Mineralocorticoid Receptor Antagonist Effect on Aldosterone to Renin Ratio in Patients with Primary Aldosteronism

Mineralocorticoid receptor antagonists effect on Aldosterone to REnin ratio for primary Aldosteronism: the MAREA study.

Alessio Pecori[1]*, Fabrizio Buffolo[1]*, Jacopo Burrello[1], Giulio Mengozzi[2], Francesca Rumbolo[2], Valeria Avataneo[3], Antonio D'Avolio[3], Franco Rabbia[1], Chiara Bertello[1], Franco Veglio[1], Paolo Mulatero[1#], and Silvia Monticone[1#].

[1]Division of Internal Medicine and Hypertension Unit, Department of Medical Sciences, University of Torino, Torino, Italy.

[2]Department of Laboratory Medicine, AOU Città della Salute e della Scienza, Turin, Italy.

[3]Laboratory of Clinical Pharmacology and Pharmacogenetics, Department of Medical Sciences, University of Turin, Amedeo di Savoia Hospital, Turin, Italy.

*These authors contributed equally to this work and should be considered as joint first authors.

[#]These authors contributed equally to this work and should be considered as joint last authors.

Correspondence: Paolo Mulatero, MD, Division of Internal Medicine and Hypertension, Department of Medical Sciences, University of Torino, Via Genova 3, 10126 Torino, Italy. Tel: +39 116336997; fax: +39 116336931; e-mail: paolo.mulatero@unito.it

Keywords: mineralocorticoid receptor antagonists; canrenone; aldosterone; renin; primary aldosteronism; hypertension.

All authors have read and approved submission of the manuscript. Material in the manuscript has not been published and is not being considered for publication elsewhere.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abstract

Purpose: We aimed to evaluate the effect of mineralocorticoid receptor antagonists on aldosterone-to-renin ratio in patients with primary aldosteronism.

Methods: We prospectively enrolled 121 patients with confirmed primary aldosteronism who started a mineralocorticoid receptor antagonist (canrenone) treatment. Eighteen patients (11 with unilateral and 7 with bilateral primary aldosteronism) composed the short-term study cohort and underwent aldosterone, renin and potassium measurement after 2 and 8 weeks of canrenone therapy. The long-term cohort comprised 102 patients (16 with unilateral and 67 with bilateral primary aldosteronism, and 19 with undetermined subtype) who underwent hormonal and biochemical re-assessment after 2 to 12 months of canrenone therapy.

Results: Renin and potassium levels showed a significant increase, and aldosterone-to-renin ratio displayed a significant reduction compared with baseline after both a short and long-term treatment. These effects were progressively more evident with higher doses of canrenone and after longer periods of treatment. We demonstrated that canrenone exerted a deep impact on the diagnostic accuracy of the screening test for primary aldosteronism: the rate of false-negative tests raised to 16.7%, 38.9%, 54.5% and 72.5% after 2 weeks, 8 weeks, 2-6 months and 7-12 months of mineralocorticoid receptor antagonist treatment, respectively.

Conclusions: Mineralocorticoid receptor antagonists should be avoided in patients with hypertension before measurement of renin and aldosterone for screening of primary aldosteronism.

Introduction

Primary aldosteronism (PA) is the most frequent form of endocrine hypertension and it is diagnosed in 5.9% of the general hypertensive population (1) and in 11.2% of patients admitted to hypertension referral centers (2). Patients with PA display an increased risk for cerebrovascular, cardiovascular and renal complications compared with individuals with essential hypertension (3,4).

Despite being a highly prevalent and curable form of hypertension, PA remains largely underdiagnosed and undertreated (5,6). According to current guidelines, around 50% of hypertensive individuals display a substantial probability of being affected by PA, and screening should be offered to these patients (7,8). Measurement of plasma renin activity (PRA) and aldosterone concentration (AC), and calculation of the aldosterone-to-renin ratio (ARR) are the mainstay of PA screening work-up. In order to avoid false positive or negative ARR results, the current guidelines recommend to withdraw drugs interfering with renin-angiotensin-aldosterone system (RAAS) before ARR assessment (7,8). In particular, mineralocorticoid receptor antagonists (MRAs), by means of natriuresis and contraction of plasma volume, stimulate RAAS activation, thus renin elevation and ARR reduction (9). Because of the potential of MRA-related false negative results, present recommendations are to stop MRAs for up to 4 to 6 weeks before screening test for PA is performed (7,8). Nevertheless, recent reports suggested that diagnosis and subtyping of PA might be achieved in selected patients with florid PA in whom MRA withdrawal is not feasible because of uncontrolled hypertension and/or severe hypokalemia, and in whom renin levels remain suppressed despite ongoing MRA treatment (10-12).

From another standpoint, Hundemer et al recently reported that patients with PA treated with an MRA and displaying increase of renin activity showed a reduced risk of mortality, cardiovascular events and incident atrial fibrillation compared with PA patients whose PRA remained low despite MRA therapy (13,14). Therefore, elevation of renin levels might represent a biochemical marker of adequate mineralocorticoid receptor (MR) blockade (15).

The aim of our study was to investigate the effects of MRA treatment on the accuracy of PA screening test in a cohort of patients with confirmed PA and the time and dose-dependent effects of MRA on ARR.

Materials and Methods

1. Study Design and Patients

The research protocol was approved by our local Ethics Committee. All recruited patients gave written informed consent.

Patients referred to the University of Torino Hypertension Center were prospectively enrolled. Inclusion criteria were: (1) age between 18 and 75 years; (2) confirmed diagnosis of PA; (3) medical treatment with an MRA (canrenone). Exclusion criteria were: (1) concomitant intake of RAAS-interfering drugs, such as beta-blockers, diuretics, angiotensin-converting enzyme inhibitors (ACE-Is) and angiotensin II-receptor blockers (ARBs); (2) patient refusal.

