SUPPLEMENTAL MATERIALS

Mineralocorticoid receptor antagonist effect on aldosterone to renin ratio in patients with primary aldosteronism.

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Summary

Supplementary Table 1 – Clinical and biochemical parameters of the short-term MRA treatment cohort.

Variables	Baseline (n=18)	2 weeks (n=18)	8 weeks (n=18)	Overall *P*-value	Pairwise comparisons		
					B *vs.* 2W	B *vs.* 8W	2 *vs.* 8 W
MRA dose (mg)	N.A.	25 [12.5; 25]	25 [12.5; 25]	-	-	-	-
SBP (mmHg)	150 ± 15.7	143 ± 17.8	136 ± 18.3	**0.008**	0.189	**0.015**	0.189
DBP (mmHg)	88 ± 9.3	86 ± 9.0	82 ± 11.3	**0.038**	0.491	**0.046**	0.146
DDD	3.0 [2.5; 3.3]	2.9 [2.3; 3.3]	2.9 [2.3; 3.4]	0.209	-	-	-
Potassium (mmol/L)	3.5 ± 0.53	3.8 ± 0.36	4.0 ± 0.36	**0.011**	**0.008**	**0.025**	0.233
PRA (ng/mL/h)	0.30 [0.16; 0.24]	0.47 [0.39; 0.78]	0.72 [0.48; 1.08]	**<0.001**	**0.008**	**<0.001**	0.999
AC (ng/dL)	23.4 [17.9; 34.0]	32.7 [24.3; 44.7]	29.0 [24.1; 51.1]	0.056	-	-	-
ARR (ng/dL/ng/mL/h)	77 [63; 184]	71 [49; 99]	52 [28; 92]	0.154	-	-	-
ARR interpretation							
Positive test	18 (100.0)	15 (83.3)	11 (61.1)				
False negative test	0 (0.0)	3 (16.7)	7 (38.9)	**0.010**	0.229	**0.008**	0.264
FN UPA	N.A.	2 (11.1)	6 (33.3)				
vs. total UPA		2 of 11 (18.2)	6 of 11 (54.5)				

Clinical and biochemical characteristics of patients with PA from the short-term MRA treatment cohort (18 patients). Blood pressure measurements, clinical evaluation and biochemical assessments were obtained at baseline and after both 2 weeks and 8 weeks of canrenone therapy.

Values are mean ± SD, median [IQR], or absolute number (%).

Abbreviations: AC, aldosterone concentration; ARR, aldosterone-to-renin ratio; B, baseline; DBP, diastolic blood pressure; DDD, daily defined dose; FN, false negative; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure; UPA, unilateral primary aldosteronism; W, weeks.

Supplementary Table 2 – Clinical and biochemical parameters of the long-term MRA treatment cohort.

Variables	Baseline (n=102)	2-6 months (n=33)	7-12 months (n=69)	Overall *P*-value	Pairwise comparisons		
					B *vs.* ≤ 6 M	B *vs.* 7-12 M	≤ 6 M *vs.* 7-12 M
Follow-up (months)	N.A.	3 [2; 6]	12 [11; 12]	-	-	-	**<0.001**
MRA dose (mg)	N.A.	25 [25; 50]	50 [25; 50]	-	-	-	**0.047**
SBP (mmHg)	157 ± 21.2	137 ± 16.1	133 ± 15.4	**<0.001**	**<0.001**	**<0.001**	0.779
DBP (mmHg)	98 ± 12.1	85 ± 8.6	84 ± 9.4	**<0.001**	**<0.001**	**<0.001**	1.000
DDD	2.0 [1.0; 3.0]	2.3 [1.3; 2.7]	2.7 [1.3; 3.5]	0.164	-	-	-
Potassium (mmol/L)	3.7 ± 0.55	4.4 ± 0.42	4.3 ± 0.53	**<0.001**	**<0.001**	**<0.001**	1.000
PRA (ng/mL/h)	0.20 [0.10; 0.40]	0.83 [0.20; 1.70]	2.30 [0.74; 5.60]	**<0.001**	**<0.001**	**<0.001**	**0.015**
AC (ng/dL)	29.0 [19.9; 37.1]	25.1 [15.8; 43.1]	27.4 [17.1; 46.6]	0.843	-	-	-
ARR (ng/dL/ng/mL/h)	121 [67; 179]	29 [18; 91]	12 [6; 35]	**<0.001**	**<0.001**	**<0.001**	**0.041**
ARR interpretation							
Positive test	102 (100.0)	15 (45.5)	19 (27.5)				
False negative test	0 (0.0)	18 (54.5)	50 (72.5)	**<0.001**	**<0.001**	**<0.001**	0.072
FN UPA	N.A.	2 (6.1)	5 (7.2)				
vs. total UPA		2 of 4 (50.0)	5 of 12 (41.7)				

