

Comprehensive Analysis of Steroid Biomarkers for Guiding Primary Aldosteronism Subtyping

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Abstract—Adrenal vein sampling (AVS) is required to distinguish unilateral from bilateral aldosterone sources in primary aldosteronism (PA), and cortisol is used for AVS data interpretation, but cortisol has several pitfalls. In this study, we present the utility of several other steroids in PA subtyping, both during AVS, as well as in peripheral serum. We included patients with PA who underwent AVS at University of Michigan between 2012 and 2018. We used mass spectrometry to simultaneously quantify 17 steroids in adrenal veins (AV) and periphery, both at baseline and after cosyntropin administration. PA was classified as unilateral or bilateral based on a lateralization index \geq or $<$ 4, respectively, separately for baseline and post-cosyntropin administration. Of 131 participants, AV catheterizations was deemed failed in 28 (21 %) patients (36 AVs) at baseline. Eight steroids demonstrated higher AV/periphery ratios than cortisol ($P < 0.01$ for all); 11β -hydroxyandrostenedione, 11-deoxycortisol, and corticosterone rescued most failed baseline catheterizations. Lateralization was generally consistent when using these alternative steroids. Based on pre- and post-cosyntropin data, the remaining 103 patients were classified as: U/U, 37; B/B, 32; U/B, 20; B/U, 14. Discriminant analysis of multi-steroid panels from peripheral serum showed distinct profiles across the 4 groups, with highest aldosterone, 18-oxocortisol and 11-deoxycorticosterone in U/U patients. In conclusion, 11β -hydroxyandrostenedione and 11-deoxycortisol are superior to cortisol for AVS data interpretation. Single assay multi-steroid panels measured in peripheral serum are helpful in stratified PA subtyping and have the potential to circumvent AVS in a subset of patients with PA. (*Hypertension*. 2020;75:00-00. DOI: 10.1161/HYPERTENSIONAHA.119.13866.) • [Online Data Supplement](#)

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Key Words: adrenal gland ■ aldosterone ■ hypertension ■ renin

Primary aldosteronism (PA) is traditionally subtyped into unilateral forms, most commonly aldosterone-producing adenoma (APA), or bilateral hyperaldosteronism (BHA).¹ Accurate PA subtyping is essential for guiding clinical management. Unilateral PA can be cured or improved by unilateral adrenalectomy, while BHA requires life-long medical therapy.¹ Adrenal vein sampling (AVS) is recommended by expert guidelines for PA subtyping.^{1,2} AVS protocols and criteria for data interpretation have varied between referral centers, leading to heterogeneity in selecting PA surgical candidates.^{3,4}

Cortisol is used in all steps of AVS results interpretation. Disadvantages of using cortisol include longer half-life relative to aldosterone and fluctuations during the procedure, particularly important in the absence of cosyntropin stimulation. Furthermore, mild autonomous cortisol excess is relatively common in patients with PA,^{5,6} which can lead to cortisol suppression in the contralateral adrenal gland and alter AVS results. Recent studies have proposed alternative biomarkers for AVS data interpretation, including metanephrines, androstenedione (A4), dehydroepiandrosterone, 17α -hydroxyprogesterone

(17OHP), and 11-deoxycortisol (11dF).⁷⁻¹¹ A4, dehydroepiandrosterone and 17OHP, however, are also produced by the gonads, and the latter has cyclical variations in reproductive age women. In contrast, 11β -hydroxyandrostenedione (11OHA4) is produced primarily and abundantly in the adrenal glands,^{12,13} and we, therefore, hypothesized that 11OHA4 could be a valuable biomarker for adrenal vein catheterization.

Beyond the variability in protocols and data interpretation among expert centers, additional AVS drawbacks, including its high cost, scarce availability and technical challenges, have recently driven efforts to develop noninvasive PA subtyping methods, such as steroid biomarkers measured in peripheral serum.^{8,14} Peripheral 18-oxocortisol (18oxoF) and 18-hydroxycortisol (18OHF) have been shown to perform well in identifying APAs in Asian patients, who have a high prevalence of *KCNJ5* mutations.¹⁴ The utility of these hybrid steroids was, however, poor when used alone in Europeans,⁸ who display a variety of somatic aldosterone-driver mutations.¹⁵ In such populations, multi-steroid panels hold more promise in PA subtyping based on peripheral blood tests.⁸ Herein, we present

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Table 1. Characteristics of Study Participants

Patient Characteristics	Unilateral, N=37	Bilateral, N=32	Discordant, N=34	Total, N=131	P Value
Sex, M/%	21 (57%)	19 (59%)	25 (74%)	81 (62%)	0.30
Age	52 [45–58]	50 [45–61]	50 [46–60]	57 [42–65]	0.98
sBP, mm Hg	150 [139–161]	160 [139–189]	156 [137–171]	156 [140–174]	0.15
dBp, mm Hg	90 [78–99]	89 [79–94]	86 [80–93]	88 [79–96]	0.50
ATC/DDD index	4.3 [2.3–5.6]	5.2 [1.7–7.1]	6.2 [3.4–8.4]	5 [2.7–7.1]	0.05
H/o hypokalemia (%)	97%	84%	94%	89%	0.12
PAC, ng/dL	36 [25–61]	24* [18–33]	26* [22–40]	28 [22–40]	0.0007
PRA, ng/mL per h	0.2 [0.1–0.6]	0.3 [0.1–0.7]	0.4 [0.1–0.6]	0.3 [0.1–0.6]	0.86
ARR	105 [53–371]	86 [33–185]	74 [51–219]	89 [45–245]	0.24

Data are expressed as medians [interquartile range]. ARR indicates PAC/PRA ratio; ATC/DDD index, standardized daily defined dose of antihypertensive medications; dBp, diastolic blood pressure; PAC, plasma aldosterone concentration; PRA, plasma renin activity; and sBP, systolic blood pressure.

