OPEN

Long-term effect of specific treatment of primary aldosteronism on carotid intima–media thickness

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Background: Aldosterone has been shown to substantially contribute to the accumulation of different types of collagen fibres and growth factors in the arterial wall, thus increasing wall thickness. A previous study showed reduction of increased common carotid intima–media thickness (IMT) in patients with primary aldosteronism 1 year after adrenalectomy. Our study in patients with primary aldosteronism was aimed at comparing the long-term effect of adrenalectomy vs. spironolactone therapy on common carotid IMT regression.

Method: Forty-two patients with confirmed primary aldosteronism (21 with aldosterone-producing adenoma treated by unilateral laparoscopic adrenalectomy, 21 treated with spironolactone) were investigated by carotid ultrasound at baseline and 1 and 6 years after the specific treatment.

Results: There was a decrease in common carotid IMT from 0.956 ± 0.140 to 0.900 ± 0.127 mm (−5.9%; $P < 0.05$) at 1 year and to 0.866 ± 0.130 mm (−9.4%; $P < 0.01$) at 6 years after adrenalectomy; in the spironolactone group, common carotid IMT decreased from 0.917 ± 0.151 to 0.900 ± 0.165 mm (−1.8%; NS) at 1 year and to 0.854 ± 0.176 mm (−6.8%; $P < 0.01$) at 6 years of treatment. The magnitude of improvement at 1 year was significantly higher (by 70%; $P < 0.05$) in the adrenalectomy group; however, the difference (by 27%) became nonsignificant at 6 years. Comparing the adrenalectomy and spironolactone groups, there was no significant difference in blood pressure decrease after treatment.

Conclusion: In the long term, spironolactone therapy in patients with primary aldosteronism had significant effect on regression of IMT, which was comparable to surgical treatment in patients with unilateral forms of primary aldosteronism.

Keywords: adrenalectomy, intima–media thickness, primary aldosteronism, spironolactone, ultrasound

Abbreviations: AVS, adrenal venous sampling; BP, blood pressure; CCA, common carotid artery; HDL, high-density cholesterol; IMT, intima–media thickness; LDL, low-density cholesterol; NS, not significant; PRA, plasma renin activity; SD, standard deviation

INTRODUCTION

Primary aldosteronism is the most frequent endocrine cause of secondary hypertension, affecting nearly 10% of all patients referred to specialized clinics and nearly 20% of patients referred with moderate to severe hypertension [1,2]. It is characterized by autonomous aldosterone overproduction, which is caused in most cases by adrenocortical adenoma or bilateral adrenal hyperplasia. This results (apart from hypertension) in potassium excretion, sodium reabsorption and fluid retention. However, manifest hypokalemia was reported in only nearly 50% of patients in recent studies [3].

In addition to these well known effects, there is compelling evidence that prolonged exposure to high aldosterone concentrations has a deleterious effect on cardiovascular tissues, independently of blood pressure (BP) [4,5]. Retrospective and prospective studies showed that individuals with primary aldosteronism might be at a higher risk of cardiovascular mortality than patients with essential hypertension [6,7].

The mechanisms by which aldosterone exerts its deleterious effect include chronic intravascular fluid retention, oxidative stress, endothelial dysfunction, inflammation and structural abnormalities such as myocardial and/or vascular remodelling, hypertrophy and/or fibrosis [8–10]. Compared with patients with essential hypertension, patients with primary aldosteronism present more frequently with increased left ventricular mass and significant impairment of left ventricular diastolic filling [11], higher excretion of albumin in urine [12], arterial wall stiffness [13] and increased intima–media thickness (IMT) of the common carotid artery (CCA) [14].

Recently published studies indicate that elimination of aldosterone overproduction after adrenalectomy can induce

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a regression of vascular and myocardial abnormalities in 1 year [15–17]. A prospective study [17] confirmed positive long-time effect on echocardiographic parameters in both surgical and pharmacological treatment of primary aldosteronism. In the long term, adrenalectomy seems to be more effective than lifelong therapy for patients with unilateral form of primary aldosteronism [18,19]. However, long-term effect of the two therapeutic approaches in primary aldosteronism on vascular bed has not yet been investigated.

Our study in patients with primary aldosteronism was aimed at comparing the long-term effect of adrenalectomy and treatment with aldosterone antagonists on regression of increased IMT of the CCA as a surrogate marker of vascular target organ damage.

MATERIALS AND METHODS

Study population

In total, 42 patients with primary aldosteronism were included in a prospective study. The initial examination of individuals was performed at the time of the diagnosis after discontinuation of antihypertensive therapy. Patients were switched to an α-blocker (doxazosine) and/or slow-release verapamil at least 14 days before the examination to eliminate the interference of other antihypertensive drugs with the renin–angiotensin–aldosterone system.

The screening for the diagnosis of primary aldosteronism was based on an elevated aldosterone-to-renin ratio at least 40 [(ng/dl)/(ng/ml per h)] when plasma renin activity (PRA) and aldosterone levels were measured after 2-h upright position, suppressed PRA ($\leq$0.7 ng/nl per h) and elevated plasma aldosterone ($\geq$15 ng/dl). The diagnosis of primary aldosteronism was confirmed by the absence of plasma aldosterone suppression after saline infusion test (plasma aldosterone $\leq$7 ng/dl) [20,21]. Adrenal venous sampling (AVS) was used in 15 out of 21 patients in the surgically treated group. The remaining six were referred directly to surgery because of a large tumour (over 2 cm), young age and absence of aldosterone stimulation during postural testing. The group treated with spironolactone consisted of six patients who refused AVS, seven in whom AVS proved idiopathic aldosteronism and eight in whom the results of AVS were inconclusive or nonselective and who refused repeated AVS. AVS criteria were used according to a previously published study [22]: success, adrenal vein/inferior vena cava cortisol gradient more than 2; lateralization was considered to be present when the aldosterone/cortisol ratio on one side was four times greater than at the contralateral side.

