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Adrenal androgens versus cortisol for primary aldosteronism subtype determination in adrenal venous sampling

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Abstract

Objective: We examined if measurement of adrenal androgens adds to subtype diagnostics of primary aldosteronism (PA) under cosyntropin-stimulated adrenal venous sampling (AVS).

Design: A prospective pre-specified secondary endpoint analysis of 49 patients with confirmed PA, of whom 29 underwent unilateral adrenalectomy with long-term follow-up.

Methods: Concentrations of androstenedione, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) were measured during AVS in addition to aldosterone and cortisol. Subjects with lateralisation index (LI) of ≥4 were treated with unilateral adrenalectomy, and the immunohistochemical subtype was determined with CYP11B2 and CYP11B1 stains. The performance of adrenal androgens was evaluated by receiver operating characteristics (ROC) curve analyses in adrenalectomy and medical therapy groups.

Results: During AVS, the correlations between cortisol and androstenedione, DHEA and DHEAS for LI and selectivity index (SI) were highly significant. The right and left side SIs for androstenedione and DHEA were higher ($p < .001$) than for cortisol. In ROC analysis, the optimal LI cut-off values for androstenedione, DHEA and DHEAS were 4.2, 4.5 and 4.6, respectively. The performance of these LIs for adrenal androgens did not differ from that of cortisol.

Conclusions: Under cosyntropin-stimulated AVS, the measurement of androstenedione and DHEA did not improve the cannulation selectivity. The performance of cortisol and adrenal androgens are confirmatory but not superior to cortisol-based results in lateralisation diagnostics of PA.

KEYWORDS

adrenal androgens, adrenal venous sampling, aldosterone producing adenoma, bilateral adrenal hyperplasia, CYP11B2, primary aldosteronism, subtype classification of primary aldosteronism

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1 | INTRODUCTION

Primary aldosteronism (PA) is the most frequent form of endocrine hypertension and is principally caused by unilateral aldosterone-producing adenoma (APA) or bilateral hyperaldosteronism.[1,2] Surgically curable APAs account for about 30%–40% of the cases.[3,4] Accurate PA subtyping is crucial since surgical treatment is associated with higher proportion of controlled hypertension, improved quality of life, and lower all-cause mortality when compared with medical treatment.[5,6]

The method recommended by the Endocrine Society for identifying unilateral aldosterone hypersecretion is adrenal venous sampling (AVS).[1] Other techniques to determine lateralisation, such as adrenal CT and MRI have not proven sufficient specificity and the data regarding ^{11}C-metomidate positron emission tomography is even more scarce.[1,7,8] Although the AVS method lacks standardisation, the number of patients cured after AVS-based adrenalectomy remains high. In a multicentre study with more than 500 AVS-based operated patients, complete biochemical cure was achieved in 93% of patients.[9]

AVS is technically demanding and the cure rate has been shown to improve with the use of cosyntropin,[10] although arguments still exist both for and against the use of it. Since a high cortisol concentration is a definite determinant of adrenal venous (AV) blood, the success of selective sampling is assessed by the selectivity index (SI), defined as the ratio of cortisol concentrations in the AV and inferior vena cava (IVC) blood.[11] However, the use of cortisol concentration has several pitfalls. APAs may be cortisol co-producing which leads to a suppressed aldosterone/cortisol ratio and a falsely low lateralisation index (LI) below the cut-off value.[12,13] Second, cortisol secretion is pulsatile, thus physiological fluctuations or any form of stress may confound the assessment of AVS selectivity and lateralisation.[14,15] Third, the relatively long half-life of cortisol in the circulation (100 min) may result in a small AV/IVC ratio and unselective sampling.[16]

Previous studies have investigated the usefulness of other adrenal hormones or metabolites to improve the diagnostic accuracy of AVS. Androstenedione, dehydroepiandrosterone (DHEA), 11-deoxycortisol and metanephrine have been shown to be superior biomarkers to cortisol in ascertaining the selectivity of AVS without cosyntropin stimulation.[16–20] However, far fewer reports discussing the selectivity of cosyntropin-stimulated AVS have been published1.[16,21,22] In a study examining a panel of 15 adrenal steroids with and without cosyntropin stimulation (in 44% and 56% of cases, respectively), androstenedione, DHEA and 11-deoxycortisol among others were considered potentially more sensitive alternatives to cortisol for determining the selectivity of AVS,[22] yet DHEA sulphate (DHEAS) failed to enhance the SI in comparison with cortisol.