*P<0.05, compared with unilateral

the performance of a broad spectrum of steroid biomarkers in AVS data interpretation and PA subtyping.

Patients and Methods

Study Participants

We included patients who underwent AVS at the University of Michigan for PA subtyping between April, 2012 and July, 2018. The diagnosis of PA was ascertained in accordance with the Endocrine Society guidelines.¹ Additional inclusion criteria comprised: written informed consent from participants, and available serum left after completion of clinical assays. The study was conducted with University of Michigan Institutional Review Boards approval. The data that support the findings of this study are available from the corresponding author on reasonable request.

AVS-Protocol and Data Interpretation

Simultaneous AVS was performed before and after 0.25 mg cosyntropin administration, as previously described.¹⁶ Samples were first stored at 4°C for clinical assays and subsequently frozen at -80°C until used for liquid chromatography-tandem mass spectrometry (LC-MS/MS) steroid measurement. Catheterization was considered successful when the selectivity index (SI=AV/IVC cortisol concentrations) was ≥2 before and ≥5 after cosyntropin administration, respectively. PA was classified as unilateral or bilateral based on a lateralization index (LI=aldosterone/cortisol ratio between the dominant and contralateral AVs) was ≥ or <4, respectively, separately for pre- and post-cosyntropin administration. A contralateral index,

defined as (aldosterone/cortisol)_{nondominant AV}/(aldosterone/cortisol)_{IVC} <1 defined contralateral suppression. Clinical laboratory cortisol, aldosterone and plasma renin activity were measured with immunoassays, as previously reported.¹⁶

Steroid Quantification by Liquid Chromatography-Tandem Mass Spectrometry

We used LC-MS/MS to quantify 17 Δ⁴ steroids in a single assay, including: aldosterone, 18OHF, 18oxoF, 11-deoxycorticosterone (DOC), corticosterone, cortisol, cortisone, 11dF, A4, 11OHA4, 11-ketoandrostenedione, testosterone (T), 11β-hydroxytestosterone, 11-ketotestosterone, progesterone (Prog), 17OHP, and 16α-hydroxyprogesterone (16OHP). Steroid extraction and LC-MS/MS quantitation were performed as recently described.¹⁷ The assay performance for four steroids not previously published (aldosterone, 18oxoF, 18OHE, and 16OHP) are presented in Table S1 in the online-only Data Supplement. For steroid quantitation in AV serum, 5 to 10 μL aliquots were used (dilution 10 and 20-fold for pre- and post-cosyntropin, respectively); and 100 μL aliquots were used to measure steroids in peripheral serum (no dilution, both pre- and post-cosyntropin).

Statistical Analysis

Comparison of continuous variables between the dominant and contralateral AV was done with paired t test. Kruskal-Wallis with Dwass-Steel pair-wise comparison were performed for comparison of continuous variables across multiple groups. Linear correlation between continuous variables were assessed by the Spearman

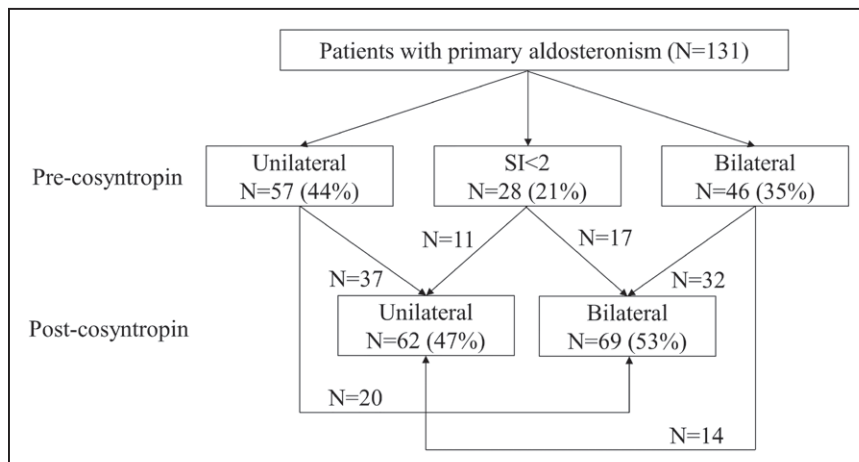


Figure 1. Aldosterone lateralization of study participants. Based on LC-MS/MS lateralization across 6/9 steroids, lateralization indices (LI) differed from clinical LI, as follows: (1) 3 patients were classified as bilateral; (2) 4 patients were classified as unilateral; and (3) 6 patients were classified as unilateral. SI indicates selectivity index.