A total of 21 patients with essential hypertension served as controls for baseline comparisons. These individuals were recruited at our hypertension clinic and were selected by frequency matching after specification of inclusion criteria to avoid age, sex, BMI and estimated duration of hypertension as potential confounding variables. In these patients, secondary causes of hypertension were excluded after an appropriate drug washout.

Blood pressure monitoring

Office BP was measured in the sitting position by using a standard sphygmomanometer, before the participant underwent the ultrasound examination. Twenty-four hour ambulatory BP monitoring was performed using an oscillometric device (SpaceLabs 90207 Medical; Redmond, Washington, USA), which was set to measure BP every 20 min during the day (from 0600 to 2200 h) and every 30 min during the night (from 2200 to 0600 h).

Laboratory

All hormonal tests were performed by radioimmunoanalysis using commercially available kits (Immunotech; Beckman Coulter Company, Prague, Czech Republic). Blood biochemistry [sodium, potassium, urea, creatinine, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol, triglycerides and glucose] was analysed using multianalysers (Modular; Roche Diagnostics, Basel, Switzerland) in the institutional central laboratory.

Carotid ultrasound and intima–media thickness measurement

High-resolution B-mode carotid ultrasound was performed with multifrequency (5–10 MHz) linear array transducer (Acuson Sequoia 512; Siemens Medical Solutions, California, USA). Standardized longitudinal B-mode images of the far wall were obtained proximally from the tip of the flow divider (zero reference point). Carotid segments (carotid bifurcation and CCA) were defined as segments between 0 and 10 mm and between 10 and 20 mm from this zero reference point, respectively. Using Meier's Carotid Arc (Meyer Medical Ultrasound, Utrecht, The Netherlands), images were taken at two angles of 90° and 150° for the right carotid artery, and 210° and 270° for the left carotid artery [14]. When an optimal image was obtained, it was frozen on the top of the R-wave of QRS complex and stored in DICOM format.

The IMT measurements were performed off-line. Frozen images were displayed on the screen of a computer using an automated edge detection program Image Pro-Plus version 4.0 (Media Cybernetics, Silver Spring, Maryland, USA). In each image, the visualized blood-intima and media-adventitia boundaries (including presented plaques) of the far wall were marked with a computer mouse-controlled calliper within the defined segment [23]. The largest distance between these two lines was considered representative IMT for each segment. The average of four IMT measurements – the mean of maximum (mean-max) IMT (two angles, two sides) – was calculated for CCA and carotid bifurcation segments (CCA-IMT, CB-IMT). This approach including IMT reproducibility was described in detail elsewhere [14]. Ultrasound examinations and the off-line IMT measurements were performed by a single sonographer and reader in one person (R.H.) blinded to participants diagnoses.

Follow-up

Twenty-one patients underwent laparoscopic adrenalectomy for unilateral adrenal aldosterone-producing adenoma (confirmed by AVS in most patients before surgery and then by normalization of plasma aldosterone, PRA, plasma potassium, BP and histology after surgery) and 21 patients were treated with spironolactone due to

bilateral adrenal hyperplasia or unknown subtype of primary aldosteronism (refusal of further investigation, age 65 and higher, unsuccessful AVS). Treatment with spironolactone was started with a dose of 50 mg/day and was titrated to achieve target BP control. Five patients who did not tolerate spironolactone treatment were switched to eplerenone and excluded from the follow-up. Clinical assessment and laboratory tests were repeated at 12 months after enrolment and every 12 months thereafter. At each visit, antihypertensive therapy was adjusted according to the physician's judgement to reach a target of 140/90 mmHg or less. The use of all antihypertensive agents was permitted; only therapy with spironolactone in essential hypertensive patients was not allowed. Carotid ultrasound was repeated after 1-year therapy of primary aldosteronism and after a minimum follow-up of 5 years in all patients.

Statistical analysis

The statistical analysis was performed by STATISTICA software version 10.1 (Statsoft, Tulsa, Oklahoma, USA). Normally distributed data were described by mean ± standard deviation (SD). Continuous variables with clearly nonnormal distributions (Shapiro–Wilks *W*-test) were described as medians (interquartile range). The paired measurements (before/after treatment) were compared using either *t*-test for dependent samples or Wilcoxon matched pairs test, as appropriate. The difference between two treatment groups was analysed by *t*-test for independent samples. Multiple-group comparisons were performed by one-way analysis of variance (ANOVA), followed by the Scheffe's multiple range test. Changes from the baseline of BP values and IMT measurement were assessed by two-way ANOVA. Pearson's correlation analysis was used to assess the relationship between the IMT and other clinical parameters as well as the relationship between their treatment-induced changes. Spearman's correlation was used for nonnormally distributed indices. A *P* value less than 0.05 was considered significant.

RESULTS

Mean follow-up was 6.0 ± 0.9 years after the adrenalectomy and 6.3 ± 1.2 years of treatment with spironolactone (final dose of 42 ± 22 mg/day, range 25–100 mg/day). The baseline characteristics of the studied groups are summarized in Table 1. Office SBP values on their chronic antihypertensive medication as well as distribution of other cardiovascular risk factors at the start of the study were similar. Only (as expected) plasma aldosterone and aldosterone-to-renin ratio were higher as well as PRA was lower in patients with primary aldosteronism than essential hypertensive patients.

During the study, four patients stopped smoking in all groups, three patients after adrenalectomy and two patients with essential hypertension developed diabetes. Other clinical relevant characteristics and laboratory data at baseline and at the end of study are summarized in Table 2. As expected, plasma potassium normalized, plasma sodium decreased (significantly more in adrenalectomy group) and plasma creatinine increased. There was no consistent improvement in serum cholesterol (total, LDL and HDL) likewise worsening in fasting plasma glucose, triglycerides and BMI in all study groups during the follow-up.