The study cohort was composed of two subgroups of patients. The first one (long-term MRA treatment group) comprised patients with PA not eligible for adrenalectomy (ADX) because of (1) bilateral primary aldosteronism (BiPA), (2) delayed or refused surgery in those affected by unilateral primary aldosteronism (UPA), or (3) patients with undetermined subtype diagnosis. PRA, AC and ARR were assessed at baseline and after 2 to 12 months of MRA treatment at follow-up visit to evaluate the treatment efficacy. The second group (short-term MRA treatment group) was composed of (1) patients with both PA subtypes who initiated MRA treatment because of surgery ineligibility, and (2) patients affected by UPA that started MRA awaiting ADX. Plasma laboratory assessments were performed after 2 and 8 weeks of MRA therapy.

We prospectively recruited 121 patients with confirmed PA who started MRA treatment (Figure 1). One patient of the short-term cohort was lost to follow-up and excluded from the analysis. The long-term MRA treatment subgroup was composed of 102 patients (67 affected by BiPA, 16 affected by UPA and 19 PA patients with an undetermined subtype diagnosis), while the short-term MRA treatment subgroup comprised 18 patients (7 with BiPA and 11 with UPA awaiting surgery). Baseline clinical and biochemical data of the included patients are reported in Table 1.

Blood pressure measurements according to European Society of Hypertension guidelines (16), clinical evaluation, and biochemical assessment of PRA, AC and potassium levels were obtained for all patients at follow-up (after 2 and 8 weeks of MRA therapy in the short-term treatment group, and after 2 to 12 months for the long-term group) (Supplementary Tables 1-2) (17).

To estimate medication adherence and its potential interference with ARR results, therapeutic drug monitoring (TDM) of canrenone was performed in a proportion of patients (37.3% of the long-term cohort and 100% of the short-term cohort, both after 2 and 8 weeks of therapy).

2. Diagnosis of Primary Aldosteronism

PA was diagnosed according to current Endocrine Society and European Society of Hypertension recommendations (7,8). Briefly, screening testing was performed after withdrawal of all RAAS-interfering antihypertensive drugs (at least for 2 weeks for beta-blockers, ACE-Is and ARBs, and 4 weeks for diuretics; no patients were under MRA) and non-interfering drugs (such as calcium-channel blockers or alpha-1 blockers) were used to control blood pressure, if necessary. The screening test was considered positive if AC $\geq$ 10 ng/dL and ARR $\geq$ 30 ng/dL/ng/mL/h. PA diagnosis confirmation was obtained by seated saline infusion test (SSIT) or captopril challenge test (CCT), whenever the former was contraindicated. Subtype diagnosis was performed by contrast-enhanced computed tomography of the adrenal glands and unstimulated and/or ACTH-stimulated adrenal vein sampling (18).

3. Biochemical measurements

Plasma renin activity and aldosterone concentration were measured as previously described (1). TDM of canrenone was performed on urine samples obtained at the same time of blood collection for follow-up assessments, and biochemical analyses were run as previously described (19).

4. Statistical Analysis

IBM SPSS Statistics version 26.0 (IBM Corp, Armonk, New York) and GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, California) were used for statistical analyses. Data were expressed as mean ± standard deviation for continuous variables with a normal distribution, or median [interquartile range] for variables with a non-normal distribution. Normally distributed data

were compared with Student *t* test for independent variables or with one-way ANOVA with Bonferroni post hoc test in case of unpaired samples, and with Student *t* test for dependent variables or repeated measures one-way ANOVA with Holm-Sidak post hoc test in case of matched samples. Non-normally distributed data were compared with Mann–Whitney U test or with Kruskal-Wallis test and Dunn post hoc test in case of independent samples, and with Friedman test with Dunn post hoc test in case of matched samples. Categorical variables were expressed as absolute number and percentage, and compared with χ^2 test or Fisher exact test, as appropriate. P values < 0.05 were considered statistically significant.

Results

1. Short-term effects of MRA on ARR in patients with PA

Nineteen patients with PA were recruited in the short-term study cohort. One patient was lost to follow-up after the 2-week assessment and excluded from the analysis (this patient also resulted not compliant to canrenone therapy when evaluated with TDM). Of the 18 patients who underwent the short-term follow-up, 11 (61.1%) were affected by UPA and 7 by BiPA (38.9%) (Table 1). As expected, systolic and diastolic blood pressure values were significantly reduced by MRA treatment (Supplementary Table 1) (17). Potassium levels were significantly higher after both 2 and 8 weeks (+8.6% and +14.3%, respectively) of canrenone therapy compared with baseline values (p=0.011) (Figure 2A). Similarly, PRA levels showed a significant 56.7% and 140% increase after 2 and 8 weeks of MRA treatment, respectively (p<0.001) (Figure 2B). Follow-up AC was not significantly different compared with baseline (Figure 2C). As a result, ARR values were reduced by MRA treatment (Figure 2D), thus leading to an increase in the rate of false-negative screening tests (p=0.010) (Figure 3A). The rate of false negative tests was 16.7% (3/18) after 2 weeks and 38.9% (7/18; p=0.008) after 8 weeks of canrenone therapy (Figure 3A, Supplementary Table 1) (17).

2. Long-term effects of MRA on ARR in patients with PA

The long-term MRA treatment cohort comprised 102 patients: 16 affected by UPA (15.7%), 67 affected by BiPA (65.7%) and 19 (18.6%) patients with undetermined subtype (Table 1). Clinical and biochemical follow-up was performed after 2 to 12 months of canrenone therapy. To stratify the long-term effects of MRA on RAAS activity, this subgroup of patients was further divided according to the length of follow-up (2 to 6 months and 7 to 12 months). Blood pressure was reduced ($p<0.001$) (Supplementary Table 2) (17) and potassium levels significantly increased by MRA treatment ($p<0.001$) (Figure 4A). Of note, the majority of potassium values at follow-up (95%) fell within the normal range. MRA treatment led to a significant increase of PRA values both at 2-6 months (+315%) and 7-12 months (+1050%) compared with baseline ($p<0.001$) (Figure 4B). Canrenone effect on PRA levels was progressively more evident over time, with PRA values after 7-12 months significantly higher than values after 2-6 months ($p=0.015$). (Figure 4, Supplementary Table 2) (17). AC remained elevated and was not significantly affected by long-term therapy with canrenone (Figure 4C). ARR was significantly reduced by MRA long-term treatment (-76% and -90.1% after 2-6 and 7-12 months, respectively; $p<0.001$) (Figure 4D). The rate of false-negative tests considerably increased to 54.5% (18/33) and 72.5% (50/69) after 2-6 and 7-2 months of MRA treatment, respectively ($p<0.001$) (Figure 3B; Supplementary Table 2) (17).