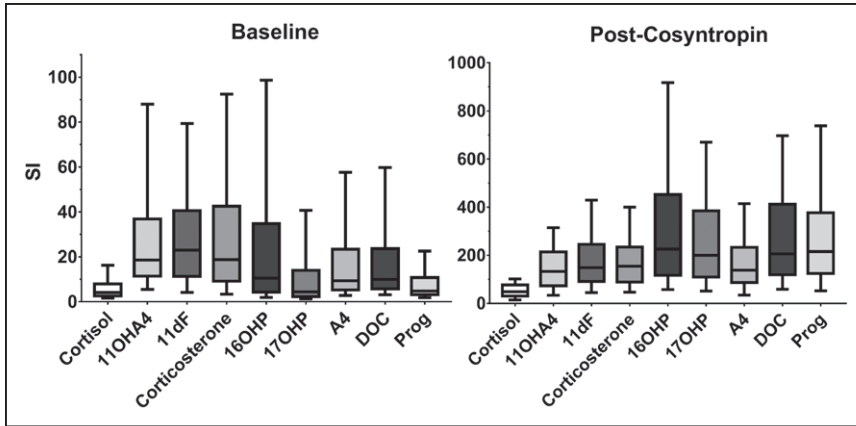


Figure 2. Comparison of selectivity index (SI) using cortisol and alternative steroids. Figure illustrates the standard-of-care, cortisol, and all steroids with SI higher than cortisol ($P < 0.01$ for all). The boxes represent the interquartile range, the horizontal line marks the median, and the whiskers mark the 10 to 90 percentiles. 11dF indicates 11-deoxycortisol; 11OHA4, 11 β -hydroxyandrostenedione; 16OHP, 16-hydroxyprogesterone; 17OHP, 17 α -hydroxyprogesterone; A4, androstenedione; DOC, 11-deoxycorticosterone; and Prog, progesterone.

correlation test. Predictions of binary responses using a panel of steroids were done using regularized (penalized) logistic regression analysis with elastic nets penalties. The penalty parameters were chosen by cross-validation to achieve the best prediction. Receiver-operating characteristics were used to characterize the prediction performance. Linear and quadratic discriminant analysis was used on multi-class classification problems. Analyses were performed using SAS 9.4. (Cary, NC) and R-3.4.3 (Foundation for Statistical Computing, Vienna, Austria). Statistical significance was determined at a level of 0.05 for 2-tailed tests.

Results

During the study period, 131 patients (81 men, median age 57 years) meeting all entrance criteria were recruited. Demographic and clinical characteristics of study participants are presented in Table 1. In total, 54 patients underwent unilateral adrenalectomy within our institution. Based on the PA surgical outcomes criteria,¹⁸ clinical benefit was achieved in 87% of patients: cure in 16 (30 %) and partial clinical success

in 31 (57%) patients; clinical benefit was absent in 7 (13%) patients. Biochemical follow-up was available in 47 (87 %) patients. Of these, biochemical cure was achieved in 43 (91.5%) patients; one patient had partial biochemical benefit, and 3 patients had no biochemical benefit after adrenalectomy.

Performance of Steroids as Indicators of AV Catheterization

Based on clinical data, 28 (21 %) patients had an SI <2 before using cosyntropin in at least one AV, including 8 patients with bilateral SI <2 (Figure 1). Of the 17 steroids measured, 8 steroids demonstrated significantly higher SI than cortisol, both before and after cosyntropin: 11OHA4, 11dF, corticosterone, 16OHP, 17OHP, A4, DOC, and Prog ($P < 0.01$ for all, compared with cortisol; Figure 2). Before cosyntropin administration, 11dF, corticosterone and 11OHA4 demonstrated the highest SI (medians of 23, 18.7, and 18.6, respectively), while the median baseline cortisol SI was 4.1 (Figure 2).

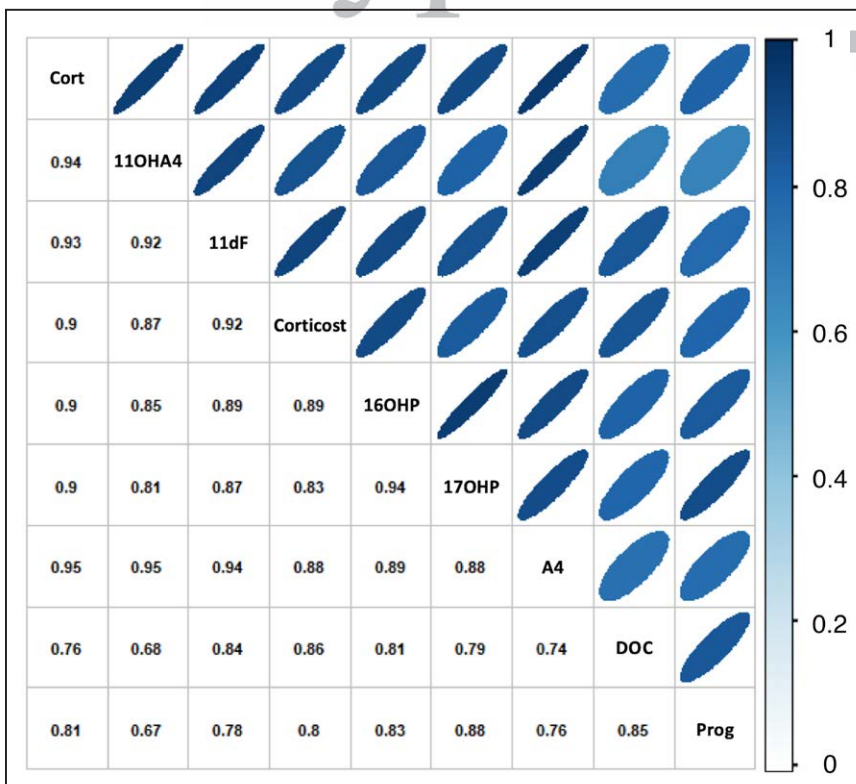


Figure 3. Correlation between lateralization indices (LI) based on correction of aldosterone to cortisol vs alternative steroids. 11dF indicates 11-deoxycortisol; 11OHA4, 11 β -hydroxyandrostenedione; 16OHP, 16-hydroxyprogesterone; 17OHP, 17 α -hydroxyprogesterone; A4, androstenedione; DOC, 11-deoxycorticosterone; and Prog, progesterone.

