients with essential hypertension (EH) (ref.[2]) and results in undesirable consequences: increased morbidity and mortality as well as healthcare costs[3]. For this reason, poor adherence to treatment should be considered in patients who fulfil the criteria for resistant hypertension.

Several methods of measuring non-adherence have been developed, and of these, chemical analysis of urine and/or blood samples are currently considered the most accurate[4-6]. A more precise method using therapeutic drug monitoring (TDM) and pharmacokinetic simulations has been proposed, to reduce the disadvantage of single drug concentration measurements[7]. However, this method is limited to drugs with a half-life long enough so that the steady-state concentrations are significantly higher than levels after single dose administration. If the parent drug does not meet this condition, it may be more accurate to use this approach for its metabolite (either active or inactive) with a longer half-life.

Spironolactone's bioavailability is estimated to be about 73% after an orally administered dose[8] and is enhanced when co-administered with food[9]. The parent drug is subjected to extensive metabolism, of which canrenone

(non-sulfur-containing metabolite) is thought to be primarily responsible for spironolactone's therapeutic effects, although other sulphur-containing metabolites add to its mineralocorticoid activity[8,10]. Spironolactone and its metabolites are more than 90% bound to plasma proteins and metabolites are excreted by the kidneys (47–57%) and in the faeces. Spironolactone's metabolites are subject to the enterohepatic circulation[11]. In a multiple dose study of 13 healthy males taking 100 mg of spironolactone for 15 days, for spironolactone was measured c_{max} 80 ± 20; t_{max} 2.6 ± 1.5 and half-life 1.4 ± 0.5 (mean, SD), and for canrenone c_{max} 181 ± 39; t_{max} 4.3 ± 1.4 and half-life 16.5 ± 6.3 (mean, SD) (ref.[10,12]). Other studies focused on canrenone determined its t_{max} 2–3.2 h and half-life 17.8–22.6 h. The pharmacokinetics appear to be linear up to 200 mg dose of spironolactone [8]. In a group of patients with resistant hypertension, sex as well as age, BMI and eGFR had no significant influence on concentration of spironolactone or canrenone and canrenone concentration was less influenced by time period between drug intake and sampling[13]. Because of a very short elimination half-life of the parent drug, serum concentrations of canrenone might be better predictors of patients' adherence, especially in an outpatient setting, in situations where time of ingestion is only estimated as reported by a patient.

In this study, we aimed to develop of a method for spironolactone adherence evaluation based on measurement of both the parent drug and its metabolite canrenone serum concentrations, that would be easy to apply during adherence assessment in regular clinical practice.

METHODS

Study design

This is a retrospective observational study using serum concentrations of spironolactone and canrenone in adult patients with resistant arterial hypertension who were examined during a visit to an outpatient hypertension clinic between January 2018 and October 2021 for adherence testing. The study was approved by the Ethics Committee of the General University Hospital in Prague under the No. 227/21 S-IV and follows the principles of the latest Declaration of Helsinki. All subjects provided written informed consent during their visit. Patients with insufficient demographic and clinical data were excluded.

Patients were divided into two groups. Group 1 consisted of conservatively treated patients with a confirmed diagnosis of primary hyperaldosteronism, previously shown by means of spironolactone and canrenone serum concentrations as highly adherent to their therapy[14]. This group data served for assessment of the relationship between spironolactone dose and canrenone level. Unselected patients examined in relation to severe arterial hypertension not responding to previous treatment were included in Group 2. They were either diagnosed with EH during subsequent visits or arterial hypertension of undetermined aetiology due to a loss of follow up. This group was assessed for adherence to spironolactone therapy.