In addition to determining the selectivity of AVS, androstenedione and DHEA have been suggested to be useful alternatives to cortisol, both in the absence and presence of cosyntropin, in assessing lateralized aldosterone production.[23] In this study, however, ROC curve analyses determining the performance of androstenedione and DHEA in assessing the LI were not performed. AVS is a difficult and costly method and new biomarkers should not increase the time used, difficulty nor costs of the procedure.

In the present study we examine the value of the adrenal cortical hormones androstenedione, DHEA and DHEAS in comparison with cortisol in demonstrating (1) the selectivity of cannulation and (2) the lateralisation of aldosterone secretion during AVS with cosyntropin stimulation. PA was accurately ascertained and patients were carefully followed up after adrenalectomy. In contrast to previous studies, we compare the performance of androstenedione, DHEA and DHEAS with cortisol in designating correct lateralisation in surgically treated patients according to the immunohistochemical analysis of aldosterone synthase (CYP11B2). We also recorded postoperative cure by improved blood pressure, number of antihypertensive medications used and correction of hypokalaemia.

2 | MATERIALS AND METHODS

2.1 | Study subjects

This prospective pre-specified secondary endpoint analysis included 49 patients with confirmed PA, who had plasma samples available from both AVs and IVC collected during the AVS procedure (Figure S1). The study design and main outcome have been reported previously.[7] All subjects were diagnosed according to the Endocrine Society guidelines[24] and underwent AVS at Tampere University Hospital as part of the diagnostic evaluation of PA. Hypercortisolism was excluded with clinical and biochemical assessments including 1 mg overnight dexamethasone test or measurement of 24-h urinary cortisol excretion (cut-points of 100 nmol/l and 144 nmol, respectively). Further inclusion and exclusion criteria were described by Soinio et al.[7] The study protocol was approved by the ethics committee of Turku University Hospital and was undertaken in accordance with the Declaration of Helsinki. Written consent was obtained from each patient after full explanation of the purpose and nature of all procedures used. The protocol was registered in the ClinicalTrials.gov database (NCT01567111).

Mineralocorticoid receptor antagonists (MRAs) were discontinued at least 6 weeks before the AVS procedures. Hypokalaemia was corrected by oral potassium supplementation. AVS was performed under continuous cosyntropin stimulation of 50 μg/h as described earlier.[7] A portion of plasma was immediately used for routine cortisol analysis (electrochemiluminescence, ECLIA) and aldosterone was analysed by liquid chromatography with tandem mass spectrometry (LC-MS/MS), while another sample fraction was frozen at −80°C until androstenedione and DHEA were measured with LC-MS/MS and DHEAS with immunoassay (Abbott Architect). One subject had inadequate samples for both androstenedione and DHEA analysis, and 10 subjects for DHEAS analysis.

Catheterisation was considered successful when the selectivity index (SI = AV/IVC cortisol concentrations) on both sides was ≥5. PA was classified unilateral based on a lateralisation index (LI = aldosterone/cortisol ratio between the dominant and contralateral AVs) ≥4.[11] We also calculated the contralateral suppression index (CSI = aldosterone/cortisol ratio between the nondominant AV and IVC) where

values <1 suggest unilateral disease. Figure S1 illustrates the division of subjects into adrenalectomy and medical therapy groups.

Because the cut-point for cortisol-based LI remains debated,[11,25] we did a post hoc evaluation of cases in which there was discrepancy between cortisol and other adrenal androgen derived indexes. All discrepancies between indexes fell into cortisol-derived LI range of 2.00–5.88, in whom we evaluated whether the use of additional adrenal androgens would have changed the diagnosis of the subtype of PA, and further in adrenalectomized patients, related this to immunohistochemical diagnosis and the cure after adrenalectomy.

2.2 | Follow-up of the patients

Among the adrenalectomy group, postoperative information was available at 3–6 months after surgery apart from the aldosterone and renin values, which were measured at variable times from a few days to 6 months postoperatively. Long-term follow-up data collected 4.3 ± 2 years after AVS were available in all subjects of the adrenalectomy group. Surgical cure was categorized as complete, partial or absent according to the Primary Aldosteronism Surgical Outcome (PASO) criteria.[26] Since aldosterone and renin measurements were not available at follow-up, biochemical cure was classified according to plasma potassium levels.