Antihypertensive drugs used at baseline and at the end of study are summarized in Table 3. One year after adrenalectomy, eight patients (38%) became normotensive and their antihypertensive therapy was permanently

TABLE 1. Baseline characteristics of the study population

	Primary aldosteronism		Essential hypertension ($n = 21$)	ANOVA *P*
	Adrenalectomy ($n = 21$)	Spironolactone ($n = 21$)		
Clinical characteristics				
Age (years)	51.4 ± 9.9	51.3 ± 8.4	55.6 ± 9.3	NS
Sex [F/M (%F)]	8/13 (38)	10/11 (48)	8/13 (38)	NS
Weight (kg)	89.2 ± 17.1	90.2 ± 18.0	90.3 ± 15.7	NS
Height (cm)	174 ± 10	173 ± 7	175 ± 11	NS
Office SBP (mmHg)	163 ± 19	161 ± 19	163 ± 23	NS
Office DBP (mmHg)	97 ± 9	101 ± 6	100 ± 17	NS
Mean 24-h SBP (mmHg)	151 ± 17	153 ± 13	148 ± 17	NS
Mean 24-h DBP (mmHg)	92 ± 9	95 ± 9	87 ± 13	NS
Estimated duration of hypertension (years)	12 ± 8	17 ± 11	12 ± 8	NS
Current cigarette smoking [*n* (%)]	5 (24)	8 (38)	6 (29)	NS
Diabetes mellitus [*n* (%)]	5 (24)	7 (33)	4 (19)	NS
Laboratatory data				
Fasting plasma glucose (mmol/l)	5.0 (4.4–5.3)	5.1 (4.8–5.3)	5.5 (5.0–6.2)	NS
Urine potassium/day (mmol/24 h)	84 (62–132)	47 (41–66)	ND	–
Urine sodium/day (mmol/24 h)	109 (89–200)	115 (64–158)	ND	–
Plasma aldosterone – upright (ng/l)	570 (353–922)**	326 (248–687)*	133 (64–199)	<0.001
Plasma renin activity – upright (ng/ml per h)	0.35 (0.23–0.52)*	0.36 (0.27–0.53)*	0.65 (0.29–0.76)	<0.05
Aldosterone to plasma renin activity ratio – upright (ng/100 ml)/(ng/ml per h)	177 (132–233)**	91 (63–146)*,***	25 (16–27)	<0.001
CCA-IMT mean-max (mm)	0.956 ± 0.140	0.917 ± 0.151	0.848 ± 0.163	NS
CB-IMT mean-max (mm)	1.079 ± 0.186	1.151 ± 0.356	1.103 ± 0.269	NS

Variables are shown as means ± SD, medians (interquartile range) or absolute numbers and percentages. ND, not done; NS, not significant.
*$P < 0.05$ vs. essential hypertension.
**$P < 0.001$ vs. essential hypertension.
***$P < 0.05$ vs. adrenalectomy.

TABLE 2. Clinical characteristics and laboratory data at baseline and at the end of study

	Primary aldosteronism						Essential hypertension		
	Adrenalectomy			Spironolactone					
	Baseline ($n=21$)	End of study ($n=21$)	P	Baseline ($n=21$)	End of study ($n=21$)	P	Baseline ($n=21$)	End of study ($n=21$)	P
BMI (kg/m^2)	29.2 ± 4.3	29.5 ± 4.9	NS	29.8 ± 4.7	31.1 ± 5.1	<0.05	29.2 ± 3.3	30.3 ± 4.8	<0.05
Plasma cholesterol (mmol/l)	4.83 ± 0.77	4.69 ± 0.88	NS	4.95 ± 1.18	4.71 ± 0.73	NS	5.21 ± 0.99	4.62 ± 0.81	<0.05
LDL-cholesterol (mmol/l)	2.81 ± 0.65	2.52 ± 0.81	<0.05	2.99 ± 0.87	3.06 ± 0.77	NS	3.21 ± 0.86	2.59 ± 0.72	<0.05
HDL-cholesterol (mmol/l)	1.28 ± 0.23	1.35 ± 0.24	NS	1.34 ± 0.55	1.26 ± 0.38	NS	1.25 ± 0.29	1.29 ± 0.41	NS
Triglycerides (mmol/l)	1.63 ± 0.68	1.76 ± 0.79	<0.05	1.63 ± 0.71	1.94 ± 0.88	<0.05	1.67 ± 0.56	1.69 ± 0.92	NS
Fasting plasma glucose (mmol/l)	5.0 (4.4–5.3)	5.9 (4.9–8.4)	<0.001	5.1 (4.8–5.3)	6.2 (5.6–7.9)	<0.001	5.5 (5.0–6.2)	5.5 (5.0–6.4)	NS
Plasma potassium (mmol/l)	3.5 ± 0.4**	4.5 ± 0.3	<0.001	3.5 ± 0.5**	4.5 ± 0.4	<0.001	4.0 ± 0.4	4.2 ± 0.5	NS
Plasma sodium (mmol/l)	144 ± 2	140 ± 2	<0.001	142 ± 3*	141 ± 2	NS	140 ± 2	140 ± 4*	NS
Plasma creatinine (μmol/l)	83.8 ± 19.6	92.8 ± 27.5	<0.05	83.9 ± 19.8	103.9 ± 27.4	<0.001	82.0 ± 16.2	89.0 ± 24.8	<0.05

Variables are shown as means ± SD, or absolute numbers and percentages. HDL, high-density lipoprotein; LDL, low density lipoprotein; NS, not significant.
*$P<0.05$, vs. adrenalectomy at baseline.
**$P<0.01$, vs. essential hypertension at baseline.

discontinued. In all remaining patients ($n=13$), hypertension improved and BP decreased to 140/90 mmHg or less when they were receiving antihypertensive therapy during the whole follow-up period, although at lower doses and with a fewer number of agents than before adrenalectomy.