Bioanalytical assay

Blood samples were refrigerated until the time of analysis (within 24 h). The drug concentration analysis was performed in the Toxicology Laboratory of the Institute of Forensic Medicine and Toxicology by means of liquid chromatography–tandem mass spectrometry (LC–MS/MS). The chromatographic separation was performed on a 1200 RRLC (Agilent, Waldbronn, Germany), consisting of a degasser, binary pump, autosampler and column compartment with controlled temperature. The mass spectrometry analysis was performed using a 3200 Q-trap triple quadrupole/linear ion trap mass spectrometer with a TurboIonSpray source (MDS Sciex, Ontario, Canada). LC–MS/MS with electrospray ionisation method was used for the simultaneous determination of spironolactone and its active metabolite, canrenone in human serum. Serum samples were prepared by liquid-liquid extraction with tert-butyl methyl ether. Spironolactone, canrenone and internal standard (isotopic labelled canrenone-d6) were separated on a Phenomenex (Kinetex C18 (50 mm × 2.1mm ID 2.6µ 100 A) column, protected by a C18 security guard ULTRA cartridge (UHPLC 2 × 2.1 mm ID C18). A chromatographic run based on a gradient elution with 10 mM ammonium formate with 0.2% formic acid (A) and methanol (B) at a flow rate of 0.5 mL/min was performed 7 min. The quantification of spironolactone and canrenone was determined in a positive ion multiple reaction monitoring (MRM) mode using [M+H]+ ions m/z 417.1 → 341.1 for spironolactone, m/z 341.1 → 107.0 for canrenone and m/z 347.1 → 107.0 for the internal standard canrenone-d6. An extensive method validation was carried out in accordance with FDA guidelines. Calibration curves of spironolactone and canrenone were linear over the range 1-200 ng/mL (R>0.99). QC samples were prepared at 30 and 100 ng/mL. The intra- and inter- accuracy and precision were within ± 15% acceptance limit across all concentrations. LOD=0.5 ng/mL, LOQ=1 ng/mL (ref.[15-18]).

Development of a two-step approach for analysing spironolactone treatment adherence

Step 1. Serum concentration-time profiles of spironolactone and canrenone after per oral administration of spironolactone in dose of 50 mg every 24 h to a 70 kg man were simulated using Simulx software version 2021 (Lixoft SAS, Antony, France). For simulation, parent-metabolite model with one compartment for both parent drug and metabolite, first order absorption for parent drug, and first order elimination of both parent drug and metabolite, and unidirectional transformation from parent drug to metabolite was applied. Population PK parameters of both spironolactone and canrenone weres set as follows: spironolactone bioavailability (F) = 70%, spironolactone absorption rate constant (K_a) = 0.294 h^{-1}, spironolactone volume of distribution (Vd) = 612 L (8.74 L/kg), spironolactone elimination rate constant (K_e) = 0.533 h^{-1}, spironolactone to canrenone conversion = 20%, parent-metabolite rate constant = 0.694 h^{-1}, canrenone Vd = 94 L (1.34 L/kg) and canrenone K_e = 0.042 h^{-1}. K_e and K_a val-

ues were calculated from elimination half-life ($t_{1/2}$) and time to maximal plasma concentration (t_{max}) values using standard equations:

$$K_e = 0.693 / t_{1/2}$$
$$\ln (K_a / K_e) = t_{max} \times (K_a / K_e).$$

Spironolactone to canrenone conversion, $t_{1/2}$ and t_{max} values were adopted from the Summary of Product Characteristics (Verospiron) (ref.[12]). Canrenone Vd was calculated from pharmacokinetic data after intravenous injection of canrenoate as Vd = dose injected / plasma concentration extrapolated at t=0 (ref.[19]). Spironolactone Vd was calculated from pharmacokinetic data after per oral administration of spironolactone as Vd = (dose × F × $t_{1/2}$) / (AUC × 0.693) (ref.[10]). Careful attention was paid to the analytic methods used in published studies, because previously used fluorometric methods were shown to be unspecific and overestimated canrenone serum concentrations compared to the HPLC used today and cited in this text[8]. The PK model is depicted in Fig. 1 which clearly shows that in steady state, canrenone levels must always be higher than spironolactone levels.

Furthermore, Fig. 2 shows serum concentrations of spironolactone and canrenone after the 1st dose reveals that only during at the outset of the treatment after the 1st dose the spironolactone level can be higher than canrenone level. This allows us to consider the patients with higher spironolactone than canrenone concentrations as "white-coat adherent" (i. e. they used the drug only once shortly before the visit).