2.3 | Histological and immunohistochemical analysis

Diagnostic hematoxylin-and-eosin-stained adrenal slides were reviewed centrally in the Helsinki University Hospital by a single pathologist with special expertise in endocrine pathology (Helena Leijon). One or two representative blocks per case were selected with following criteria: (a) adenoma coupled with normal adrenal cortex and (b) hyperplasia presenting with a dominant nodule. Immunohistochemical labelling was performed with previously described primary antibodies CYP11B1 (11β-hydroxylase, dilution 1:5) and CYP11B2 (aldosterone synthase, dilution 1:3000).[27] Each sample was categorized as APA or aldosterone-producing nodules (APN, also described as non-APA) based on immunohistochemistry (Figure S1) as described previously.[7,28,29] Immunoreactivity was semiquantitatively assessed with H-scoring system based on the staining intensity and the percentage of stained cells as described by Nakamura et al. and McCarty et al.[28,30] CYP11B1 H-score was analysed to investigate the role of subclinical cortisol co-secretion from APAs.[31]

2.4 | Statistical analysis

Results are expressed as either number and percentage, mean and standard deviation or median and interquartile range, as appropriate. Comparisons of independent samples were performed with either independent-samples *t*-test or Mann–Whitney *U*-test, as appropriate. Wilcoxon test was used for within-patient comparisons, as no transformation of skewed variables was applied. The relationships between the SIs and LIs with cortisol, androstenedione, DHEA, DHEAS and aldosterone were assessed using two-tailed Spearman's correlation coefficients. A receiver operating characteristics (ROC) curve was constructed from the pairs of sensitivity and specificity measured for LI calculated using cortisol, androstenedione, DHEA and DHEAS. The ROC analysis was conducted using allocation to operation or medical therapy as the standard. One patient chose not to have adrenalectomy despite having lateralisation in AVS and was therefore excluded from the ROC analyses. One patient in the medical treatment group had failed cannulation according to SI with androstenedione and DHEAS and was excluded from the ROC analysis of these two hormones. SPSS Statistics (version 27 for MAC) was used for statistical analyses.

3 | RESULTS

3.1 | Patient characteristics

Patient characteristics are displayed in Table 1. Most patients presented with normokalaemia, but total of 17 were on MRAs, while 29 were using potassium supplements. According to the AVS results, 29 patients had a unilateral disease and consequently underwent unilateral adrenalectomy. The division to the APA and APN subgroups was based on CYP11B2 staining of the histological samples (Figure S1).

3.2 | Outcome of the adrenalectomy and long-term cure

Clinical data post-adrenalectomy in the APA and APN subgroups is presented in Table 2. No significant differences in blood pressure, daily defined dose (DDD) of antihypertensive medications, postoperative plasma potassium concentration or aldosterone-to-renin ratio (ARR) were observed between the groups.

In accordance with the PASO criteria,[26] complete or partial biochemical cure 3–6 months after adrenalectomy was achieved in all 29 patients. Postoperative aldosterone and renin analyses were missing from two patients but based on the resolution of their hypokalaemia they were classified as biochemically cured. Complete clinical cure was achieved in 8 (27.6%) patients, partial clinical cure in 19 (65.5%) patients and clinical cure was absent in 2 (6.9%) patients (Table 2).

Patients in the adrenalectomy group were followed up to 7 years (mean 4.3 ± 2 years). The APA subgroup retained normokalaemia without potassium supplements suggesting biochemical remission, while in the APN subgroup three (33.3%) patients lost the postoperative improvement and presented with absent clinical improvement during the long-term follow-up. Two of the patients had low potassium level (<3.3 mmol/l) and one had restarted