Six patients treated conservatively remained on spironolactone only, whereas 15 patients required combined antihypertensive therapy with fewer antihypertensive agents than baseline. During the whole follow-up, all 21 essential hypertensive patients remained on combined antihypertensive therapy. On the contrary, at the end of the follow-up, the number of antihypertensive agents used by patients treated for primary aldosteronism has decreased (the difference of -1.3 ± 1.9 after adrenalectomy vs. -0.9 ± 1.5 on spironolactone; NS) and the number of antihypertensive agents in essential hypertensive patients has increased (the difference of +0.5 ± 1.8; $P<0.001$). At yearly visits, values of BP and the number of antihypertensive drugs were comparable to that at the end of the follow-up.

Laboratory data, BP values, IMT measurements at baseline, after 1 year and at the end of study are summarized in Table 4. As expected, we observed normalization of aldosterone levels and PRA after surgery and increase of aldosterone levels and PRA after spironolactone treatment in all patients. All BP variables (office SBP and DBP as well as mean 24-h SBP and DBP) decreased comparably in both groups of patients treated for primary aldosteronism already at the first year of follow-up and there was further trend in BP drop towards the end of 6-year follow-up. At interim yearly investigations, the BP readings did not deviate significantly from the BP trend at reported 1-year and 6-year visits throughout the study. The decrease in BP in essential hypertensive patients at the end of the study was also significant but, compared with both groups of patients treated for primary aldosteronism, fewer differences were achieved (Table 5).

Although CCA-IMT significantly decreased after adrenalectomy as well as on spironolactone therapy at the end of the study, no significant decrease was observed in essential hypertensive patients. A significant decrease in CCA-IMT as early as at first-year assessment has been observed in surgical group only (Fig. 1) and the improvement in

TABLE 3. Use of antihypertensive and hypolipidemic drugs at baseline and at end of study

	Primary aldosteronism				Essential hypertension	
	Adrenalectomy		Spironolactone			
	Baseline ($n=21$)	End of study ($n=21$)	Baseline ($n=21$)	End of study ($n=21$)	Baseline ($n=21$)	End of study ($n=21$)
Chronic antihypertensive therapy						
Diuretics [n (%)]	7 (33)	7 (33)	15 (72)	15 (72)	17 (81)	17 (81)
β-blockers [n (%)]	10 (48)	4 (19)	15 (72)	5 (24)	12 (59)	11 (52)
Calcium channel blockers [n (%)]	16 (76)	11 (53)	16 (76)	17 (81)	15 (71)	17 (81)
Angiotensin- converting enzyme inhibitors [n (%)]	11 (53)	11 (53)	12 (58)	8 (38)	12 (58)	12 (58)
Angiotensin receptor blockers [n (%)]	12 (53)	3 (15)	11 (53)	3 (15)	6 (28)	10 (48)
α-blockers [n (%)]	6 (29)	4 (19)	5 (24)	4 (19)	5 (24)	10 (48)
Central agonists [n (%)]	5 (24)	1 (5)	13 (62)	2 (10)	9 (43)	7 (33)
Aldosterone antagonists	0 (0)	0 (0)	0 (0)	21 (100)	0 (0)	0 (0)
Number of antihypertensive drugs	3.5 ± 1.2	2.0 ± 1.8*	4.5 ± 1.7*	3.5 ± 1.3**	3.8 ± 1.8*	4.3 ± 1.8***
Lipid-lowering therapy						
Statins [n (%)]	2 (10)	7 (33)	4 (19)	10 (48)	6 (29)	15 (71)
Other drugs, n [(%)]	0 (0)	0 (0)	0 (0)	1 (5)	2 (10)	4 (19)

NS, not significant.
*$P<0.05$ vs. adrenalectomy at baseline.
**$P<0.05$ vs. spironolactone at baseline.
***$P<0.05$ vs. essential hypertension at baseline.

TABLE 4. Laboratory data, blood pressure values and intima–media thickness measurement at baseline, after 1 year and at the end of study

	Primary aldosteronism								Essential hypertension		
	Adrenalectomy				Spironolactone						
	Baseline ($n=21$)	After 1 year ($n=21$)	End of study ($n=21$)	P	Baseline ($n=21$)	After 1 year ($n=21$)	End of study ($n=21$)	P	Baseline ($n=21$)	End of study ($n=21$)	P
Laboratory data											
Plasma aldosterone – upright (ng/l)	570 (353 –922)	78 (52–256)**	–	<0.001	326 (248 –687)	1056 (452 –1652)*	–	<0.05	133 (64–199)	–	–
Plasma renin activity – upright (ng/ml per h)	0.35 (0.23 –0.52)	2.26 (1.06–4.02)**	–	<0.001	0.36 (0.27 – 0.53)	2.13 (1.03 – 3.06)**	–	<0.001	0.65 (0.29 – 0.76)	–	–
Aldosterone to plasma renin activity ratio – upright (ng/100 ml)/(ng/ml per h)	177 (132 –233)	4 (3–11)**	–	<0.001	91 (63–146)	43 (32–124)	–	NS	25 (16–27)	–	–
Blood pressure											
Office SBP (mm Hg)	163 ± 19	145 ± 17**	140 ± 18**	<0.001	161 ± 19	152 ± 16**	139 ± 19**	<0.001	163 ± 23	154 ± 15	<0.05
Office DBP (mmHg)	97 ± 9	87 ± 12**	85 ± 10**	<0.001	101 ± 6	93 ± 10*	87 ± 12*	<0.01	100 ± 17	89 ± 11	<0.01
Mean 24-h SBP (mmHg)	151 ± 17	130 ± 12**	126 ± 11**	<0.001	153 ± 13	135 ± 10**	127 ± 11**	<0.001	148 ± 17	141 ± 14	<0.01
Mean 24-h DBP (mmHg)	92 ± 9	80 ± 7**	76 ± 7**	<0.001	95 ± 9	85 ± 7**	80 ± 7**	<0.001	87 ± 13	81 ± 11	<0.001
IMT measurement											
CCA-IMT mean-max (mm)	0.956 ± 0.140	0.904 ± 0.140*	0.866 ± 0.130**	<0.001	0.917 ± 0.151	0.892 ± 0.179	0.854 ± 0.176**	<0.01	0.848 ± 0.163	0.848 ± 0.175	NS
CB-IMT mean-max (mm)	1.079 ± 0.186	1.059 ± 0.261	1.172 ± 0.390	NS	1.151 ± 0.356	1.182 ± 0.371	1.234 ± 0.370	NS	1.103 ± 0.269	1.138 ± 0.255	NS

CB, carotid bifurcation; CCA, common carotid artery; IMT, intima–media thickness.
*$P < 0.05$, vs. baseline.
**$P < 0.01$, vs. baseline.