3. Effects of different MRA doses on ARR in patients with PA

We also evaluated the effect of different doses of canrenone on the ARR. Potassium levels and PRA showed a significant progressive increase compared with baseline in patients treated with increasing MRA doses ($p< 0.001$) (Figure 5A-B) (Supplementary Table 3) (17). In particular, PRA levels showed an increase of 75% to 1650% compared with baseline in patients treated with 12.5 mg to 100 mg/day of canrenone, respectively. Despite a moderate increase in AC levels (Figure 5C), ARR values progressively decreased with increasing MRA doses (-52.9% to -92.4%) (Figure 5D). Accordingly, we observed a progressive increase in the rate of false-negative screening tests with

higher doses of canrenone, up to 86.7% in patients with PA treated with 100 mg/day of canrenone (Figure 3C; Supplementary Table 3) (17).

4. Effects of drug adherence on ARR

Canrenone TDM was performed on a proportion of patients to evaluate treatment compliance and effects of non-adherence on biochemical parameters. Seventy-five TDM tests were performed: 1 patient from the short-term cohort was lost to follow-up and excluded from the analysis (urinary canrenone was absent in this patient). Of the remaining 74 TDM tests, 38 were performed on patients of the long-term cohort and 36 in patients of the short-term cohort (all patients were evaluated after 2 and 8 weeks of MRA therapy). TDM showed non-adherence to canrenone in 12.2% of cases (9/74). We observed a significantly lower PRA (p=0.010) and markedly higher ARR values (p=0.003) in patients with a negative TDM compared with fully compliant patients (Supplementary Table 4) (17).

Discussion

The present study demonstrated a time and dose effect of MRA therapy on the ARR and a deep impact on the diagnostic accuracy as a screening test for PA.

Current guidelines recommend to withdraw antihypertensive medications potentially affecting renin and/or aldosterone levels before the performance of screening test for PA, to prevent false-negative or -positive results (7,8). MRAs induce volume contraction and increase of potassium levels, leading to RAAS activation with renin elevation and ARR reduction (9). Because of the potential of false negative PA screening tests, present recommendations are to stop MRAs for up to 4 to 6 weeks beforehand (7,8).

Some studies displayed the possibility of subtype diagnosis determination by adrenal vein sampling in patients with florid phenotypes, even without MRA withdrawal (10,11), as long as renin levels are still suppressed before the procedure (20). A recent study performed in 42 patients (32 with

206 UPA and 10 with BiPA) reported that 1-month therapy with the MRA canrenone did not interfere
207 significantly with ARR values and with interpretation of the screening (2/42 false negative results,
208 5%); after addition of olmesartan to canrenone for further 4 weeks the rate of false negative
209 diagnoses increased to 8/34 (23.5%) (12). From these data it was not possible to determine if the
210 effect on the ARR after two months was due to the addition of olmesartan or the longer duration of
211 canrenone therapy or both.
212 The aim of the present study was to investigate the effects of MRA treatment on ARR in patients
213 with confirmed PA, and the impact on the accuracy of the screening test.
214 We observed a significant increase of PRA levels already after 2 weeks of MRA therapy and this
215 effect was further magnified by the duration of the treatment up to 12 months. Since aldosterone
216 levels were only marginally affected, ARR displayed a progressive decrease depending on the
217 duration of the MRA therapy. As a result, the rate of false-negative screening tests increased from
218 16.7 to 72.5% after 2 weeks to 12 months of therapy. As expected, the ARR also decreased
219 progressively with the increase of MRA dosage from 30.8% with 12.5 mg of canrenone to 86.7%
220 with 100 mg/day.
221 Our results are in agreement with previous studies (21-25), showing a significant and early increase
222 in PRA levels in patients with PA treated with MRA, resulting in a significant increase in the rate of
223 false-negative screening tests.
224 Even though these effects were evident after just two weeks of MRA therapy, the reduction of ARR
225 appeared to be progressive and incremental over time (21,25). Several explanations were proposed
226 for the gradual increase of renin levels during MRA treatment. First, complete abrogation of the
227 negative feed-back of chronic hyperaldosteronism on RAAS activity might take some time to be
228 observed: the increase of renin release caused by tubuloglomerular feedback activation, in turn
229 driven by MRA-related volume depletion, might be apparent over time (26). Second, canrenone
230 might require a discrete period of time to fully exert its MR blockade effects for the relatively lower
231 affinity for the mineralocorticoid receptor compared with spironolactone (27).

Discrepancies between our findings and results from the EMIRA study (12) might be potentially related to its design: persistent suppression of renin might result from the limited time interval (1 month) between MRA initiation and hormonal follow-up, and the recruitment of patients affected by florid PA phenotype requiring high doses of MRA and prolonged treatment to achieve complete MR blockade.

We also investigated MRA drug adherence in a proportion of recruited patients, demonstrating 12.2% of absent compliance to the treatment. This is not surprising since partial/total non-adherence was observed in 20-60% of patients with hypertension (28). As expected, PRA levels were significantly lower and ARR was significantly higher in non-adherent patients compared with fully compliant. We may speculate that part of the lack of the effect of MRA therapy on renin levels in some patients with PA might be due variable degrees of reduced treatment compliance.

Hundemer et al recently demonstrated that increase of renin levels was related to significant reduction in mortality risk, cardiovascular events rate and atrial fibrillation incidence in patients with PA on MRA treatment (13,14). Renin elevation and ARR reduction might then serve as biochemical markers of adequate MR blockade and potential predictors of favorable clinical outcome (15).