TABLE 1 Patient characteristics at baseline

Variable (reference range, unit)	All subjects *n* = 49	Adrenalectomy			Medical therapy *n* = 20
		Total, *n* = 29	APA, *n* = 20	APN, *n* = 9	
Number (male/female)	49 (37/12)	29 (20/9)	20 (12/8)	9 (8/1)	20 (17/3)
Age (y)	53.2 ± 8.6	53.2 ± 9.1	52.1 ± 9.0	55.9 ± 9.2	53.1 ± 8.2
BMI (kg/m^2)	30.6 ± 5.6	30.0 ± 6.0	28.7 ± 6.0	32.9 ± 5.0	31.4 ± 5.1
Systolic BP (mmHg)	154 ± 20	155 ± 19	158 ± 21	148 ± 7	154 ± 21
Diastolic BP (mmHg)	94 ± 10	94 ± 10	95 ± 12	90 ± 6	94 ± 10
Lowest plasma K^+ (3.3–5.2 mmol/l)	3.0 ± 0.4	2.8 ± 0.3	2.7 ± 0.3	3.1 ± 0.3	3.2 ± 0.4**
Plasma K^+ (3.3–5.2 mmol/l)	3.5 ± 0.4	3.3 ± 0.4	3.3 ± 0.4	3.5 ± 0.3	3.7 ± 0.3*
24 h U-K^+ (60–90 mmol)	129 ± 41	131 (104-172)	143 ± 39	132 ± 40	106 (86-127)*
Serum aldosterone (<520 pmol/l)	568 (432–829)	685 (484–1116)	715 (552–1328)	497 (347–749)	531 (371–628)*
PRA (1.5–5.7 μg/l/h)	0.2 (<0.2–0.3)	0.2 (<0.2–0.2)	0.2 (<0.2–0.2)	0.2 (0.2–0.6)	0.2 (0.2–0.2)
DRC (4.4–46 mU/l)	2.5 (*n* = 3)	2.1 (*n* = 2)	2.1 (*n* = 2)	(*n* = 0)	10.1 (*n* = 1)
ARR, PRA	1983 (1480–3350)	2660 (1645–4105)	3433 (2480–5118)	1825 (1183–2188)	1655 (1390–2645)*
ARR, DRC	338 (N/a)	1055 (N/a)	1055 (N/a)	N/a	119 (N/a)
24 h U-aldosterone (<40 nmol)	60 (51–90)	71 (57–114)	73 (57–137)	57 (56–71)	56 (47–64)*
Antihypertensive medication, DDD	4.2 ± 2.6	4 ± 2.4	4.5 ± 2.3	4.1 ± 2.7	4.1 ± 2.9

Note: The adrenalectomy group was divided into APA and APN groups according to the adrenal pathology. Asterisks indicate significant differences between medical therapy versus adrenalectomy at baseline.

Abbreviations: APA, aldosterone-producing adenoma; APN, aldosterone-producing nodules; ARR, aldosterone–renin ratio; BMI, body mass index; BP, blood pressure; DDD, daily defined dose; DRC, direct renin concentration; N/a, not available; PRA, plasma renin activity; U, urine.

Number (*n*), mean and standard deviation, or median and interquartile range.

*p < .05; **p < .001.

spironolactone due to hypertension and hypokalaemia. All the remaining patients in the APN subgroup used antihypertensive medication other than MRA at follow-up, but with a lower DDD than before adrenalectomy, and thus they were classified as having reached partial clinical cure. In the APA subgroup, 40% of the patients had no need of antihypertensive medication and presented with complete clinical cure.

3.3 | Selectivity index in AVS

Medians for cortisol, androstenedione, DHEA and DHEAS from the left and right AV and IVC, as well as SI values calculated for each hormone are displayed in Table 3. Expectedly, the step-up between DHEAS from each AV compared with IVC was modest but significant (p < .001), resulting in low SI-values when compared with the three other hormones. The right and left SIs for androstenedione and DHEA were significantly higher (p < .001) than for cortisol.

Significant positive correlations (p < .001) were observed between the SI values for cortisol and adrenal androgens, both in the right (r = .639, .689 and .563 for androstenedione, DHEA and DHEAS, respectively) and left (r = .532, .672, and .554 for androstenedione, DHEA and DHEAS, respectively) sides (Figure S2). The concentrations of adrenal androgens and cortisol did not differ in the AVs when the left and right or the dominant and the nondominant sides were compared (Tables 3 and 4). The same applied for subgroup analysis in the adrenalectomy and the medical therapy groups. In one patient, the right-side SI values for both androstenedione and DHEA were low (2.48 and 3.25, respectively), whereas with cortisol the SI was 20.3.

3.4 | Lateralisation index and contralateral suppression index in AVS

The LI values and CSI values are presented in Table 4. There were strong positive correlations between LIs corrected with androstenedione, DHEA and DHEAS in comparison with cortisol-corrected LI (r = .905, .873 and .936, respectively, p < .001 for all, Figure S3). In the adrenalectomy group, no statistically significant differences were detected between LIs corrected with each adrenal androgen when compared with cortisol. However, in the APA subgroup, DHEAS-corrected LI was significantly higher than that with cortisol (p = .035), while in the APN subgroup no such differences were observed.