Clinical and biochemical characteristics of patients with PA from the long-term MRA treatment cohort (102 patients). Blood pressure measurements, clinical evaluation and biochemical assessments were obtained at baseline, and after 2-6 and 7-12 months of canrenone therapy in 33 and 69 patients, respectively.

Values are mean ± SD, median [IQR], or absolute number (%).

Abbreviations: AC, aldosterone concentration; ARR, aldosterone-to-renin ratio; B, baseline; DBP, diastolic blood pressure; DDD, daily defined dose; FN, false negative; M, months; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure; UPA, unilateral primary aldosteronism.

Supplementary Table 3 – Clinical and biochemical parameters of patients according with MRA dosage.

Variables	Baseline (n=120)	12.5 mg (n=13)	25 mg (n=69)	50 mg (n=41)	100 mg (n=15)	*P*-value
Follow-up (months)	N.A.	2 [0.5; 2]	4 [2; 12]	12 [6; 12]	12 [9; 12]	**<0.001**
SBP (mmHg)	156 ± 20.6	135 ± 14.1	133 ± 14.7	137 ± 19.0	144 ± 16.8	**<0.001**
DBP (mmHg)	97 ± 12.3	85 ± 8.0	82 ± 8.2	86 ± 10.2	91 ± 10.4	**<0.001**
DDD	2.0 [1.0; 3.0]	2.7 [1.2; 4.2]	2.3 [1.3; 2.9]	2.7 [1.7; 3.7]	3.3 [2.8; 5.3]	**0.005**
Potassium (mmol/L)	3.7 ± 0.55	4.1 ± 0.38	4.1 ± 0.49	4.3 ± 0.54	4.6 ± 0.36	**<0.001**
PRA (ng/mL/h)	0.28 [0.10; 0.40]	0.49 [0.21; 0.73]	0.74 [0.42; 1.72]	1.70 [0.80; 6.01]	4.90 [2.60; 9.40]	**<0.001**
AC (ng/dL)	28.1 [18.3; 37.0]	25.8 [21.2; 35.1]	25.1 [20.0; 45.8]	34.6 [15.3; 46.6]	42.1 [33.3; 71.7]	**0.037**
ARR (ng/dL/ng/mL/h)	119 [66; 178]	56 [29; 147]	36 [14; 94]	18 [5; 36]	9 [3; 21]	**<0.001**
ARR interpretation						
Positive test	120 (100.0)	9 (69.2)	37 (53.6)	12 (29.3)	2 (13.3)	**<0.001**
False negative test	0 (0.0)	4 (30.8)	32 (46.4)	29 (70.7)	13 (86.7)	

Clinical and biochemical characteristics of patients with PA on MRA according with canrenone dosage. For each dose category, the summation of patients receiving that particular dosage at each follow-up interval has been considered (clinical and biochemical data of patients from the short-term MRA cohort at both 2 and 8 weeks were counted).

Values are mean ± SD, median [IQR], or absolute number (%).

Abbreviations: AC, aldosterone concentration; ARR, aldosterone-to-renin ratio; DBP, diastolic blood pressure; DDD, daily defined dose; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure.

Supplementary Table 4 – Effect of duration of follow-up, MRA dose and baseline biochemical parameters on ARR at follow-up.