Even though our study design and the limited duration of our follow-up were not appropriate to investigate clinical outcomes of PA patients treated with MRAs, we believe our results are supportive of some important insights. First, renin levels should be monitored and MRA treatment up-titrated by the physician to reach the required dose not only to control hypertension and hypokalemia but also to obtain increase of renin levels. Second, TDM may be considered to assess patient compliance to MRA treatment and to guide therapy optimization when renin levels remain suppressed. Lastly, PRA and ARR re-assessment should be performed after an appropriate time span from MRA initiation in order to allow the completion of MR blockade effects and subsequently adjust antihypertensive therapy whenever necessary.

The current study has some limitations. We did not recruit a control group of patients with PA on antihypertensive treatment without MR blockade to evaluate potential MRA-independent ARR modifications over time. We also did not assess MRA compliance in the whole study cohort, although our TDM estimation might suggest that drug adherence was around 88%, thus likely not interfering our conclusions on ARR assessments. Finally, in the long-term cohort the time of the follow-up and the dose of MRA were non standardized and left to the clinicians' judgement: however, this represents a "real life" situation of what happens during the long-term medical treatment of patients with PA.

In conclusion, the results of the present study indicate that MRAs display a marked and rapid effect on ARR and therefore, the screening test should be performed after appropriate withdrawal of MRAs. Moreover, our findings support that renin elevation should be used as a biochemical marker of efficient MR blockade.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request. Supplemental materials (17) are available at the following link: https://github.com/CentroIpertenUnito/MAREA/raw/main/Supplemental%20materials.pdf.

References

1. Monticone S, Burrello J, Tizzani D, et al. Prevalence and clinical manifestations of primary aldosteronism encountered in primary care practice. *J Am Coll Cardiol*. 2017;69:1811-1820.
2. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1125 hypertensive patients. *J Am Coll Cardiol*. 2006;48:2293-2300.

3. Monticone S, D'Ascenzo F, Moretti C, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: A systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. 2018;6:41-50.
4. Monticone S, Sconfienza E, D'Ascenzo F, et al. Renal damage in primary aldosteronism: A systematic review and meta-analysis. *J Hypertens*. 2020;38:3-12.
5. Mulatero P, Monticone S, Burrello J, Veglio F, Williams TA, Funder J. Guidelines for primary aldosteronism: uptake by primary care physicians in Europe. *J Hypertens*. 2016;34:2253-2257.
6. Cohen JB, Cohen DL, Herman DS, Leppert JT, Byrd JB, Bhalla V. Testing for Primary Aldosteronism and Mineralocorticoid Receptor Antagonist Use Among U.S. Veterans: A Retrospective Cohort Study. *Ann Intern Med*. 2020;Epub ahead of print. doi:10.7326/M20-4873.
7. Mulatero P, Monticone S, Deinum J, et al. Genetics, prevalence, screening and confirmation of primary aldosteronism: a position statement and consensus of the Working Group on Endocrine Hypertension of The European Society of Hypertension. *J Hypertens*. 2020;38(10):1919-1928.
8. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2016;101:1889-1916.
9. Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the aldosterone/renin ratio. *Horm Metab Res*. 2012;44:170-176.
10. Haase M, Riester A, Kröpil P, et al. Outcome of adrenal vein sampling performed during concurrent mineralocorticoid receptor antagonist therapy. *J Clin Endocrinol Metab*. 2014;99(12):4397-4402.

11. Nanba AT, Wannachalee T, Shields JJ, et al. Adrenal vein sampling lateralization despite mineralocorticoid receptor antagonists exposure in primary aldosteronism. *J Clin Endocrinol Metab*. 2019;104(2):487-492.
12. Rossi GP, Ceolotto G, Rossitto G, Maiolino G, Cesari M, Seccia TM. Effects of Mineralocorticoid and AT1 Receptor Antagonism on The Aldosterone-Renin Ratio In Primary Aldosteronism-the EMIRA Study. *J Clin Endocrinol Metab*. 2020;105(6):dgaa080.
13. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes Endocrinol*. 2018;6(1):51-59.
14. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Incidence of Atrial Fibrillation and Mineralocorticoid Receptor Activity in Patients With Medically and Surgically Treated Primary Aldosteronism. *JAMA Cardiol*. 2018;3(8):768-774.
15. Mulatero P, Sechi LA, Williams TA, et al. Subtype diagnosis, treatment, complications and outcomes of primary aldosteronism and future direction of research: a position statement and consensus of the Working Group on Endocrine Hypertension of the European Society of Hypertension. *J Hypertens*. 2020;38(10):1929-1936.
16. Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J*. 2018;39(33):3021-3104.
17. Pecori A, Buffolo F, Burrello J, et al. Data from: Mineralocorticoid receptor antagonists effect on Aldosterone to REnin ratio for primary Aldosteronism: the MAREA study. *J Clin Endocrinol Metab*. 2021 https://github.com/CentroIpertenUnito/MAREA. Accessed March 15, 2021.
18. Burrello J, Burrello A, Pieroni J, et al. Development and Validation of Prediction Models for Subtype Diagnosis of Patients With Primary Aldosteronism. *J Clin Endocrinol Metab*. 2020;105(10):dgaa379.