TABLE 2 Clinical and biochemical cure in the adrenalectomy group according to the PASO criteria[23]

Variable (reference range, unit)	All adrenalectomized, *n* = 29		APA, *n* = 20		APN, *n* = 9	
	Postoperative	Follow-up	Postoperative	Follow-up	Postoperative	Follow-up
Biochemical cure						
Complete, *n* (%)	27 (93.1)	N/a	18(90)	N/a	9 (100)	N/a
Partial, *n* (%)	2(6.9)	N/a	2(10)	N/a	0 (0)	N/a
Absent, *n* (%)	0 (0)	N/a	0 (0)	N/a	0 (0)	N/a
Clinical cure						
Complete, *n* (%)	8 (27.6)	8 (27.6)	6 (30.3)	8 (40.0)	2 (22.2)	0 (0)
Partial, *n* (%)	19 (65.5)	18 (62.1)	14 (73.7)	12 (60.0)	5 (55.6)	6 (66.7)
Absent, *n* (%)	2 (6.9)	3 (10.3)	0 (0)	0 (0)	2 (22.2)	3 (33.3)
Systolic BP (mmHg)	133 ± 13	133 ± 10	133 ± 14.4	133 ± 9	132 ± 10	132 ± 14
Diastolic BP (mmHg)	80 ± 9	82 ± 9	80 ± 10	83 ± 9	80 ± 8	79 ± 8
Plasma K^+ (3.3–5.2 mmol/l)	4.0 ± 0.5	4.0 ± 0.4	4.1 ± 0.5	4.1 ± 0.3	3.8 ± 0.3	3.8 ± 0.4
Serum aldosterone (<520 pmol/l)	76 (30–208)	N/a	71 (30–178)	N/a	164 (44–213)	N/a
PRA (1.5–5.7 μg/l/h), *n* = 15	0.6 (0.2–2.4)	N/a	0.7 (0.2–5.2)	N/a	0. (0.2–2.0)	N/a
DRC (4.4–46 mU/l), *n* = 12	10.2 (5.3–19.5)	N/a	10.4 (5.0–21.0)	N/a	10.0 (6.1–37.5)	N/a
ARR, PRA, *n* = 15	140 (33–297)	N/a	140 (17–297)	N/a	118 (85–603)	N/a
ARR, DRC, *n* = 12	11.9 (6.5–19.7)	N/a	12.3 (7.9–21.4)	N/a	11.5 (4.4–17.8)	N/a
Antihypertensive medication, DDD	2.3 ± 2.3	1.9 ± 2.0	2.1 ± 2.3	1.1 ± 1.5	3.0 ± 2.3	3.7 ± 2.0

Note: Postoperative clinical data and potassium values are collected at 3–6 months postoperatively. The sampling times for aldosterone and renin concentrations varied from a few days to 6 months postoperatively, and thus, some individuals show suppressed renin, but normalisation of ARR. The follow-up data were collected at 4.3 ± 2.0 years after surgery. The APA and APN groups were defined according to the adrenal CYP11B2 staining (see Figure S1). Number (*n*) and (percentage), mean ± standard deviation, or median (interquartile range).

Abbreviations: APA, aldosterone-producing adenoma; APN, aldosterone-producing nodules; ARR, aldosterone–renin ratio; BP, blood pressure; DDD, daily defined dose; DRC, direct renin concentration; N/a, not available; PRA, plasma renin activity.

TABLE 3 Concentrations and selectivity indexes of adrenal androgens and cortisol

	Right AV	Left AV	IVC	SI right side	SI left side
Androstenedione	531.5 (360.8–653)	360.5 (263.5.–484.5)	5.0 (3.7–6.5)	96 (78–144)*	75 (45–102)*
DHEA	2210 (1388–3595)	1229 (750–1823)	19 (11–30)	121 (86–163)*	66 (48–93)*
DHEAS	5.4 (3.9–7.7)	4.6 (3.4–7.1)	3.5 (2.5–5.1)	1.5 (1.4–1.7)*	1.3 (1.3–1.5)*
Cortisol	29,696 (21,622–39,995)	18,790 (13,462–26,999)	864 (724–994)	36 (26–43)	21 (16–31)

Note: Asterisks indicate significant differences in SI between adrenal androgens and cortisol. Median (interquartile range).

Abbreviations: AV, adrenal vein; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulphate; IVC, inferior vena cava; SI, selectivity index.

*$p < .001$.

Similarly, there were strong positive correlations between CSIs with cortisol and adrenal androgens across all patients ($r = .907$, .963 and .959 for androstenedione, DHEA and DHEAS, respectively, $p < .001$ for all, data not shown).