Table 2. Comparison of Baseline and Cosyntropin-Stimulated Steroid Concentrations in the Adrenal Veins (AV) and Peripheral Serum of Patients With PA Across Lateralization Groups

Steroid	Baseline Concentrations, Dominant AV (ng/dL for all, except for cortisol, mcg/dL)				P Value
	B/B	B/U	U/B	U/U	
18OHF	5361 [2254–13039]	3024 [1289–6617]	4506 [2362–10354]	11 936 [3854–58 500]	0.009
18oxoF	61 [19–155]	40 [13–153]	91 [44–230]	294 [74–1732]	0.0004
Aldosterone	461 [237–1052]	179 [56–650]	1141 [485–2719]	2309 [801–6529]	<.0001
DOC	52 [29–163]	32 [22–75]	132 [56–241]	259 [65–1285]	0.0002
Cortisol	25 [19–56]	18 [13–28]	27 [18–41]	28 [18–101]	0.12
Cortisone	1968 [1483–3033]	1513 [1195–2500]	1408 [1255–2598]	2182 [1252–4015]	0.28
11OHT	59 [33–101]	54 [26–132]	32 [15–79]	52 [31–83]	0.33
11KT	45 [30–55]	37 [23–53]	27 [18–43]	36 [27–58]	0.44
11OHA4	5705 [3019–11709]	2875 [1278–8972]	4737 [2613–11 089]	5250 [3090–11 840]	0.28
11KA4	170 [74–343]	99 [50–181]	90 [66–164]	103 [59–126]	0.04
11dF	603 [319–1323]	348 [240–494]	815 [346–994]	648 [418–3197]	0.04
Corticosterone	2797 [1285–5884]	1235 [381–2317]	3093 [1585–4070]	2624 [1428–18 386]	0.02
16OHP	134 [57–631]	71 [39–112]	166 [93–331]	151 [58–947]	0.09
17OHP	348 [163–991]	210 [147–257]	426 [243–907]	280 [192–1790]	0.09
T	203 [68–373]	252 [178–479]	170 [46–344]	166 [91–287]	0.31
A4	565 [419–1420]	348 [244–675]	578 [316–1127]	632 [351–3618]	0.19
Progesterone	35 [20–81]	20 [16–40]	73 [46–101]	87 [29–308]	0.0007
Steroid	Baseline Concentrations, Contralateral AV, (ng/dL for all, except for cortisol, mcg/dL)				P Value
	B/B	B/U	U/B	U/U	
18OHF	1879 [1382–7724]	3084 [968–5401]	1632 [1131–3842]	2426 [1784–8629]	0.19
18oxoF	20 [12–53]	25 [8–63]	19 [7–31]	21 [8–51]	0.54
Aldosterone	152 [74–455]	121 [67–272]	95 [39–248]	80 [41–283]	0.26
DOC	46 [27–77]	30 [25–81]	37 [24–50]	48 [31–92]	0.44
Cortisol	21 [15–46]	18 [15–32]	19 [13–27]	27 [18–97]	0.11
Cortisone	1789 [1328–2490]	1791 [1158–2692]	1259 [1045–2632]	2267 [1508–5628]	0.045
11OHT	41 [20–91]	79 [28–131]	35 [18–69]	53 [29–93]	0.19
11KT	38 [25–55]	38 [24–65]	29 [17–42]	39 [25–56]	0.24
11OHA4	3634 [2133–10 879]	3751 [2181–9319]	2855 [2212–11 273]	5819 [2880–17521]	0.27
11KA4	95 [63–311]	162 [56–180]	89 [54–148]	79 [49–134]	0.19
11dF	508 [277–941]	378 [294–725]	453 [306–780]	481 [352–1729]	0.48
Corticosterone	1465 [977–4329]	1335 [623–3440]	1027 [668–3346]	1909 [1040–8286]	0.14
16OHP	129 [62–379]	80 [45–137]	107 [54–330]	129 [55–903]	0.53
17OHP	306 [169–729]	224 [114–448]	287 [189–709]	285 [154–1815]	0.71
T	165 [52–362]	263 [201–465]	147 [37–326]	215 [112–392]	0.09
A4	447 [303–875]	431 [316–996]	446 [325–830]	677 [367–4915]	0.29
Progesterone	28 [16–61]	24 [16–45]	28 [19–41]	29 [17–204]	0.95

(Continued)

Importantly, 30/36 (83%) AV samples with a clinical precosyntropin SI <2 were above the threshold of 2 when using 11OHA4 and corticosterone, and in 29/36 (80.6%) AV samples SI was minimum 2 when using 11dF. After cosyntropin administration, SI was amplified in all of these 8 steroids, and achieved the highest values for 16OHP, Prog, DOC, and

17OHP, all with median SI >200, while the median SI for cortisol was 49 (Figure 2).

PA Lateralization

Of the 103 patients in whom catheterization was deemed successful both before and after cosyntropin based on