19. Avataneo V, De Nicolò A, Rabbia F, et al. A simple UHPLC-PDA method with a fast dilute-and-shot sample preparation for the quantification of canrenone and its prodrug spironolactone in human urine samples. *J Pharmacol Toxicol Methods*. 2018;94(Pt 2):29-35.
20. Monticone S, Viola A, Rossato D, et al. Adrenal vein sampling in primary aldosteronism: towards a standardised protocol. *Lancet Diabetes Endocrinol*. 2015;3(4):296-303.
21. Sakamoto H, Ichikawa S, Sakamaki T, et al. Time-related changes in plasma adrenal steroids during treatment with spironolactone in primary aldosteronism. *Am J Hypertens*. 1990;3(7):533-537.
22. Seifarth C, Trenkel S, Schobel H, Hahn EG, Hensen J. Influence of antihypertensive medication on aldosterone and renin concentration in the differential diagnosis of essential hypertension and primary aldosteronism. *Clin Endocrinol (Oxf)*. 2002;57(4):457-465.
23. Parthasarathy HK, Ménard J, White WB, et al. A double-blind, randomized study comparing the antihypertensive effect of eplerenone and spironolactone in patients with hypertension and evidence of primary aldosteronism. *J Hypertens*. 2011;29(5):980-990.
24. Pilz S, Trummer C, Verheyen N, et al. Mineralocorticoid Receptor Blockers and Aldosterone to Renin Ratio: A Randomized Controlled Trial and Observational Data. *Horm Metab Res*. 2018;50(5):375-382.
25. Tezuka Y, Turcu AF. Mineralocorticoid Receptor Antagonists Decrease the Rates of Positive Screening for Primary Aldosteronism. *Endocr Pract*. 2020;26(12):1416-1424.
26. Armanini D, Sabbadin C, Boscaro M. Primary aldosteronism: considerations about the evaluation of the aldosterone to renin ratio during canrenone treatment. *J Endocrinol Invest*. 2021;Epub ahead of print. doi:10.1007/s40618-021-01500-z.
27. Armanini D, Sabbadin C, Donà G, Clari G, Bordin L. Aldosterone receptor blockers spironolactone and canrenone: two multivalent drugs. *Expert Opin Pharmacother*. 2014;15(7):909-912.

28. Berra E, Azizi M, Capron A, et al. Evaluation of Adherence Should Become an Integral Part of Assessment of Patients With Apparently Treatment-Resistant Hypertension. *Hypertension*. 2016;68(2):297-306.

Legends for Figures and Tables

Figure 1 – Study design and flowchart of study cohort. Abbreviations: MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; RAAS, renin-angiotensin-aldosterone system.

Figure 2 – Short-term effects of MRA on potassium and hormonal parameters in patients with PA. Potassium levels (K+) (**Panel A**), plasma renin activity (PRA) (**Panel B**), aldosterone concentration (AC) (**Panel C**) and aldosterone-to-renin ratio (ARR) (**Panel D**) after 2 and 8 weeks (W) of mineralocorticoid receptor antagonist (MRA) therapy compared with baseline (B). Shadowed area indicates normal values for potassium, unsuppressed renin for PRA (> 1 ng/mL/h) and negative screening test for ARR (< 30 ng/dL/ng/mL/h). Solid horizontal lines represent median [IQR] for PRA, AC and ARR, and mean ± SD for potassium levels. Levels of significance are reported as follows: $0.01<p<0.05$ (*); $p<0.01$ (**); $p<0.001$ (***).

Figure 3 – Screening test results in patients with PA under MRA treatment. Rate of false negative screening tests for primary aldosteronism (PA) (in grey) after 2 and 8 weeks (W) (**Panel A**) and after 2-6 and 7-12 months (M) of canrenone therapy (**Panel B**). Rate of false negative screening tests for primary aldosteronism (PA) (in grey) with different doses of canrenone (12.5 to 100 mg/day) (**Panel C**).

Figure 4 – Long-term effects of MRA on potassium and hormonal parameters in patients with PA. Potassium levels (K+) (**Panel A**), plasma renin activity (PRA) (**Panel B**), aldosterone concentration (AC) (**Panel C**) and aldosterone-to-renin ratio (ARR) (**Panel D**) after 2-to-6 months (2-6 M) and 7-

to-12 months (7-12 M) of mineralocorticoid receptor antagonist (MRA) therapy compared with baseline (B). Shadowed area indicates normal values for potassium, unsuppressed renin for PRA (> 1 ng/mL/h) and negative screening test for ARR (<30 ng/dL/ng/mL/h). Solid horizontal lines represent median [IQR] for PRA, AC and ARR, and mean ± SD for potassium levels. Levels of significance are reported as follows: $0.01<p<0.05$ (*); $p<0.01$ (**); $p<0.001$ (***).

Figure 5 – Effects of different MRA doses on ARR in patients with PA. Potassium levels (K+) (**Panel A**), plasma renin activity (PRA) (**Panel B**), aldosterone concentration (AC) (**Panel C**) and aldosterone-to-renin ratio (ARR) (**Panel D**) after different doses (12.5 to 100 mg/day) of mineralocorticoid receptor antagonist (MRA) therapy compared with baseline (B). Shadowed area indicates normal values for potassium, unsuppressed renin for PRA (> 1 ng/mL/h) and negative screening test for ARR (<30 ng/dL/ng/mL/h). Solid horizontal lines represent median [IQR] for PRA, AC and ARR, and mean ± SD for potassium levels. Levels of significance are reported as follows: $0.01<p<0.05$ (*); $p<0.01$ (**); $p<0.001$ (***).

Table 1 - Values are mean ± SD, median [IQR], or absolute number (%). Abbreviations: AC, aldosterone concentration; ARR, aldosterone-to-renin ratio; BiPA, bilateral primary aldosteronism; DBP, diastolic blood pressure; DDD, daily defined dose; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure; UPA, unilateral primary aldosteronism.

Table 1 – Clinical and biochemical parameters of patients.