Step 2. Patients took spironolactone in the morning (between 7 and 8 a.m.) and samples were drawn between 1 and 2 p.m. Therefore, for PK analysis, time between ingestion of the drug and sampling was determined as 6 hours. Based on the concentrations of spironolactone and canrenone in group of highly adherent patients (Group 1), a body weight-normalised spironolactone dose / canrenone level nomogram was developed (Fig. 3). When the canrenone serum concentration was under 90% of expected level (lower dotted line in Fig. 3), the concentration was drawn during the cumulative phase rather than during steady state and the patient was regarded as non-adherent or "white-coat adherence".

The scheme of the final proposed approach combined from Step 1 and Step 2 is depicted in Fig. 4.

Application of two-step approach to a group of unselected patients

We analysed spironolactone and canrenone serum concentrations of patients with EH or arterial hypertension of undetermined aetiology (Group 2) and determined their adherence to therapy using the two-step approach described above. Results were compared with a traditional approach, where patients are considered adherent to therapy when the concentration of either spironolactone or canrenone is at least at the lowest detectable level. Finally, we performed a subanalysis of patients with a diagnosis of EH followed up by periodical visits to our clinic with patients that were assessed during a single visit and lost from follow up.

Statistics

Descriptive parameters mean, standard deviation, coefficient of variation, median, and interquartile range (IQR) were calculated using MS Excel 2010 (Microsoft Corporation, Redmond, USA). Wilcoxon matched-pair signed rank test was used for comparison of spironolactone and canrenone serum concentrations. Relationship between body weight-normalised spironolactone daily dose and canrenone serum concentration was explored using linear regression model. Fisher's exact test was used to determine the difference in adherence between periodically monitored patients and patients without regular monitoring. GraphPad Prism 8.2.1 software (GraphPad Inc., La Jolla, USA) was used for all comparisons, and *P*-values < 0.05 were considered as statistically significant.

Table 1. Demographic and clinical data of Group 1 and 2.

	Group 1 n=21, males 12 (57%)	**Group 2** n=71, males 37 (52%)
age (years)		
mean ± SD	63 ± 10	58 ± 13
median (IQR)	65 (56–70)	58 (48–69)
min.–max.	40–81	28–81
weight (kg)		
mean ± SD	100 ± 19	99 ± 22
median (IQR)	96 (89–107)	98 (87–116)
min.–max.	63–136	50–160
height (cm)		
mean ± SD	174 ± 9	171 ± 11
median (IQR)	175 (168–179)	170 (164–181)
min.–max.	163–186	147–189
BMI (kg/m²)		
mean ± SD	33.3 ± 6.3	33.8 ± 6.7
median (IQR)	31.8 (29.7–34.9)	33.0 (29.4–37.2)
min.–max.	23.1–48.9	20.9–51.2
BSA (m²)		
mean ± SD	2.19 ± 0.22	2.16 ± 0.28
median (IQR)	2.15 (2.07–2.30)	2.20 (1.98–2.36)
min.–max.	1.73–2.59	1.52–2.80
serum creatinine (μmol/L)		
mean ± SD	96 ± 28	89 ± 29
median (IQR)	87 (81–107)	86 (68–101)
min.–max.	63–183	41–187
eGFR (mL/min.)		
mean ± SD	93 ± 30	93 ± 43
median (IQR)	98 (73–111)	91 (67–119)
min.–max.	41–161	42–174

BMI: body mass index; BSA: body surface area (Mosteller); eGFR: estimated glomerular filtration rate (CKD-EPI_{creat} 2021); SD: standard deviation.

RESULTS

Demographics and clinical characteristics of patients are summarised in Table 1.

Twenty-one patients (12 males) were enrolled in Group 1. Their spironolactone daily dose ranged from 12.5 to 100 mg with a median of 50 mg and median (IQR) serum spironolactone and canrenone concentrations 6 hours after spironolactone administration 4.4 (2.3–9.0) ng/mL and 44.2 (31.6–65.5) ng/mL, respectively. Median (IQR) metabolite ratio (canrenone/spironolactone) was 10.6 (4.8–13.7). Canrenone serum levels measured 6 h after spironolactone administration were significantly higher than spironolactone levels (P<0.0001). This is in line with the PK model that shows that if the patient is adherent, they must have a canrenone serum level higher than the spironolactone serum level at steady state, 6 hours after administration (Fig. 1).