We used ROC analysis to estimate the optimal LI cut-off values for the adrenal androgens. These were 4.2, 4.5 and 4.6 for androstenedione, DHEA and DHEAS, respectively (Figure 1). There were no differences between the AUCs of the ROC curves between the adrenal androgens and cortisol.

3.5 | Analysis of subjects with discrepant results between cortisol and adrenal androgens

We evaluated whether using the determined LI cut-off values for androstenedione, DHEA and DHEAS would have resulted in a different choice of treatment in any of the patients. Eight cases with cortisol-corrected LI from 2.00 to 5.88 had discrepant LI with adrenal androgens compared to cortisol. Table S1 displays all 13 subjects who fell into this LI range. Single adrenal androgens performed variably

TABLE 4 Lateralisation indexes (LIs) and contralateral suppression indexes (CSIs) of adrenal androgens and cortisol

	Medical therapy, *n* = 20				Adrenalectomy, *n* = 29			
	Dominant AV	Contralateral AV	LI	CSI	Dominant AV	Contralateral AV	LI	CSI
Cortisol (nmol/l)	30,564	30,281	1.6	2.2	18,880	22,800	14.2	0.4
	(15,688–23,419)	(23,419–38,614)	(1.3–2.1)	(1.6–2.9)	(12,540–34,386)	(16,275–27,695)	(6.7–77.4)	(0.5–0.8)
A4 (nmol/l)	518	538	1.7	0.8	379	364	12.0	0.1
	(286–678)	(398–620)	(1.3–3.3)	(0.4–0.9)**	(263–519)	(248–559)	(6.5–74.7)	(0.04–0.3)**
DHEA (nmol/l)	1362	2170	2.4	0.7	1400	1454	14.5	0.1
	(615–3020)	(1583–3595)	(1.6–3.8)	(0.5–0.9)**	(870–2740)	(884–2410)	(6.7–87.3)	(0.03–0.2)**
DHEAS (μmol/l)	6.6	6.4	1.6	46.7	4.5	4.8	16.3	6.6
	(3.8–7.7)	(4.4–9.2)	(1.3–2.5)	(36.6–70.8)**	(3.1–7.3)	(3.2–7.6)	(8.0–76.6)	(1.6–13.0)**
	APN, *n* = 9				APA, *n* = 20			
	Dominant AV	Contralateral AV	LI	CSI	Dominant AV	Contralateral AV	LI	CSI
Cortisol (nmol/l)	21,290	17,481	7.1	0.7	18,295	23,302	41.7	0.2
	(13,692–36,731)	(15,515–26,486)	(5.2–11.8)	(0.4–1.2)	(11,072–26,020)	(16,484–27,960)	(10.1–103.1)	(0.1–0.7)
A4 (nmol/l)	578	358	9.1	0.3	366	377	27.1	0.05
	(201–904)	(236–564)	(5.2–10.8)	(0.1–0.5)*	(263–487)	(255–559)	(8.4–94.3)	(0.03–0.2)**
DHEA (nmol/l)	1584	999	6.1	0.3	1325	1579	35.1	0.1
	(693–2920)	(719–2251)	(3.9–11.8)	(0.1–0.4)*	(871–2635)	(884–2455)	(10.5–98.9)	(0.02–0.2)**
DHEAS (μmol/l)	3.9	3.3	10.7	13.6	4.5	5.3	56.9	2.0
	(2–6.5)	(1.8–6.2)	(5.2–16.5)	(6.5–21.3)*	(3.8–10.1)	(3.7–11.2)	(10.0–105.7)*	(1.3–7.2)**

Note: Asterisks denote significant differences in LI or CSI between adrenal androgens and cortisol. Median (interquartile range); dominant AV, adrenal vein draining the dominant aldosterone-producing adrenal; contralateral AV, adrenal vein draining the nondominant adrenal.

Abbreviations: A4, androstenedione; APA, aldosterone-producing adenoma; APN, aldosterone-producing nodules; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulphate.

*$p < .05$; **$p < .001$.

when compared with cortisol. In 10 patients with complete analyses available, at least 2 out of the 3 measured adrenal androgen-corrected LIs were concordant with the cortisol-corrected LIs. In the remaining three patients with missing DHEAS analyses, at least one out of two LIs were also concordant with the cortisol-corrected LIs. Similar findings were observed in those patients who underwent adrenalectomy, among whom the long-term cure was retained in four subjects (two with APA and two with APN) and lost in two subjects with APN.