Table 2. Continued

Cosyntropin-Stimulated Concentrations, Dominant AV (ng/dL)				
B/B	B/U	U/B	U/U	P Value
73 349 [40 577–147 242]	69 421 [39 342–129 379]	63 714 [41 582–121 160]	84 488 [54 549–145 029]	0.69
588 [234–1474]	1044 [493–3213]	503 [328–966]	2235 [1073–4700]	0.0002
4597 [2440–9261]	8392 [4496–14212]	4434 [2503–9163]	18 878 [7973–32 221]	<.0001
5727 [2950–10010]	5371 [3856–8759]	5751 [3991–10622]	11 682 [5627–17 538]	0.0015
947 [528–1264]	634 [407–1003]	961 [615–1138]	719 [525–1059]	0.38
30 416 [11 313–49 079]	19 174 [4289–31 223]	25 823 [14 733–41 557]	17 142 [11 260–41 048]	0.83
288 [150–463]	212 [113–392]	298 [198–388]	213 [118–333]	0.45
97 [59–141]	75 [39–118]	101 [60–169]	73 [56–105]	0.45
67 597 [39 870–102 234]	51 730 [33 216–81 854]	690 06 [48 205–103 691]	53 477 [30 143–66 649]	0.17
463 [239–1514]	400 [162–861]	813 [385–1202]	317 [179–584]	0.051
27 912 [15 860–36 632]	21 060 [12 217–27 370]	25 862 [18 437–38 522]	25 107 [17 825–34 616]	0.37
244 267 [113 286–415 793]	192 696 [75 742–259 351]	223 477 [156 043–264 565]	211 714 [14 3397–319 911]	0.49
23 582 [11 527–40 850]	17 458 [9189–28 057]	31 107 [16 821–46 469]	20 499 [12 361–32 502]	0.12
47 590 [22 766–81 813]	28405 [19059–55290]	71 899 [43 643–108 654]	42 390 [22 838–61 863]	0.01
500 [323–732]	489 [345–759]	464 [204–725]	436 [289–545]	0.72
14 961 [10 106–24 745]	12 449 [8841–20 879]	11 744 [9824–22 868]	13 423 [8871–18 793]	0.70
6359 [4102–12 525]	4310 [2648–8723]	11 600 [4876–16 463]	6565 [4410–12 737]	0.11
Cosyntropin-Stimulated Concentrations, Contralateral AV, ng/dL				
B/B	B/U	U/B	U/U	P Value
79 929 [46 433–155 073]	48 992 [42 991–11 1783]	46 846 [37 231–78 585]	43601 [22112–77795]	0.036
613 [180–1148]	173 [125–549]	248 [198–420]	131 [60–327]	0.0005
4328 [1460–6752]	1004 [325–2083]	1948 [1179–3479]	635 [236–1648]	<.0001
6271 [3580–10 194]	3222 [2488–5505]	5374 [3545–8268]	4673 [2707–6541]	0.062
982 [795–1226]	747 [713–1718]	727 [507–1099]	757 [536–1086]	0.23
29 128 [14 027–53 349]	27 490 [22 668–74 159]	20 999 [13 781–28 681]	22 594 [11 919–44 734]	0.33
269 [189–490]	321 [216–445]	268 [115–417]	214 [144–320]	0.14
93 [52–176]	99 [76–140]	75 [55–134]	75 [50–113]	0.28
70 963 [44 376–119 969]	69827 [54693–108958]	51 296 [38 890–102 088]	50 179 [32 084–81 350]	0.10
634 [232–1561]	490 [280–861]	479 [312–1111]	286 [151–627]	0.03
27 653 [15 200–42 550]	18 193 [15 402–30 975]	24 580 [18 520–36 577]	19 159 [10 963–27 952]	0.17
300 606 [196 666–371 580]	202 337 [153 672–371 142]	204 575 [132 741–248 671]	203 525 [13 3218–244 159]	0.04
27 244 [15 386–41 119]	20 732 [13 885–35 962]	29 541 [12 319–42 774]	18 291 [11 251–42 384]	0.69
45 355 [32 521–79 222]	30 474 [17 127–92 238]	60 553 [32 943–112 855]	35 833 [17 547–87 247]	0.23
530 [348–787]	599 [427–1004]	446 [245–685]	425 [231–610]	0.054
16651 [9766–24 995]	15 574 [10 470–17 948]	12 305 [7655–21 359]	12 304 [8548–18 653]	0.21
8423 [5437–14 218]	6332 [2895–13 347]	9586 [4562–20 034]	5657 [3618–10 104]	0.09

(Continued)

clinical laboratory data, lateralization was concordant pre- and post-cosyntropin stimulation in 69 patients (37 U/U and 32 B/B), while 34 patients had discrepant lateralization results (20 U/B and 14 B/U; Figure 1). Of the 28 patients with failed catheterization precosyntropin based on clinical laboratory cortisol, 17 patients were diagnosed

with bilateral PA and 11 with unilateral PA based on post-cosyntropin clinical laboratory data (Figure 1). When lateralization was adjudicated based on consistent LI for at least 6 of 9 LC-MS/MS-measured steroids, including cortisol and the 8 steroids with SI performance superior to cortisol, results differed from clinical laboratory data-based

Table 2. Continued

	Baseline Concentrations, Peripheral Serum, ng/dL				P Value
	B/B	B/U	U/B	U/U	
18OHF	372 [236–612]	418 [234–557]	298 [105–398]	421 [202–893]	0.26
18oxoF	2 [1–4]	2 [1–8]	2 [1–2]	5 [2–16]	0.002
Aldosterone	14 [7–27]	18 [8–20]	12 [7–22]	34 [10–62]	0.026
DOC	4 [3–6]	4 [3–11]	3 [3–5]	7 [4–18]	0.006
Cortisol	6 [4–11]	6 [4–8]	5 [4–7]	6 [4–10]	0.79
Cortisone	1057 [743–1422]	917 [713–1452]	877 [744–1177]	1097 [687–1502]	0.89
11OHT	15 [9–22]	16 [12–31]	11 [8–21]	13 [8–20]	0.38
11KT	21 [13–39]	22 [12–35]	19 [13–27]	16 [11–30]	0.62
11OHA4	211 [150–416]	186 [142–368]	187 [150–290]	158 [119–291]	0.20
11KA4	36 [20–68]	22 [15–34]	39 [20–59]	22 [14–33]	0.005
11dF	25 [14–41]	28 [13–41]	18 [12–36]	23 [12–58]	0.81
Corticosterone	91 [52–199]	71 [36–355]	69 [38–169]	128 [58–361]	0.32
16OHP	12 [5–35]	10 [6–19]	14 [7–19]	12 [2–21]	0.74
17OHP	70 [35–131]	58 [41–118]	74 [37–137]	61 [36–89]	0.82
T	183 [25–329]	331 [161–521]	275 [26–411]	223 [22–428]	0.19
A4	51 [42–60]	50 [35–67]	48 [35–74]	50 [33–87]	0.96
Progesterone	6 [4–10]	6 [4–11]	6 [5–8]	6 [5–15]	0.61