TABLE 5. Significant differences in values of blood pressure and intima–media thickness between baseline and the end of study

	Primary aldosteronism		Essential hypertension	ANOVA
	Adrenalectomy ($n=21$)	Spironolactone ($n=21$)	($n=21$)	*P*
Blood pressure				
Office SBP (mmHg)	$-33\pm24^{*}$	$-29\pm30^{*}$	-9 ± 21	0.008
Office DBP (mmHg)	-17 ± 16	-14 ± 17	-11 ± 16	NS
Mean 24-h SBP (mmHg)	$-27\pm16^{**}$	$-26\pm19^{**}$	-8 ± 13	<0.001
Mean 24-h DBP (mmHg)	$-16\pm10^{*}$	$-15\pm11^{*}$	-6 ± 8	0.008
IMT measurement				
CCA-IMT mean-max (mm)	$-0.090\pm0.093^{*}$	-0.063 ± 0.122	-0.001 ± 0.072	0.013
CB-IMT mean-max (mm)	0.037 ± 0.470	0.083 ± 0.282	0.087 ± 0.291	NS

CB, carotid bifurcation; CCA, common carotid artery; IMT, intima–media thickness.
$^{*}P<0.05$, vs. essential hypertension.
$^{**}P<0.01$, vs. essential hypertension.

CCA-IMT did not differ significantly between patients who were treated by surgery alone vs. those who required adjuvant antihypertensive therapy. CCA-IMT measured at baseline was significantly positively correlated with age ($r=0.42$, $P<0.05$), BMI ($r=0.38$, $P<0.05$), SBP ($r=0.36$, $P<0.05$), 24-h SBP ($r=0.41$, $P<0.05$) and negatively with HDL cholesterol ($r=$-0.45, $P<0.05$). No significant correlations were found between the difference in CCA-IMT before/after treatment and the decrease in BP or other treatment-induced effects. No significant changes were observed in CB-IMT.

None of study patients experienced cardiovascular or cerebrovascular event during the 6-year follow-up.

DISCUSSION

Increased common carotid IMT has been reported in patients with primary aldosteronism and might predispose these patients to a higher incidence of cardiovascular complications than essential hypertensive patients [14]. Our study has examined the long-term effect of appropriate treatment on carotid IMT in a cohort of patients with primary aldosteronism. It demonstrated that adrenalectomy and medical therapy by mineralocorticoid antagonist spironolactone were both effective in reduction of carotid IMT, although this effect occurred earlier after surgical treatment.

On the contrary, we observed only modest BP reduction associated with nonsignificant IMT change in group of essential hypertensive patients, which was intentionally composed of those not treated by mineralocorticoid antagonists. This suggests that amelioration of aldosterone excess may play a key role not only in patients with primary aldosteronism but also in patients with essential hypertension [24]. Whether the significant IMT change is mediated by substantial BP reduction, by some unknown direct effect of spironolactone, or both, remains to be elucidated.

Different antihypertensive medications may have different effects on carotid IMT reduction [25]. In a meta-analysis of randomized controlled trials evaluating the impact of antihypertensive drugs on carotid IMT reduction, the effect of calcium-channel blockers was superior to that of angiotensin-converting enzyme inhibitors, followed by angiotensin II receptor blockers and α-blockers, and the least effective diuretics or β-blockers despite comparable BP reductions [25]. The effect of spironolactone therapy on carotid IMT has not been investigated in general population of hypertensive patients. There is only one double-blind study in a special population of 53 patients receiving haemodialysis who were randomized to spironolactone or placebo treatment. Favourable effect of spironolactone on carotid IMT progression was observed during 2-year follow-up [26]. Adjuvant antihypertensive medications were

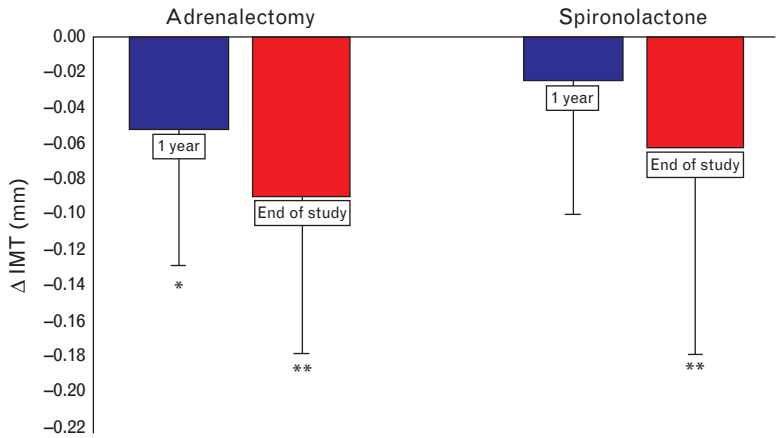

FIGURE 1 Changes in common carotid IMT in the short-term and long-term follow-up in patients with primary aldosteronism who were treated with adrenalectomy ($n=21$) or spironolactone ($n=21$). Short-term and long-term follow-up measurements were performed after 1 year and after an average period of 6.0 years or 6.3 years, respectively. $^{*}P\leq0.05$ vs. baseline; $^{**}P\leq0.01$ vs. baseline.

comparable in our patients, did not alter much during the study and can be therefore hardly taken responsible for the observed effects.