Entire Cohort	**n=120**
Age (years)	50 ± 9.3
Sex (male)	75 (62.5)
Subtype Diagnosis	
UPA	27 (22.5)
BiPA	74 (61.7)
SBP (mmHg)	156 ± 20.6
DBP (mmHg)	97 ± 12.3
DDD	2.0 [1.0; 3.0]
Potassium (mmol/L)	3.7 ± 0.55
PRA (ng/mL/h)	0.28 [0.10; 0.40]
AC (ng/dL)	28.1 [18.3; 37.0]
ARR (ng/dL/ng/mL/h)	119 [66; 178]

Short-term Cohort	**n=18**
Age (years)	54 ± 10.2
Sex (male)	16 (88.9)
Subtype Diagnosis	
UPA	11 (61.1)
BiPA	7 (38.9)
SBP (mmHg)	150 ± 15.7
DBP (mmHg)	88 ± 9.3
DDD	3.0 [2.5; 3.3]
Potassium (mmol/L)	3.5 ± 0.53
PRA (ng/mL/h)	0.30 [0.16; 0.34]
AC (ng/dL)	23.4 [17.9; 34.0]
ARR (ng/dL/ng/mL/h)	77 [63; 184]

Long-term Cohort	**n=102**
Age (years)	50 ± 9.1
Sex (male)	59 (57.8)
Subtype Diagnosis	
UPA	16 (15.7)
BiPA	67 (65.7)
SBP (mmHg)	157 ± 21.2
DBP (mmHg)	98 ± 12.1
DDD	2.0 [1.0; 3.0]
Potassium (mmol/L)	3.7 ± 0.55
PRA (ng/mL/h)	0.20 [0.10; 0.40]
AC (ng/dL)	29.0 [19.9; 37.1]
ARR (ng/dL/ng/mL/h)	121 [67; 179]

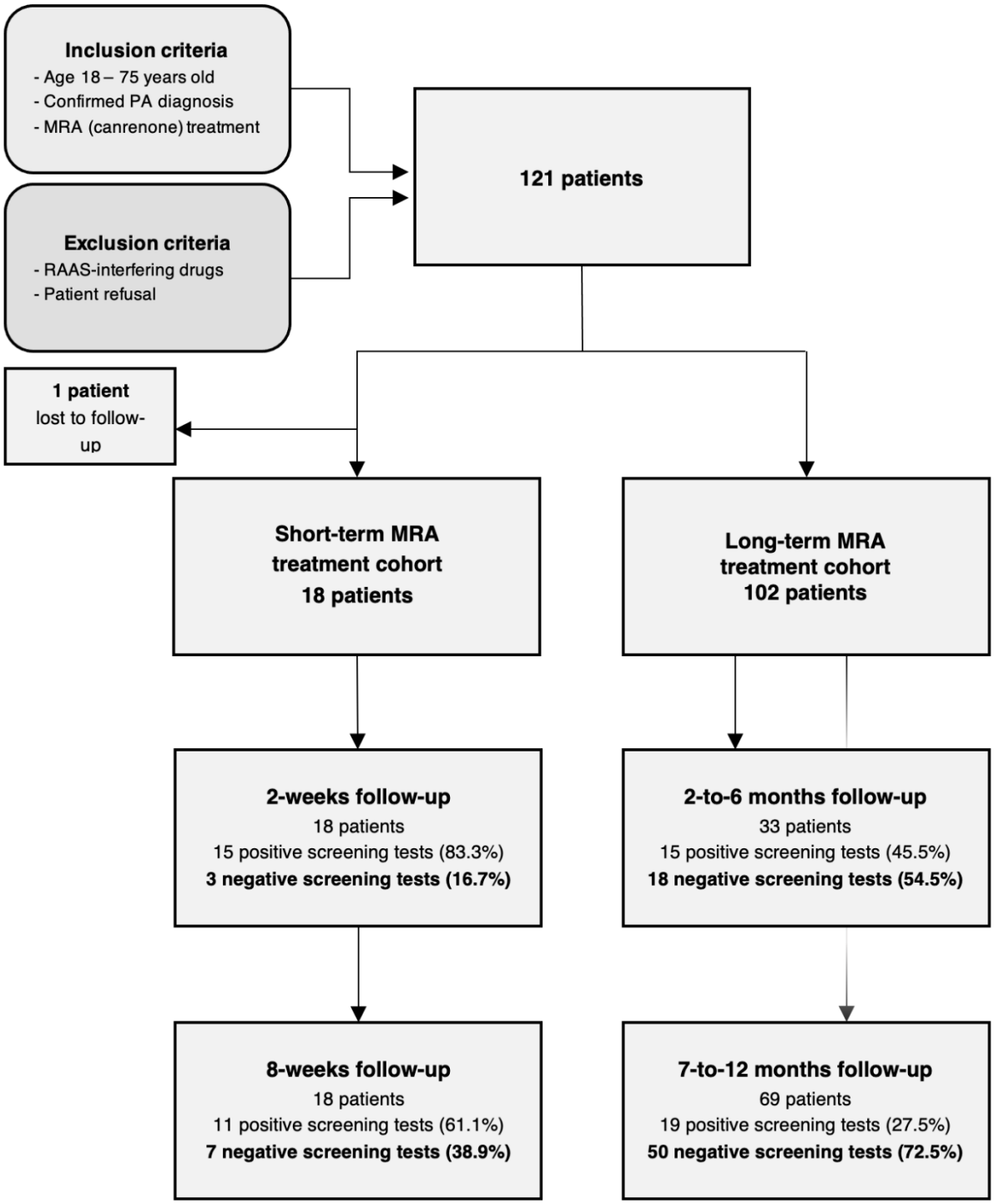
Inclusion criteria
- Age 18 – 75 years old
- Confirmed PA diagnosis
- MRA (canrenone) treatment
Exclusion criteria
- RAAS-interfering drugs
- Patient refusal
121 patients
1 patient
lost to follow-up
Short-term MRA
treatment cohort
18 patients
Long-term MRA
treatment cohort
102 patients
2-weeks follow-up
18 patients
15 positive screening tests (83.3%)
3 negative screening tests (16.7%)
2-to-6 months follow-up
33 patients
15 positive screening tests (45.5%)
18 negative screening tests (54.5%)
8-weeks follow-up
18 patients
11 positive screening tests (61.1%)
7 negative screening tests (38.9%)
7-to-12 months follow-up
69 patients
19 positive screening tests (27.5%)
50 negative screening tests (72.5%)