As expected, we observed a strong relationship (r^2 = 0.6742) between spironolactone body weight-normalised daily dose administered before measurement and canrenone serum level (Fig. 3). Serum level of spironolactone was also associated with its normalised dose, but the relationship was weaker (r^2 = 0.4552) due to the fast elimination of spironolactone. The dotted lines in Fig. 3 define the 90% prediction interval. This means that if the measured level is lower than the bottom of the 90% prediction interval and therefore it can be said with 95% assurance that the patient is non-adherent. The line defining the lower limit of the 90% prediction interval has a following function:

$$MPCL = 102.4 \times SD - 35.86$$

MPCL (minimal predicted canrenone level (ng/mL)), SD (spironolactone normalised dose (mg/kg)).

Seventy one patients were enrolled in Group 2. In this group we identified 23 patients with undetectable levels of spironolactone and 13 patients with undetectable levels of canrenone. Of those, 9 patients (12.7%) had neither detectable spironolactone nor canrenone levels and would be recognised as non-adherent by traditional approaches.

Using the first step of our approach, these patients are still considered nonadherent and we detected 6 more patients with lower levels of canrenone than of spironolactone. This renders 15 (21.1%) of non-adherent (Fig. 4). Additional 3 patients whose canrenone level did not fall into expected levels as simulated in Fig. 3 were detected in the second step. Therefore, we identified 53 (74.6%) adherent, 9 (12.7%) completely non-adherent patients and 9 (12.7%) patients with masked non-adherence making together 25.4% non-adherent, which is twice as many as would be unmasked by the most sensitive traditional approach.

Moreover, 13 patients (18.3%) had undetectable levels of spironolactone most probably due to the low dose and rapid elimination but canrenone levels proved that they were adherent. Periodically monitored patients with a confirmed diagnosis of EH (n=37; 32 adherent) were

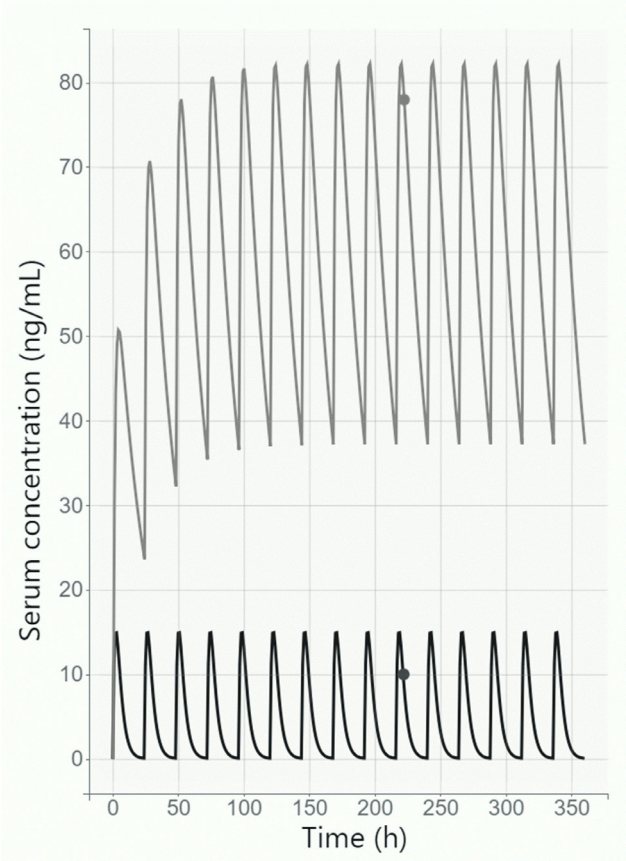

Fig. 1. Simulation of spironolactone (gray curve) and canrenone (black curve) serum concentration-time profiles after per oral administration of spironolactone in dose of 50 mg every 24 h based on population pharmacokinetic data[10,12,19].
Black dots are spironolactone (ng/mL) and canrenone (ng/mL) expected steady-state levels 6 h after spironolactone administration.