ARR at follow-up	Follow-up (months)	MRA dose (mg)	Baseline K^+ (mmol/L)	Baseline ARR
β Estimate (%)	0.915	0.986	0.797	1.001
95% CI - Lower	0.869	0.977	0.533	1.000
95% CI - Upper	0.963	0.996	1.191	1.003
***P*-value**	**0.001**	**0.007**	0.267	0.129

Multinomial linear regression for ARR changes at follow-up.

A β estimate greater than 1 means a direct association with ARR at follow-up, with an increase of X% of ARR for each 1-unit increase of the explored covariate, where "X" is calculated according to the following equation: Y = e^β * e^(β*X). A β estimate lower than 1 means an inverse association with ARR at follow-up, with a decrease of X% of ARR for each 1-unit increase of the explored covariate. β-estimates were reported together with their 95% confidence intervals.

Duration of follow-up and MRA dose were independently associated to ARR at follow-up (i.e., 9.2% decrease of average ARR for each 1-month follow-up increase, and 1.4% decrease of average ARR for each 1 mg MRA dose increase; $p<0.01$).

Abbreviations: ARR, aldosterone-to-renin ratio; K^+, potassium; MRA, mineralocorticoid receptor antagonist.

Supplementary Table 5 – Biochemical parameters of patients according with TDM results.

Variables	Positive TDM (n=65)	Negative TDM (n=9)	*P*-value
Sample characteristics at Baseline			
Potassium (mmol/L)	3.6 ± 0.54	3.9 ± 0.58	0.153
PRA (ng/mL/h)	0.30 [0.15; 0.33]	0.30 [0.10; 0.47]	0.993
AC (ng/dL)	25.9 [18.1; 36.8]	26.5 [20.6; 41.2]	0.810
ARR (ng/dL/ng/mL/h)	86 [65; 176]	89 [57; 234]	0.914
Sample characteristics at Follow-up			
Potassium (mmol/L)	4.0 ± 0.47	4.2 ± 0.36	0.331
PRA (ng/mL/h)	0.72 [0.44; 1.83]	0.20 [0.10; 0.76]	**0.010**
AC (ng/dL)	26.3 [20.1; 43.8]	40.1 [18.1; 62.4]	0.546
ARR (ng/dL/ng/mL/h)	35 [11; 91]	117 [57; 351]	**0.003**

Biochemical assessment of blood samples obtained from patients of the study cohort who underwent canrenone TDM (74 patients): 38 samples from the long-term and 36 samples from the short-term MRA treatment cohort (18 patients tested both at 2 and 8 weeks of canrenone therapy). Values are mean ± SD, or median [IQR].

Abbreviations: AC, plasma aldosterone concentration; ARR, aldosterone-to-renin ratio; PRA, plasma renin activity; TDM, therapeutic drug monitoring.

Supplementary Table 6 – Clinical and biochemical parameters of the long-term MRA treatment cohort after exclusion of non-compliant patients.

Variables	Baseline (n=93)	2-6 months (n=26)	7-12 months (n=67)	Overall *P*-value	Pairwise comparisons		
					B *vs.* ≤ 6 M	B *vs.* 7-12 M	≤ 6 M *vs.* 7-12 M
Follow-up (months)	N.A.	3 [2; 6]	12 [11; 12]	-	-	-	-
MRA dose (mg)	N.A.	50 [25; 50]	50 [25; 50]	-	-	-	-
SBP (mmHg)	157 ± 20.9	136 ± 9.0	133 ± 15.6	**<0.001**	**<0.001**	**<0.001**	1.000
DBP (mmHg)	98 ± 12.4	84 ± 6.1	84 ± 9.5	**<0.001**	**<0.001**	**<0.001**	1.000
DDD	2.0 [1.0; 3.0]	2.3 [1.3; 2.7]	2.7 [1.3; 3.7]	0.211	-	-	-
Potassium (mmol/L)	3.7 ± 0.55	4.4 ± 0.41	4.3 ± 0.53	**<0.001**	**<0.001**	**<0.001**	1.000
PRA (ng/mL/h)	0.20 [0.10; 0.40]	1.15 [0.30; 2.01]	2.30 [0.74; 5.70]	**<0.001**	**<0.001**	**<0.001**	0.259
AC (ng/dL)	29.5 [19.5; 37.0]	25.4 [15.9; 39.3]	26.0 [16.3; 44.6]	0.894	-	-	-
ARR (ng/dL/ng/mL/h)	121 [68; 177]	23 [15; 59]	11 [6; 33]	**<0.001**	**<0.001**	**<0.001**	0.476
ARR interpretation							
Positive test	93 (100.0)	8 (30.8)	17 (25.4)	**<0.001**	**<0.001**	**<0.001**	0.597
False negative test	0 (0.0)	18 (69.2)	50 (74.6)				