Figure 1

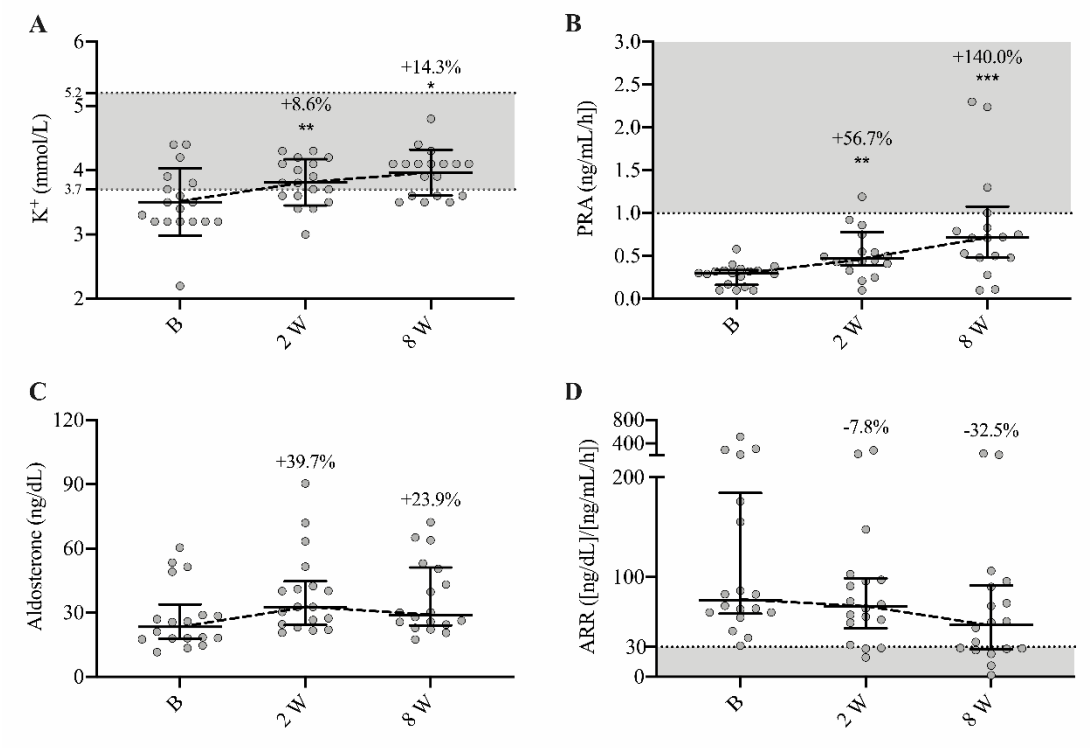

Figure 2

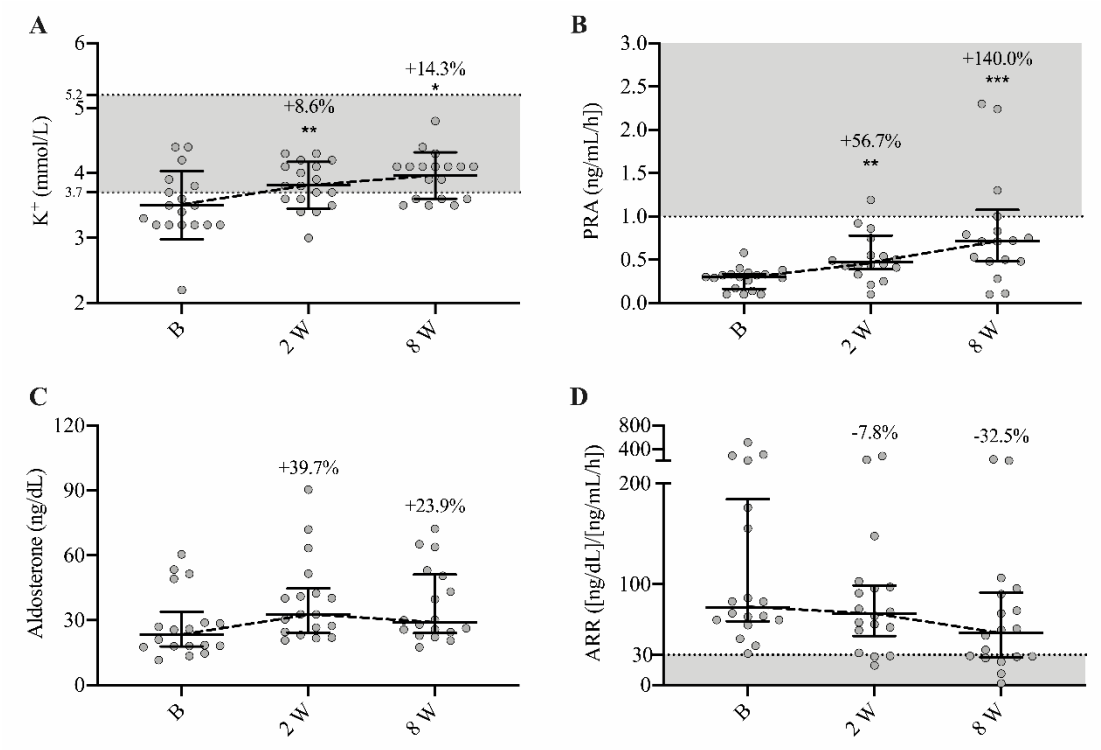