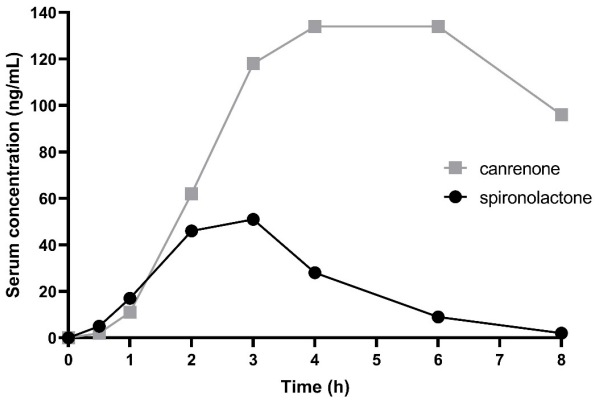

Fig. 2. Simulation of spironolactone (black curve) and canrenone (gray curve) serum concentration-time profiles in first 8 h after per oral single dose administration of spironolactone 100 mg (adapted from[10]).

significantly more adherent (P<0.0277) than single-visit patients without regular follow up (n=34, 21 adherent).

DISCUSSION

In the traditional approach to adherence assessment by chemical analysis of urine and/or blood samples, those with detectable drug concentrations are identified as adherent to therapy[5]. This assumption has its limits especially when it comes to masked non-adherence where a drug is ingested just once before a scheduled appointment and a sample drawn or generally when the drug is taken in an irregular pattern.

Our method shows that roughly one quarter of patients with resistant hypertension was not adherent to treatment. This is in line with recent systematic review and meta-analysis of studies investigating non-adherence to antihypertensive drugs. The authors found out prevalence of 27–40% depending on the method used[20]. Assessment of drug adherence by drug detection in blood is currently considered as most accurate and objective method of adherence testing. Nevertheless, studies using biochemical assays revealed lower prevalence of non-adherence than other methods, which might be influenced by their use in high-income countries where better adherence is reported or a bias caused by irregular usage[2,20]. Our study shows that simple detection of the drug or its metabolites in blood does not fully reveal white-coat adherence effect and that drug's pharmacokinetics should be considered when interpreting the measured concentration. As drug detection in blood samples belongs to more expensive methods, it is desirable to improve its sensitivity in non-adherence testing with more precise evaluation.

Our study is targeted at improvement of adherence control in patients treated for arterial hypertension with spironolactone by means of pharmacokinetic methods that are used for interpretation of parent drug and metabolite levels. For this purpose we built a PK model with PK data obtained from the SmPC and the literature[10,12,19] which confirms that during repeated administration, canrenone may never exhibit lower absolute serum concentrations than its parent drug spironolactone due to its longer half-life (Fig. 1). This allows direct detection of patients who self-administered spironolactone only once not long before sampling and fail to metabolise most of the spironolactone to canrenone in this short time. We further analysed the concentrations of spironolactone and canrenone in a group of adherent patients (Group 1) and found high correlation of weight-normalised administered dose with canrenone concentration with low interpatient variability. This allows us to create a body weight-normalized spironolactone dose / canrenone level nomogram (Fig. 3) which can be used to further evaluate whether the measured canrenone concentration represents the real value obtainable in steady state during long-term treatment with given spironolactone dose or whether it represents cumulative PK phase after single or irregular drug self-administration.

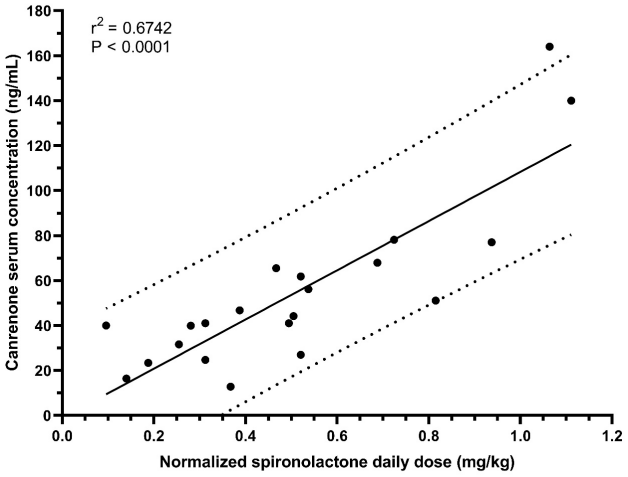

Fig. 3. Relationship between canrenone serum levels (blood collection 6 h after spironolactone administration) and body weight-normalized spironolactone daily dose administered before measurement. Dotted lines define the 90% prediction interval.