Clinical and biochemical characteristics of treatment-compliant patients with PA from the long-term MRA cohort (93 patients). Patients who resulted non-adherent to MRA treatment at TDM (n=9) were excluded from this sub-analysis. Blood pressure measurements, clinical evaluation and biochemical assessments were obtained at baseline, and after 2-6 and 7-12 months of canrenone therapy in 26 and 67 patients, respectively.
Values are mean ± SD, median [IQR], or absolute number (%).
Abbreviations: AC, aldosterone concentration; ARR, aldosterone-to-renin ratio; B, baseline; DBP, diastolic blood pressure; DDD, daily defined dose; M, months; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure; TDM, therapeutic drug monitoring.

Supplementary Table 7 – Clinical and biochemical parameters of patients according with MRA dosage after exclusion of non-compliant patients.

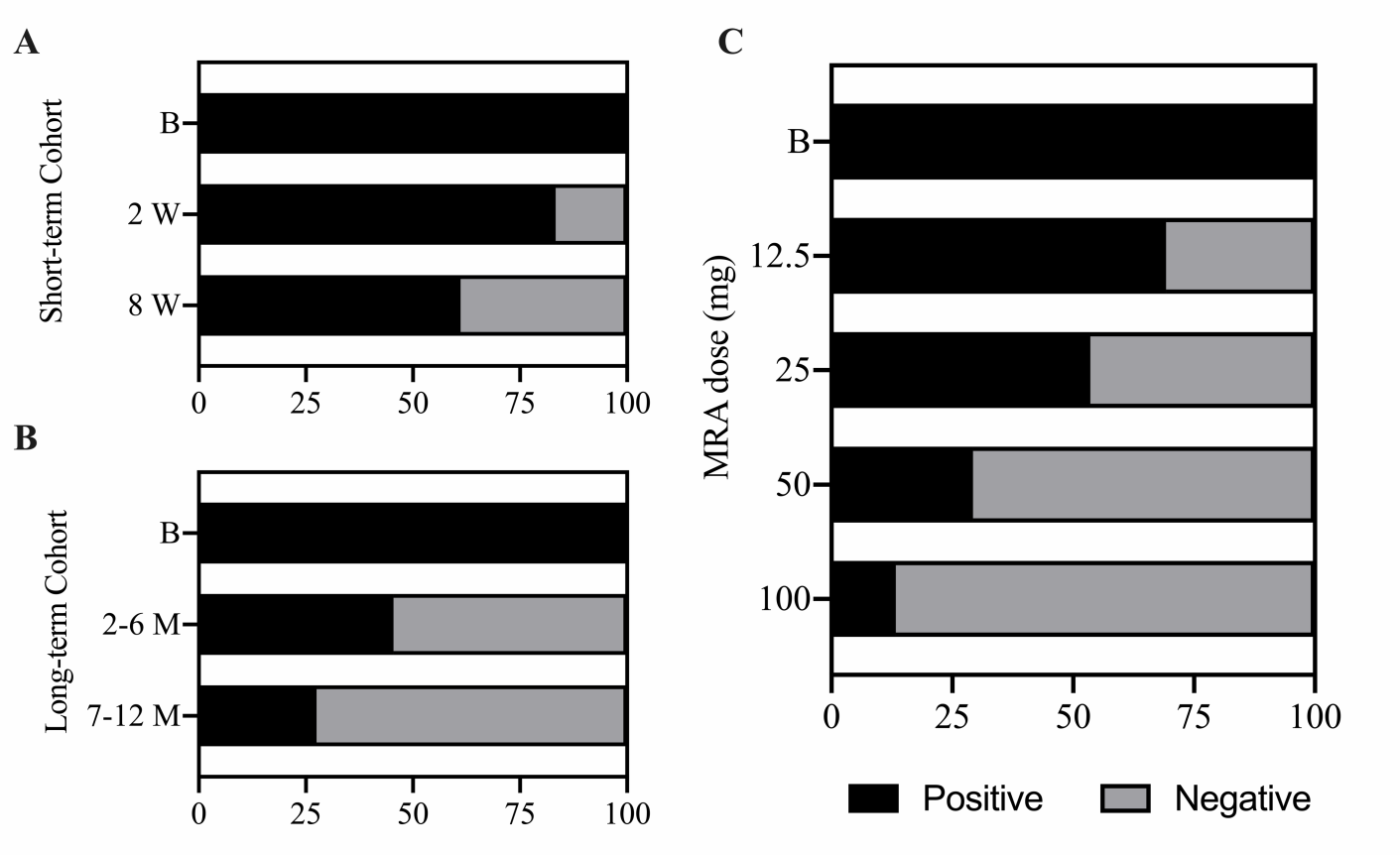
Variables	Baseline (n=111)	12.5 mg (n=12)	25 mg (n=62)	50 mg (n=40)	100 mg (n=15)	*P*-value
Follow-up (months)	N.A.	2 [0.5; 2]	4 [2; 12]	12 [6; 12]	12 [9; 12]	**<0.001**
SBP (mmHg)	156 ± 20.3	135 ± 14.4	134 ± 15.2	135 ± 15.1	144 ± 16.8	**<0.001**
DBP (mmHg)	97 ± 12.5	84 ± 7.8	82 ± 8.5	85 ± 8.7	91 ± 10.4	**<0.001**
DDD	2.0 [1.0; 3.0]	2.9 [1.2; 4.2]	2.3 [1.3; 3.3]	2.7 [1.7; 3.5]	3.3 [2.8; 5.3]	**0.006**