Only one study described a regression of common carotid IMT in patients with aldosterone-producing adenoma 1 year after adrenalectomy [16] and this was the first human study demonstrating the reversibility of vascular organ damage. This finding was corroborated by an improvement of arterial stiffness, assessed by pulse wave propagation velocity.

The first proof of arterial stiffness improvement after adrenalectomy was published by Štrauch *et al.* [15]. In a control group of patients treated by spironolactone, only nonsignificant decrease in pulse wave velocity was observed, which was mainly explained by the nonsignificant BP decrease in patients on medical therapy.

Similarly as in our study, Catena *et al.* [17] demonstrated that adrenalectomy and spironolactone were both effective in decreasing the left ventricle mass and that this effect occurs earlier (at one year) after surgical treatment. Unlike echocardiographic effects in this study, we did not observe correlation between the regression of CCA-IMT and plasma aldosterone upright concentration at baseline. This finding could be explained by the more complex background of IMT that represents a surrogate marker of generalized atherosclerosis [27]. In patients with accumulation of risk factors for atherosclerosis, a correlation between carotid IMT and particular competitive risk factors (including aldosterone level) can disappear.

The superior effect of adrenalectomy compared with spironolactone treatment was explained by persistent hyperaldosteronemia in case of medical therapy with possible involvement of nongenomic effects of circulating aldosterone as a cause of delayed regression of left ventricular hypertrophy [28]. Mineralocorticoid receptors have been demonstrated in human vascular smooth muscle cells [29] and their activation (similarly as in the heart) might play a role in tunica media hypertrophy and vascular remodelling in patients with primary aldosteronism via mechanisms that include modulation of ionic movements [30] and accelerated fibrosis [31]. Interruption of these receptor-mediated mechanisms might explain why, in the long term, both adrenalectomy and spironolactone treatment have comparable effects in terms of regression of IMT thickening, although this response may occur later in case of pharmacological treatment due to nongenomic effects of circulating aldosterone [32–34].

Another explanation for the difference in IMT-related effects between surgical and medical therapy is the relatively low starting dose of spironolactone unable to induce regression of thickening regardless of significant BP decrease. It might be caused by our fear of frequent dose-dependent side effects, predominantly gynecomastia [35]. In a study published by Mulatero *et al.* [36], because of side effects, spironolactone treatment was discontinued in 28% of patients with primary aldosteronism (compared with 24% of patients in our study). Therefore, for the prevention of side effects, in pharmacological treatment of patients with primary aldosteronism, we did prefer a lower but still effective dose of spironolactone in combination with other antihypertensive drugs. In our opinion, a supposed lower compliance to spironolactone at start of treatment poses a bigger problem than the loading therapeutic dose (specifically 30% in the outpatients group in our previous study concerning patients with resistant hypertension) [37].

No regression of CB-IMT was found. This discrepancy can be explained by the following mechanisms speculatively: the density/affinity of aldosterone receptors can be expected to be lower in the region of CB than in the straight part of the carotid artery. Then, the decrease of fibroblast proliferation in tunica media potentially caused by excessive aldosterone production may not be marked despite an appropriate therapy of primary aldosteronism. Another explanation considers a stronger influence of risk factors in arterial bifurcation due to different physical forces of blood stream on the arterial wall than in other arterial segments. Some studies described lower wall shear stress in the carotid bulb than with the CCA 20–30 mm upstream [38]. The wall shear stress correlated negatively with the IMT [39]. Therefore, a beneficial effect of specific therapy on tunica media can be masked by thickening of atherosclerotic plaques in the region of carotid bulb due to spontaneous ageing.

Surprisingly, an increase in plasma glucose levels has been recorded, apart from patients on medical treatment, also in patients after adrenalectomy showing no concomitant increase in body weight. According to a recently published meta-analysis, prevalence of abnormal glucose metabolism is more frequent in patients with primary aldosteronism; however, the favourable effect of specific treatment of that disease on glucose metabolism has not been demonstrated [40,41]. The differences in fasting plasma glucose levels did not disappear even after the exclusion of diabetic patients and patients treated by statins. It is plausible to speculate that an increase in blood glucose in primary aldosteronism patients originates, at least in part, from different conditions under which the plasma glucose levels were measured. The baseline sampling was performed in patients on modified hypertension therapy including only the metabolically neutral calcium channel blockers and α-blockers. In contrast, the glycaemia at the end of the study was measured in patients with ongoing antihypertensive medication with a considerable percentage of diuretics and beta-blockers, both enhancing the fasting plasma glucose levels. This is in agreement, with no change of plasma glucose in patients with essential hypertension in whom metabolic active antihypertensive medication was not discontinued at the time of baseline examination.

Proportion of patients with adenoma, who were cured by surgery alone, is in agreement with the study by Blumenfeld *et al.* [42] in 82 patients with primary aldosteronism 5 years after adrenalectomy. Similarly, a comparable effect of adrenalectomy on CCA-IMT regression in subgroups of patients with and without subsequent medical treatment of residual hypertension in our study confirms the data reported by Lin *et al.* [16] in patients 1 year after adrenalectomy.

Our study was not designed to investigate the effect of intervention on the incidence cardiovascular or cerebrovascular events. Some insight may be derived from the meta-analysis of observational studies by Lorenz *et al.* [43],

which offered the estimates on relative reduction of myocardial infarction and stroke per normalized change in CCA-IMT. Consequently, considerable treatment-induced CCA-IMT change in patients with primary aldosteronism overall suggests substantial long-term reduction in clinical events. It remains however unknown whether patients will benefit comparably from medical vs. surgical treatment in terms of cardiovascular morbidity.