All steroid concentrations are expressed in ng/dL, except cortisol, which is expressed in mcg/dL. Values represent medians [interquartile range]. 18OHF indicates 18-hydroxycortisol; 18oxoF, 18-oxocortisol; DOC, 11-deoxycorticosterone; 11OHT, 11 β -hydroxytestosterone; 11KT, 11-ketotestosterone; 11OHA4, 11 β -hydroxyandrostenedione; 11KTA, 11-ketoandrostenedione; 11dF, 11-deoxycortisol; 16OHP, 16 α -hydroxyprogesterone; 17OHP, 17 α -hydroxyprogesterone; T, testosterone; A4, androstenedione.

lateralization in 12 patients. Of these, a single case with B/B clinical data classification was considered U/U based on steroids measured by mass spectrometry, and this patient did not undergo adrenalectomy. The other 11 cases had partially discordant classification (only pre- or post-cosyntropin) between clinical and LC-MS/MS-derived LI. Three such patients, all with LC-MS/MS unilateral classification (versus bilateral based on clinical data LI), underwent surgery and all 3 had complete biochemical resolution of PA.

Overall, clinical laboratory LI correlated tightly with LI based on LC-MS/MS measurements using as denominators cortisol, and the 8 steroids with SI superior to cortisol ($r^2 \geq 0.75$, $P < 0.001$ for all, and the correlation of cortisol-based LI was tightest with A4, 11OHA4 and 11dF (Figure 3). Paired comparison of LI across steroids, showed that without cosyntropin, 11OHA4 and A4 were significantly higher than clinical LI ($P = 0.02$ and 0.04 , respectively), while after cosyntropin, LI was overall similar across all steroids used.

Steroid Profiles in PA Subtypes

Within-group comparison of steroids in the dominant versus the contralateral AV revealed that in the U/U group not only aldosterone, but also 18oxoF, 18OHF, and DOC were higher in the dominant AV both pre- and post-cosyntropin stimulation (Table 2). Conversely, in the U/B, the same 4 steroids were significantly higher in the dominant AV only at baseline, while in the B/U group, they were

significantly higher only after cosyntropin stimulation (Table 2).

Across-group comparisons of steroids showed that aldosterone and 18oxoF were consistently highest in the U/U group, not only in the dominant AV (pre- and post-cosyntropin), but also in peripheral serum, both at baseline and after cosyntropin stimulation (Table 2). DOC and 18OHF were also overall highest in the U/U group, reaching statistical significance when compared with the B/B patients in the dominant AV, both before and after cosyntropin stimulation (Table 2). These results suggest a gradient of PA severity across groups, ranging from most severe PA in U/U cases to mildest in the B/B cases. Conversely, in the contralateral AV, cosyntropin-stimulated aldosterone, 18OHF and 18oxoF were highest in the B/B group as compared with the other 3 groups (Table 2), consistent with a mechanism of contralateral suppression for unilateral cases.

Discriminant analysis incorporating all steroids measured in peripheral serum demonstrated specific profiles to the 4 groups, as shown in Figure 4A. Multi-steroid receiver-operating characteristic curve analysis showed poor discriminatory power of peripheral steroids measured precosyntropin between patients classified as either U or B based on precosyntropin data alone (area under the curve = 0.586). When PA subtyping incorporated both pre- and post-cosyntropin AVS data, the discriminatory power between the U/U and B/B groups of the same steroid panel in peripheral serum was improved to an area

Table 2. Continued

Cosyntropin–Stimulated Concentrations, Peripheral Serum, ng/dL				
B/B	B/U	U/B	U/U	P Value
552 [314–1151]	594 [480–1340]	491 [302–711]	515 [420–1835]	0.29
5 [3–7]	7 [2–22]	4 [3–6]	10 [7–22]	0.0005
37 [18–68]	54 [38–87]	31 [20–43]	60 [42–100]	0.008
26 [13–46]	21 [14–43]	24 [16–40]	33 [18–54]	0.66
17 [13–21]	15 [10–21]	15 [14–19]	15 [12–22]	0.79
1120 [812–1549]	1082 [732–1739]	1006 [817–1288]	1283 [1051–1762]	0.13
15 [11–18]	17 [8–28]	13 [9–18]	14 [10–18]	0.65
20 [14–32]	24 [9–34]	18 [14–24]	20 [13–28]	0.84
476 [323–1090]	412 [259–633]	539 [292–783]	317 [199–500]	0.027
35 [20–120]	35 [18–59]	42 [22–66]	22 [14–30]	0.042
153 [75–344]	123 [91–204]	174 [121–220]	120 [84–284]	0.72
1434 [1097–2277]	1456 [818–2022]	1291 [934–2057]	1116 [709–1853]	0.35
113 [45–251]	69 [43–126]	145 [96–199]	56 [34–107]	0.059
217 [119–393]	156 [92–267]	373 [166–547]	151 [93–305]	0.06
187 [39–390]	333 [222–456]	280 [21–440]	238 [27–415]	0.28
92 [67–158]	93 [76–114]	87 [61–157]	84 [51–109]	0.69
30 [21–51]	22 [16–38]	52 [30–78]	27 [14–51]	0.03

All steroid concentrations are expressed in ng/dL, as medians [interquartile range]. 11dF indicates 11-deoxycortisol; 11OHA4, 11β-hydroxyandrostenedione; 16OHP, 16-hydroxyprogesterone; 17OHP, 17α-hydroxyprogesterone; A4, androstenedione; DOC, 11-deoxycorticosterone; and Prog, progesterone.

under the curve of 0.768 at baseline and 0.907 after cosyntropin stimulation (Figure 4B).