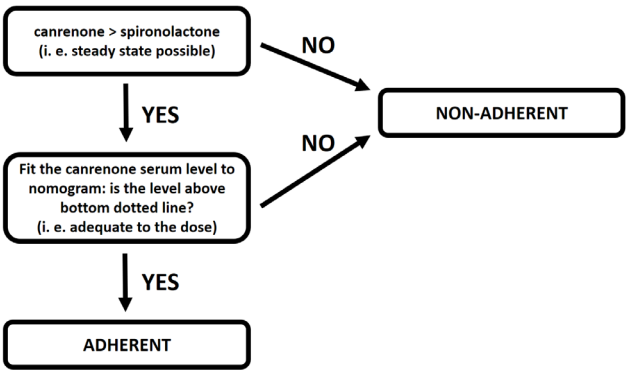

Fig. 4. A two-step approach to assessment of adherence to spironolactone therapy. Patient is considered non-adherent if canrenone level is lower than spironolactone (including both levels being non-measurable) or if canrenone levels do not match expected values according to normalized spironolactone dose.

We combined both approaches into a simple two-step approach by which we unveiled the non-adherence in twice as high a number of patients compared to traditional approach where undetectable level of drug and metabolite only was considered. We therefore confirmed that pharmacokinetic modelling may significantly improve the notice value of drug samples and that monitoring of drug metabolites may even further improve this method at least for drugs with short half-life. Moreover, we also confirmed the importance of measuring both spironolactone and canrenone concentrations – patients that had expected levels of canrenone even though their level of spironolactone was unmeasurable might have been falsely regarded as non-adherent without canrenone concentration measurement. Measuring only canrenone, on the other hand, would not reveal masked non-adherence in patients through comparison with parental drug level.

A similar approach may also be useful for many other drugs used in preventive medicine as they frequently have metabolites with long half-lives which may help during evaluation of a patient's adherence. This is true for example for atorvastatin and its lactone[21], ezetimibe and its glucuronide[22] etc. It can be simply said that the longer the half-life (of a drug or a metabolite), the more the difference between serum concentrations after single dose and multiple dose administration.

Our study has some limitations – step 2 of the adherence assessment (Fig. 3) may not reveal non-adherence with 95% assurance in individuals with a daily dose of spironolactone less than 0.35 mg/kg. Nevertheless, despite this theoretical expectation, all such patients from Group 2 had measurable canrenone concentrations.

Furthermore, the levels were drawn during a similar daily time and the spironolactone ingestion was not thoroughly controlled and may even in adherent patients, vary as only "morning" administration was recommended and medication was self-administered. This may bring some variability into the measured levels. Nevertheless, this possible small irregularity did not affect the levels of canrenone from having a high correlation with normalised administered spironolactone dose ($r^2 = 0.6742$) in Group 1 patients that served for development of nomogram for adherence assessment. This also shows that when a drug or a metabolite has a long half-life and consequently low plasma level fluctuations in steady state, the imprecision in sample draw time does not hamper its use for further analysis which again makes our approach of using metabolites with the longest possible half-lives even more practical for regular clinical use.

CONCLUSION

During our study we developed a robust and reliable method of evaluating spironolactone adherence. Our method was shown as more sensitive than standard approaches based on parent drug concentrations only without PK considerations. For further studies of drug adherence, not only concentrations of parent drugs but also their metabolites should be used as they may allow more precise determination of drug use behavior and thus reveal potential white-coat adherence in clinical practice.

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Author contributions: AP, JMH: formulated the method and prepared the manuscript for publication; MS: performed pharmacokinetic simulation and statistical analysis including nomogram; OS: provided pharmacological advices; IK, VM: analyzed the samples; JW, TMPNN: designed the study and organized data collection.

Conflict of interest statement: The authors have no conflicts of interest to declare.

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