The study has undoubtedly limitations: First, true control group to patients after adrenalectomy is missing. We were not able to compose an optimum control group of patients with pharmacological treatment of unilateral form of aldosterone overproduction because in such patients – according to guidelines – adrenalectomy is invariably the method of choice. The results should be interpreted cautiously because treatment-induced metabolic and hormonal effects differ significantly between the two groups and, consequently, the study does not offer direct implications for the management of patients with unilateral aldosteronism. The same problem involved patients with essential hypertension. It was hard to find any in which simply changing therapy without adding spironolactone achieved a comparable reduction in BP as the specific treatment of primary aldosteronism. For this reason, we were unable to confirm the causality of spironolactone therapy on IMT regression. Second, some antihypertensive drugs used by patients could have different impact on IMT regression, although differences in concomitant antihypertensive medication between study groups were not significant. Also, the reduction of BP during the study does not necessarily reflect the true change in terms of long-term BP burden prior to enrolment. Third, we did not investigate laboratory inflammation markers or perform an integrated backscatter study of carotid artery to assess vascular fibrosis in this study and to provide an additional support for the improvement of vascular fibrosis after appropriate therapy of primary aldosteronism.

In conclusion, we followed a cohort of patients with primary aldosteronism long term after specific treatment. This study presents evidence that adrenalectomy and medical therapy by mineralocorticoid antagonist spironolactone were both effective in reduction of common carotid IMT, although the effect was slightly more pronounced after adrenalectomy. In order to achieve earlier regression of arterial thickness as a marker of target organ damage (and potential cure) and consequently a lower risk of cardiovascular events, surgical treatment should be preferred in all patients with unilateral forms of primary aldosteronism. Nevertheless, in the long term, spironolactone therapy achieves a comparable effect. These findings underscore the importance of a timely identification of this endocrine disorder to obtain a regression of vascular abnormalities as well as a lower risk of cardiovascular events.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Štrauch B, Zelinka T, Hampf M, Bernhardt R, Widimský J Jr. Prevalence of primary hyperaldosteronism in moderate to severe hypertension in the Central Europe region. *J Hum Hypertens* 2003; 17:349–352.
2. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies – a review of the current literature. *Horm Metab Res* 2012; 44:157–162.
3. Kim SH, Ahn JH, Hong HC, Choi HY, Kim YJ, Kim NH, *et al.* Changes in the clinical manifestations of primary aldosteronism. *Korean J Intern Med* 2014; 29:217–225.
4. Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol* 2005; 45:1243–1248.
5. Savard S, Amar L, Plouin PF, Steichen O. Cardiovascular complications associated with primary aldosteronism: a controlled cross-sectional study. *Hypertension* 2013; 62:331–336.
6. Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, Sechi LA. Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern Med* 2008; 168:80–85.
7. Reincke M, Fischer E, Gerum S, Merkle K, Schulz S, Pallauf A, *et al.* Observational study mortality in treated primary aldosteronism: the German Conn's registry. *Hypertension* 2012; 60:618–624.
8. Catena C, Colussi G, Marzano L, Sechi LA. Aldosterone and the heart: from basic research to clinical evidence. *Horm Metab Res* 2012; 44:181–187.
9. Stehr CB, Mellado R, Ocaranza MP, Carvajal CA, Mosso L, Becerra E, *et al.* Increased levels of oxidative stress, subclinical inflammation, and myocardial fibrosis markers in primary aldosteronism patients. *J Hypertens* 2010; 28:2120–2126.
10. Widimský J Jr, Štrauch B, Petrák O, Rosa J, Šomlóová Z, Zelinka T, Holaj R. Vascular disturbances in primary aldosteronism: clinical evidence. *Kidney Blood Press Res* 2012; 35:529–533.
11. Rossi GP, Sacchetto A, Visentin P, Canali C, Graniero GR, Palatini P, Pessina AC. Changes in left ventricular anatomy and function in hypertension and primary aldosteronism. *Hypertension* 1996; 27:1039–1045.
12. Rossi GP, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, *et al.* Renal damage in primary aldosteronism: results of the PAPY Study. *Hypertension* 2006; 48:232–238.
13. Štrauch B, Petrák O, Wichterle D, Zelinka T, Holaj R, Widimský J Jr. Increased arterial wall stiffness in primary aldosteronism in comparison with essential hypertension. *Am J Hypertens* 2006; 19:909–914.
14. Holaj R, Zelinka T, Wichterle D, Petrák O, Štrauch B, Widimský J Jr. Increased intima-media thickness of the common carotid artery in primary aldosteronism in comparison with essential hypertension. *J Hypertens* 2007; 25:1451–1457.
15. Štrauch B, Petrák O, Zelinka T, Wichterle D, Holaj R, Kasalický M, *et al.* Adrenalectomy improves arterial stiffness in primary aldosteronism. *Am J Hypertens* 2008; 21:1086–1092.
16. Lin YH, Lin LY, Chen A, Wu XM, Lee JK, Su TC, *et al.* Adrenalectomy improves increased carotid intima-media thickness and arterial stiffness in patients with aldosterone producing adenoma. *Atherosclerosis* 2012; 221:154–159.
17. Catena C, Colussi G, Lapenna R, Nadalini E, Chiuch A, Gianfagna P, Sechi LA. Long-term cardiac effects of adrenalectomy or mineralocorticoid antagonists in patients with primary aldosteronism. *Hypertension* 2007; 50:911–918.
18. Ghose RP, Hall PM, Bravo EL. Medical management of aldosterone-producing adenomas. *Ann Intern Med* 1999; 131:105–108.
19. Sywak M, Pasieka JL. Long-term follow-up and cost benefit of adrenalectomy in patients with primary hyperaldosteronism. *Br J Surg* 2002; 89:1587–1593.
20. Rossi GP, Belfiore A, Bernini G, Desideri G, Fabris B, Ferri C, *et al.* Prospective evaluation of the saline infusion test for excluding primary aldosteronism due to aldosterone-producing adenoma. *J Hypertens* 2007; 25:1433–1442.