4 | DISCUSSION

The determination of surgically eligible PA patients remains cumbersome and the search for the optimal methodology for the identification of lateralized aldosterone excess continues. Since improvements in survival, morbidity and on quality of life are superior with adrenalectomy as compared with MRA treatment, selection of the most appropriate treatment is fundamental.[32–35] AVS is recommended for PA subtyping, but the procedure is challenging.[11,36] Measurements of androstenedione, DHEA and DHEAS are commonly used in the endocrine practice. In the present study, we focused on the possible additional diagnostic value of determining these adrenal androgens during cosyntropin-stimulated AVS. Our study indicates that the low-cost measurement of androstenedione and DHEA may improve the assessment of cannulation selectivity, but only play a confirmatory role in the subtype diagnosis of PA.

In our study, androstenedione and DHEA performed well in assessing selectivity. We found that the right and left side SIs for androstenedione and DHEA were higher than those for cortisol, and there were strong correlations between cortisol-based SI with SIs measured for each adrenal androgen. Our results confirm those of a previous study, in which AVS was performed either with or without cosyntropin but postoperative follow-up lasted only for 12 months.[23] However, the present adrenalectomy population was examined using a more homogenous methodology combined with long-term follow-up of up to 7 years. The comparisons of basal and cosyntropin-stimulated AVS sampling by Turcu et al.[21] found that the androstenedione LI exceeded that of cortisol LI, but their study did not include DHEA analyses. We found that the concentration ratio step-up from the IVC to the AV was significant but small for DHEAS, making it less useful for measuring SI and therefore, in PA subtype diagnostics in general. The long half-life of DHEAS may explain the finding.

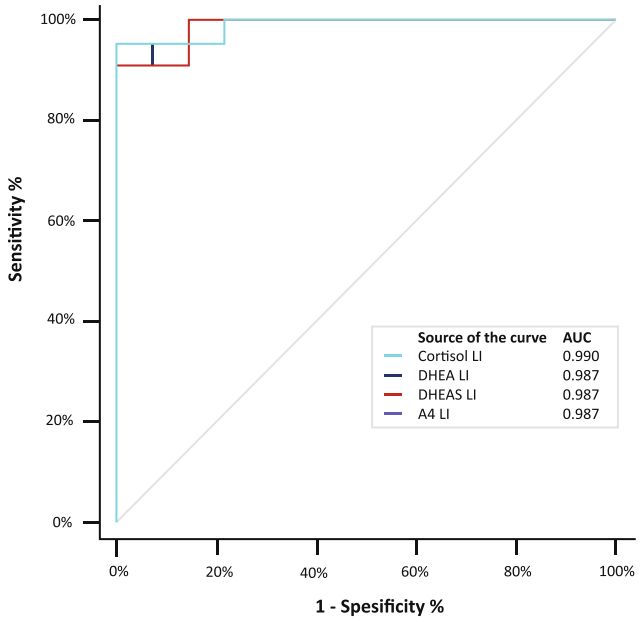

FIGURE 1 ROC curve of lateralisation indexes calculated with cortisol and androstenedione, DHEA and DHEAS. A4, androstenedione; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulphate; ROC, receiver operating characteristics [Color figure can be viewed at wileyonlinelibrary.com]

We aimed to evaluate whether the use of androstenedione, DHEA and DHEAS in calculating the LI would provide additional benefit. Only few studies have evaluated the use of biomarkers other than cortisol in assessing the lateralisation under cosyntropin stimulation.[21–23] Among these studies, cosyntropin was given as a single dose by Turcu et al.,[21] while continuous cosyntropin stimulation was administered to a subset of patients by Peitzsch et al. and Eisenhofer et al.[22,23] In the aforementioned investigations 31 and 68 patients, respectively, underwent analyses from samples taken during continuous cosyntropin stimulation. In line with the study using a single injection of cosyntropin,[21] we found a strong correlation between LI values with cortisol and adrenal androgens during cosyntropin infusion.