Discussion

The first major finding of our study is that several steroids are superior to cortisol for ascertaining AV catheterization. Of these, 11dF, 11OHA4, and corticosterone displayed the highest unstimulated gradients between AV and periphery. These findings are particularly relevant for centers that do

not use cosyntropin, as such protocols have been associated with high rates of AV catheterization failure.^{19–21} A report of 4 PA patients suggested that 11dF might be superior to cortisol in establishing AV catheterization.¹⁰ In a larger study, Eisenhofer et al⁸ had previously proposed A4 and dehydroepiandrosterone as alternatives to cortisol. In addition to A4, Ceolotto et al⁹ also proposed 17OHP, although this study was limited to a small number of patients, and steroids were measured by immunoassays. We found that 17OHP

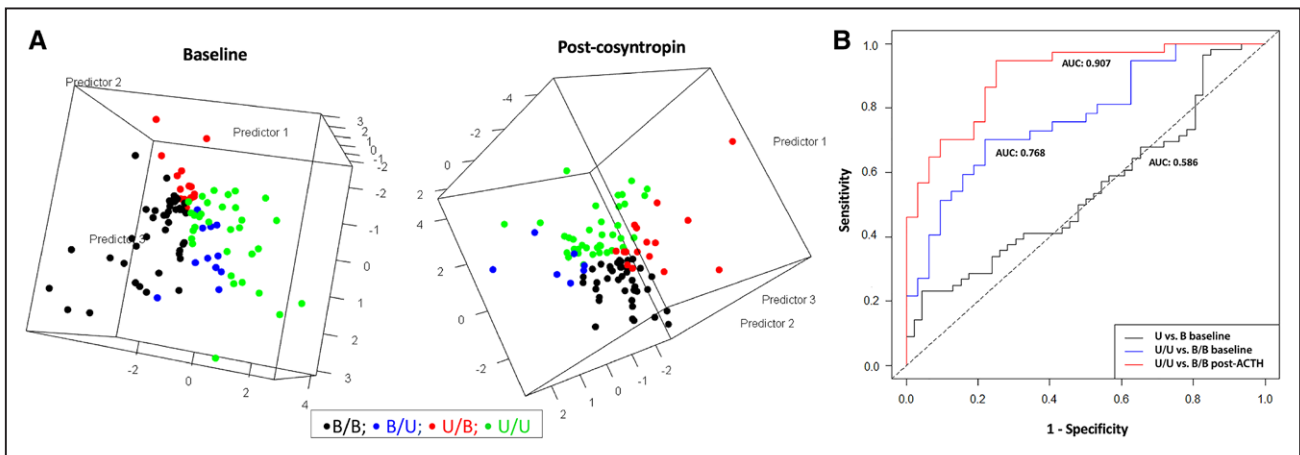


Figure 4. Performance of a 17-steroid panel measured in peripheral serum for primary aldosteronism (PA) subtyping. **A**, Discriminant analysis between 4 PA subtypes at baseline and after cosyntropin stimulation. **B**, Receiver-operating characteristics curves for PA subtyping according to baseline and cosyntropin-stimulated adrenal vein sampling (AVS) results. Gray line: baseline peripheral serum, to distinguish unilateral (U) vs bilateral (B) PA subtyping based on baseline AVS results alone. Blue line: baseline peripheral serum for distinguishing PA subtypes based on both baseline and cosyntropin AVS data (U/U vs B/B). Red line: cosyntropin-stimulated peripheral serum for distinguishing PA subtypes based on both baseline and cosyntropin AVS data (U/U vs B/B).

and 16OHP achieved particularly dramatic SI values after cosyntropin stimulation but more modest values at baseline. Other disadvantages of 17OHP are that this steroid also derives from the gonads and increases during the luteal phase in reproductive age women, which might decrease SI. We have previously shown that 11OHA4 has higher AV and peripheral circulation concentrations than A4.^{12,13} Furthermore, in contrast with its precursor A4, 11OHA4 is produced almost exclusively by the adrenals. Notably, 83% of samples deemed unsuccessful based on clinical laboratory SI <2 would have been interpretable (SI ≥2) if 11OHA4 or corticosterone were used instead of cortisol. Because, unlike corticosterone, 11OHA4 and 11dF are not within the mineralocorticoid pathway and both are adrenal-specific, we suggest these 2 steroids as the preferred candidates for AV catheter placement ascertainment during AVS, particularly so when cosyntropin is not used.

Overall, lateralization was consistent across clinical and LC-MS/MS assays for the steroids with good SI performance, both in the absence and presence of cosyntropin. A previous multi-center study, which included centers with heterogeneous AVS protocols (one with and 2 without cosyntropin) found strong positive correlations between LIs obtained from radioimmunoassays and LC-MS/MS assays of cortisol, A4, and dehydroepiandrosterone.⁸ Taken together, these results demonstrate that LI remains reliable when using alternative steroids and suggest that the optimal steroid(s) to normalize aldosterone values during AVS should be guided mainly by their performance and advantages for SI, as discussed above.