21. Giacchetti G, Ronconi V, Lucarelli G, Boscaro M, Mantero F. Analysis of screening and confirmatory tests in the diagnosis of primary aldosteronism: need for a standardized protocol. *J Hypertens* 2006; 24:737–745.
22. Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L, *et al*. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. *J Clin Endocrinol Metab* 2004; 89:1045–1050.
23. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986; 74:1399–1406.
24. Chapman N, Dobson J, Wilson S, Dahlof B, Sever PS, Wedel H, *et al*. Effect of spironolactone on blood pressure in subjects with resistant hypertension. *Hypertension* 2007; 49:839–845.
25. Wang JG, Staessen JA, Li Y, Van Bortel LM, Nawrot T, Fagard R, *et al*. Carotid intima-media thickness and antihypertensive treatment: a meta-analysis of randomized controlled trials. *Stroke* 2006; 37:1933–1940.
26. Vukusich A, Kunstmann S, Varela C, Gainza D, Bravo S, Sepulveda D, *et al*. A randomized, double-blind, placebo-controlled trial of spironolactone on carotid intima-media thickness in nondiabetic hemodialysis patients. *Clin J Am Soc Nephrol* 2010; 5:1380–1387.
27. Mancini GB, Dahlof B, Diez J. Surrogate markers for cardiovascular disease: structural markers. *Circulation* 2004; 109:IV22–IV30.
28. Rocha R, Funder JW. The pathophysiology of aldosterone in the cardiovascular system. *Ann N Y Acad Sci* 2002; 970:89–100.
29. Funder JW, Pearce PT, Smith R, Campbell J. Vascular type I aldosterone binding sites are physiological mineralocorticoid receptors. *Endocrinology* 1989; 125:2224–2226.
30. Kometiani P, Li J, Gnudi L, Kahn BB, Askari A, Xie Z. Multiple signal transduction pathways link Na+/K+-ATPase to growth-related genes in cardiac myocytes. The roles of Ras and mitogen-activated protein kinases. *J Biol Chem* 1998; 273:15249–15256.
31. Funder J. Mineralocorticoids and cardiac fibrosis: the decade in review. *Clin Exp Pharmacol Physiol* 2001; 28:1002–1006.
32. Schiffrin EL. Effects of aldosterone on the vasculature. *Hypertension* 2006; 47:312–318.
33. Callera GE, Montezano AC, Yogi A, Tostes AC, He Y, Schiffrin EL, Touyz RM. c-Src-dependent nongenomic signaling responses to aldosterone are increased in vascular myocytes from spontaneously hypertensive rats. *Hypertension* 2005; 46:1032–1038.
34. Callera GE, Yogi A, Briones AM, Montezano AC, He Y, Tostes RC, *et al*. Vascular proinflammatory responses by aldosterone are mediated via c-Src trafficking to cholesterol-rich microdomains: role of PDGFR. *Cardiovasc Res* 2011; 91:720–731.
35. Jeunemaitre X, Chatellier G, Kreft-Jais C, Charru A, DeVries C, Plouin PF, *et al*. Efficacy and tolerance of spironolactone in essential hypertension. *Am J Cardiol* 1987; 60:820–825.
36. Mulatero P, Bertello C, Rossato D, Mengozzi G, Milan A, Garrone C, *et al*. Roles of clinical criteria, computed tomography scan, and adrenal vein sampling in differential diagnosis of primary aldosteronism subtypes. *J Clin Endocrinol Metab* 2008; 93:1366–1371.
37. Štrauch B, Petrák O, Zelinka T, Rosa J, Šomlóová Z, Indra T, *et al*. Precise assessment of noncompliance with the antihypertensive therapy in patients with resistant hypertension using toxicological serum analysis. *J Hypertens* 2013; 31:2455–2461.
38. Kornet L, Lambregts J, Hoeks AP, Reneman RS. Differences in near-wall shear rate in the carotid artery within subjects are associated with different intima-media thicknesses. *Arterioscler Thromb Vasc Biol* 1998; 18:1877–1884.
39. Irace C, Cortese C, Fiaschi E, Carallo C, Farinaro E, Gnasso A. Wall shear stress is associated with intima-media thickness and carotid atherosclerosis in subjects at low coronary heart disease risk. *Stroke* 2004; 35:464–468.
40. Matrozova J, Steichen O, Amar L, Zacharieva S, Jeunemaitre X, Plouin PF. Fasting plasma glucose and serum lipids in patients with primary aldosteronism: a controlled cross-sectional study. *Hypertension* 2009; 53:605–610.
41. Štrauch B, Widimský J, Šindelka G, Škrha J. Does the treatment of primary hyperaldosteronism influence glucose tolerance? *Physiol Res* 2003; 52:503–506.
42. Blumenfeld JD, Sealey JE, Schlussel Y, Vaughan EDJr, Sos TA, Atlas SA, *et al*. Diagnosis and treatment of primary hyperaldosteronism. *Ann Intern Med* 1994; 121:877–885.
43. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007; 115:459–467.

Reviewers' Summary Evaluations

Referee 1

The manuscript by Holaj and colleagues demonstrates, for the first time, the beneficial vascular effects of treatment of aldosterone excess. They show significant regression of carotid IMT in individuals with primary aldosteronism (PA) who have undergone either medical or surgical treatment of their aldosterone excess. This effect is not simply mediated by blood pressure lowering as significant IMT regression was not found in a cohort of essential hypertensive controls. These data further illustrate that simply lowering the blood pressure in patients with PA is insufficient and specific treatment of aldosterone excess (medical or surgical methods equally efficacious) is required in order to reduce the substantial cardiovascular morbidity that accompanies this increasingly common condition.

Referee 2

The authors of this study found that in patients with primary aldosteronism, improvement of intima media thickness (IMT) of the common carotid artery occurred earlier after adrenalectomy than after medical treatment. A strong point of this study is the long-term follow-up in a considerable number of patients. However, a weak point is that these data cannot definitely exclude that this differential time effect is due to a difference in effects on blood pressure. In addition, it is not clear whether this earlier improvement of IMT after adrenalectomy translates in better cardiovascular outcomes. Nevertheless, these data support the contention that adrenalectomy is the preferred mode of treatment for unilateral aldosterone excess.