All adrenalectomized patients showed biochemical cure and most showed clinical cure according to the PASO criteria at 3–6 months postoperatively, which is consistent with the literature.[9] We related our LI data to the adrenalectomy outcome and performed a ROC analysis to assess LI cut-offs for different adrenal androgens. Using these cut-offs, we observed that when cortisol-corrected LI was above 5.88 or below 2.00, adrenal androgen-corrected LIs were always concordant with the cortisol-based LIs. However, seven of the 13 patients with cortisol-corrected LI ranging from 2.00 to 5.88 demonstrated some disagreement between cortisol and adrenal androgen-based lateralisation (Table S1). Two of the six adrenalectomized subjects with LI up to 5.88 lost the initial clinical and biochemical cure during follow-up, most probably due to progression of hyperplasia in the remaining adrenal gland. Earlier Turcu et al.[21] found a discrepancy between pre- and post-cosyntropin results in patients with intermediate disease severity when compared with clear concordance in patients with robust lateralisation. This probably reflects the same hard-to-define population as in our study. Neither cortisol nor any single adrenal androgen-corrected LI was able to perfectly distinguish between long-term cure and failure in the operated patients. Based on these findings, the performance of adrenal androgens was confirmatory but not superior to the cortisol-based LI in the lateralisation diagnostics of PA when continuous cosyntropin was used.

We could further characterize the adrenalectomy group to those with CYP11B2-positive APA and those with APN, that is, patients with CYP11B2-positive hyperplastic nodules, microadenomas or multiple aldosterone-producing micronodules (APMs)[37] While the adrenal androgens performed well in the APA subgroup, the significantly higher LI values for DHEAS as compared to those with cortisol must be interpreted together with the problems detected in DHEAS-based SI, which impede its value on the subtype diagnostic of PA. Furthermore, androstenedione and DHEA showed similar ratios of CSI in adrenalectomy and medical therapy groups as well as APA and APN subgroups as compared to cortisol whereas DHEAS performed less accurately.

Cosyntropin stimulation during AVS aims to improve the selectivity of cannulation and may improve the cure rate up to 20%–30%.[36,38] However, it has been suggested to potentially reduce the LI value erroneously below the lateralisation limit.[38] One probable reason for this is aberrant melanocortin 2 receptor (MC2R) expression in APA, which may in some cases mask true lateralisation, or even invert the side of lateralisation.[39] In our study, conformity of lateralisation with cortisol-based and other adrenal steroid-based results suggests against major effect by cosyntropin stimulation.

The strengths of our study are its prospective design, long-term follow-up of the adrenalectomy group, use of CYP11B1 and CYP11B2 staining, and detailed analysis of the value of adrenal androgens in aiding the cure following AVS and the choice of therapy. However, our study has some limitations. The statistical power was primarily planned for comparing the performance of AVS compared with ^{11}C-metomidate positron emission tomography and the sample size for this secondary analysis is rather low considering small differences found between variables especially since the blood samples were inadequate for analysing DHEAS in all subjects. Additionally, because the ROC analysis was performed on the grounds of treatment choices based on cortisol-corrected LI, the cut-offs derived from the analysis must be addressed with caution. On hindsight, we can speculate that the comparison of adrenocortical hormone-corrected LIs to adrenal medullary metanephrine-corrected LI would have been interesting. We cannot rule out the possibility of unilateral disease in cases without surgical treatment which leaves the open question whether cosyntropin stimulation causes a bias in subtype categorisation. Finally, evaluation of postoperative biochemical cure was not perfectly timed and the long-term follow-up included potassium but lacked measuring renin and aldosterone which would have given more solid proof of cure.

In summary, in a well-defined group of PA patients, we report that the analyses of androstenedione and DHEA, but not of DHEAS, may improve the cannulation selectivity under cosyntropin-infusion stimulated AVS. For the lateralisation diagnostics, the performance of adrenal androgens is confirmatory but not superior to cortisol-based decision making, which proved to be reliable when compared with long-term cure in adrenalectomized subjects. Furthermore, single adrenal androgen measurements perform impeccably when cortisol-corrected LI is above 5.88 or below 2.00, but do not improve cortisol-based subtyping. Whether analyses of multiple adrenal androgens support decision-making between surgery and medical treatment in case of borderline LI values deserves further studying. However, these results do not support the implementation of additional adrenal androgens to contemporary practice.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Eeva Kokko and Marianna Viukari reviewed the literature and wrote the original version of the manuscript. Marianna Viukari performed statistical analyses. Ilkka Pörsti, Pasi I. Nevalainen, and Niina Matikainen participated in the design of technical details and methodology of the study. All authors contributed to the discussion and editing of the manuscript. Niina Matikainen and Pasi I. Nevalainen were responsible for designing and conducting the study. All authors take the responsibility for the contents of the manuscript

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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