Potassium (mmol/L)	3.7 ± 0.55	4.1 ± 0.39	4.1 ± 0.50	4.3 ± 0.55	4.6 ± 0.36	**<0.001**
PRA (ng/mL/h)	0.27 [0.10; 0.40]	0.51 [0.23; 0.74]	0.76 [0.44; 2.20]	1.81 [0.81; 6.11]	4.90 [2.60; 9.40]	**<0.001**
AC (ng/dL)	28.5 [18.2; 37.0]	26.2 [22.0; 37.4]	25.0 [19.8; 42.6]	32.8 [15.2; 47.0]	42.1 [33.3; 71.7]	**0.041**
ARR (ng/dL/ng/mL/h)	119 [66; 176]	55 [29; 129]	29 [13; 79]	16 [5; 35]	9 [3; 21]	**<0.001**
ARR interpretation						
Positive test	111 (100.0)	8 (66.7)	30 (48.4)	11 (27.5)	2 (13.3)	**<0.001**
False negative test	0 (0.0)	4 (33.3)	32 (51.6)	29 (72.5)	13 (86.7)	

Clinical and biochemical characteristics of treatment-compliant patients with PA on MRA according with canrenone dosage. Patients who resulted non-adherent to MRA treatment at TDM (n=9) were excluded from this sub-analysis. For each dose category, the summation of patients receiving that particular dosage at each follow-up interval has been considered (clinical and biochemical data of patients from the short-term MRA cohort at both 2 and 8 weeks were counted).

Values are mean ± SD, median [IQR], or absolute number (%).

Abbreviations: AC, aldosterone concentration; ARR, aldosterone-to-renin ratio; DBP, diastolic blood pressure; DDD, daily defined dose; MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure.; TDM, therapeutic drug monitoring.

Figure S1 – 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**).