Figure 3

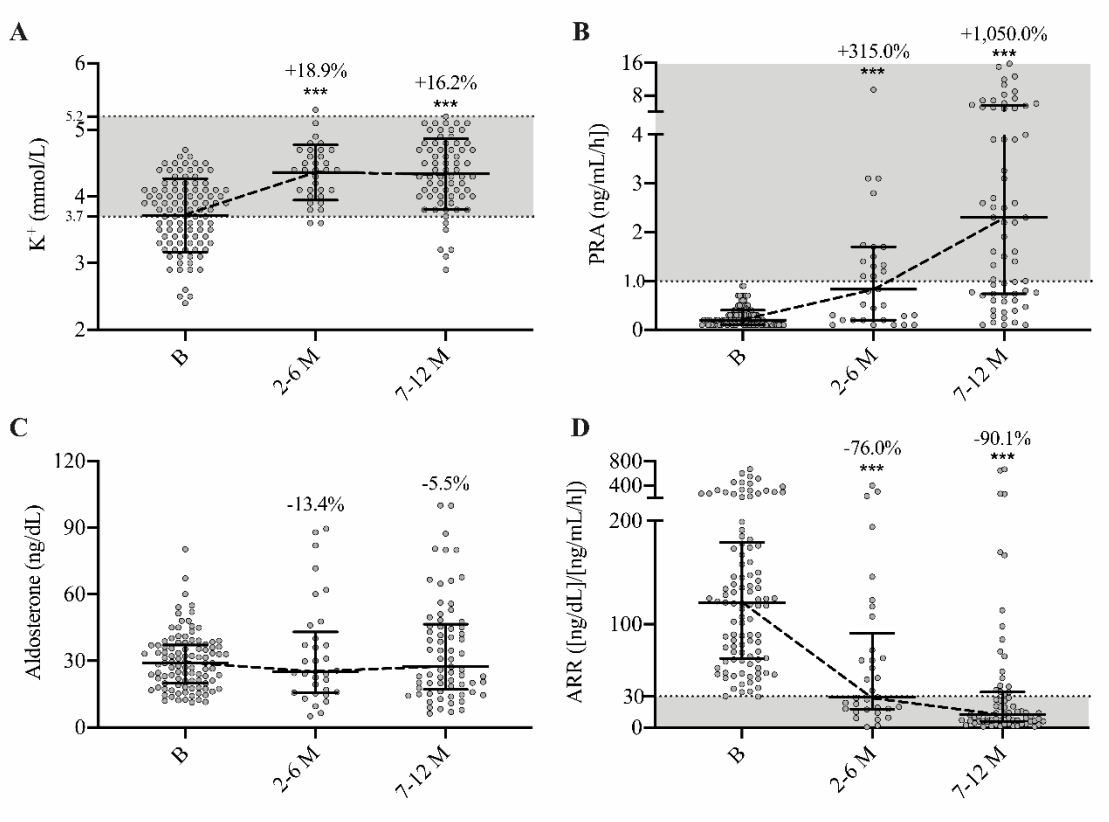

Figure 4

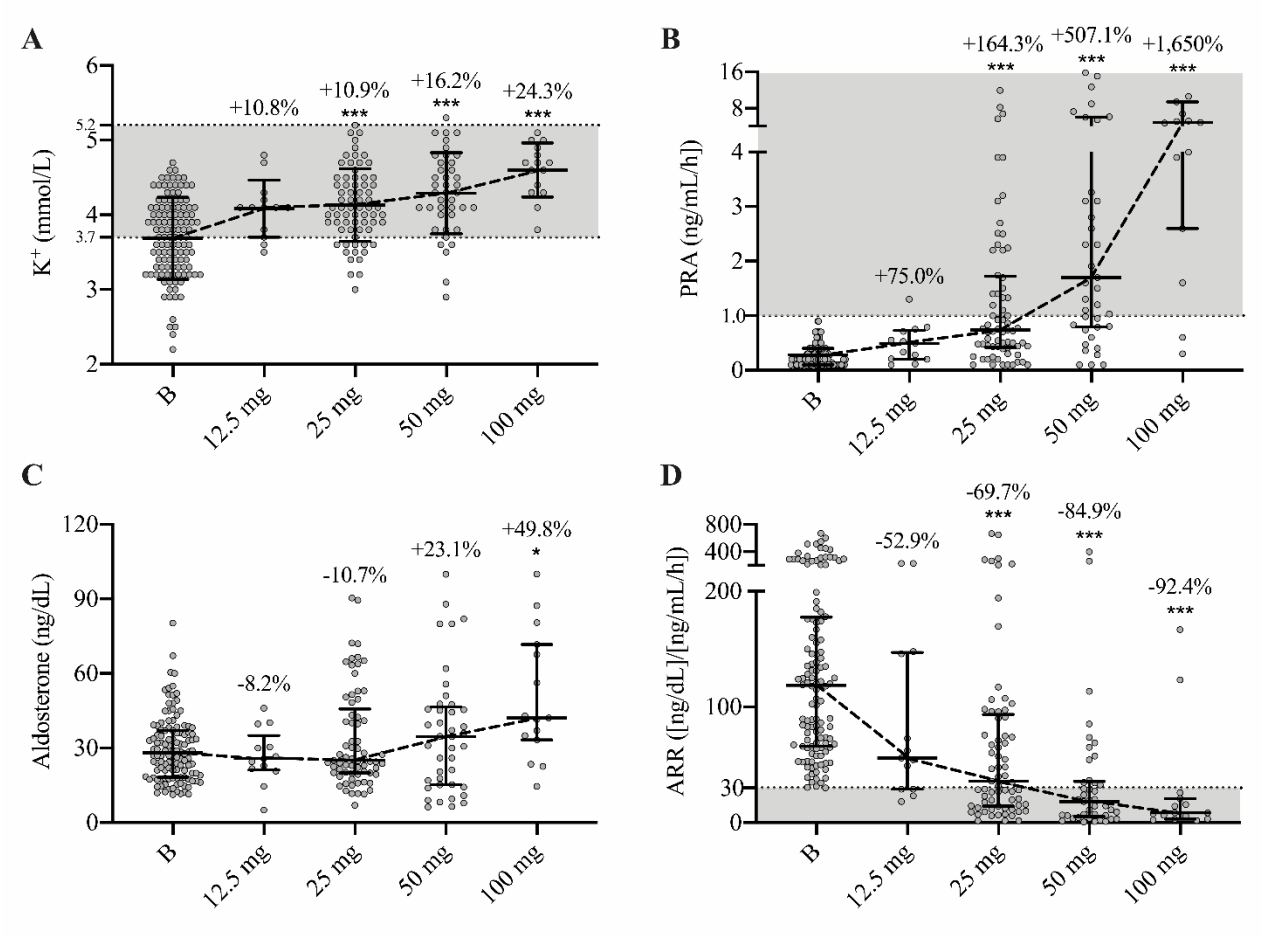

Figure 5