The second important finding of our study is the performance of multi-steroid panels measured in peripheral serum for PA subtyping. The hybrid steroids 18oxoF and 18OHF have been previously proposed as biomarkers of APA and showed promising sensitivity and specificity (0.83 and 0.99, respectively) in Japanese patients with PA.¹⁴ These steroids, however, had rather modest discriminatory power between APA and BHA in a European population.⁸ It is now recognized that the synthesis of 18oxoF and 18OHF is highest in patients with APAs harboring *KCNJ5* mutations,²² due to the co-expression of *CYP11B2* and *CYP17A1* in such tumors.²³ *KCNJ5* mutations are highly prevalent in Japanese and East Asian patients with PA,^{24–26} while European and American populations display a wider variety of aldosterone-driver mutations,^{23,27} explaining the differences in APA/BHA discriminatory power differences among these populations. Expanding to a 12-steroid panel measured in peripheral serum, Eisenhofer et al⁸ reported accurate APA versus BHA discrimination is 80% of 216 European patients with PA. An important caveat of this initial study is the different AVS protocols used in the participating centers, either only without or only with cosyntropin. As discussed above, we and others have found that some patients display discrepant lateralization results during AVS performed with and without cosyntropin.^{16,19} Our study suggests that patients with PA with discrepant pre- and post-cosyntropin lateralization have intermediate disease severity as compared with patients with robust lateralization regardless of the protocol used, and that these groups display distinct steroid profiles in

peripheral serum. Furthermore, logistic regression analysis demonstrated poor performance of peripheral serum steroids for distinguishing between cases classified as unilateral or bilateral based on precosyntropin AVS data alone; in contrast, the discrimination was greatly improved for subtypes with consistent AVS lateralization pre- and post-cosyntropin. Notably, the highest discriminatory performance between U/U and B/B groups was achieved by peripheral measurement of cosyntropin-stimulated steroids (area under the curve of 0.9). Previous studies conducted in China²⁸ and Japan²⁹ used cosyntropin stimulation after dexamethasone suppression and demonstrated less overlap of aldosterone concentrations between patients with APA or BHA than that observed at baseline. A small Japanese study subsequently reported that the distinction between APA and BHA based on peripheral serum-aldosterone concentrations alone is better after cosyntropin stimulation, without prior dexamethasone suppression.³⁰ Noninvasive dynamic testing for PA subtyping appears promising but needs to be confirmed in larger, ideally prospective studies. The identification of BHA with a peripheral blood test would circumvent the need for AVS in many PA patients.

In summary, we have developed a multi-steroid panel that could offer 2 advantages to PA subtyping. First, we propose 11OHA4 and 11dF, 2 adrenal-specific steroids, as superior to cortisol for reliably establishing AV catheterization during AVS, particularly for centers that do not use cosyntropin and in cases where cortisol-based SI is inadequate. Second, we found that cases with inconsistent lateralization between pre- and post-cosyntropin appear to be overall less severe than those with consistent unilateral aldosterone dominance and display distinct steroid profiles in peripheral serum. While the long-term evolution and surgical benefit for these intermediate subtypes remains to be determined, incorporating both pre- and post-cosyntropin data for PA subtyping is likely to be valuable, and adds yet another need for more reliable SI parameters. The main limitations to our study are its retrospective design, limited number of cases with discrepant lateralization and incomplete post-operative follow-up data. Nonetheless, our study is the first to incorporate both pre- and post-cosyntropin AV and peripheral serum data sets, which along with multi-steroid profiling using LC-MS/MS reveals 4-tiered rather than binary PA subtypes. Larger, multi-center studies, with prospective follow-up of patients, including a large number of patients with PA with discordant pre and post-cosyntropin AVS lateralization are needed to establish the clinical significance of the 4-tiered system of PA subtyping and the best management approach for each subtype.

Perspectives

AVS is currently an essential tool for identifying patients with PA who could benefit from unilateral adrenalectomy. Cortisol has been used for AVS data interpretation, but its numerous pitfalls can lead to unreliable results in many patients. Steroid panels measured with highly accurate mass spectrometry assays can serve dual purposes: (1) adrenal-specific steroids that are not normally produced by APAs, such as 11β-hydroxyandrostenedione, could facilitate accurate AVS

data interpretation; (2) after validation in larger studies, steroid fingerprints measured in peripheral serum will be able to identify patients who would benefit from adrenalectomy, and as such, eliminate the need for invasive and costly tests in all other patients with PA.

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Disclosures

None.

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Novelty and Significance

What Is New?

- We have used a panel of 17 steroids measured in a single small serum aliquot to assist with subtyping of primary aldosteronism (PA).
- We have found that the adrenal specific steroids 11 β -hydroxyandrostenedione, 11-deoxycortisol, and corticosterone are superior to cortisol as indicators of adrenal vein catheterization. Using these 3 steroids, 80% of cases deemed failed based on the current standard-of-care, cortisol, are valid.
- We have found that based on baseline and cosyntropin-stimulated adrenal vein sampling results, PA subtyping can be stratified in 4 groups with distinct steroid profiles, both in adrenal veins, as well as in periphery.

What Is Relevant?

- 11 β -hydroxyandrostenedione and 11-deoxycortisol are the ideal candidates for adrenal vein data interpretation in patients with PA.
- Multi-steroid profiling can assist in PA subtyping and, in future, will likely circumvent the need for adrenal vein sampling in many patients with PA.

Summary

Using mass spectrometry, we demonstrate the utility of a multi-steroid panel in PA subtyping. Compared with cortisol, 11 β -hydroxyandrostenedione and 11-deoxycortisol have superior adrenal vein/periphery gradients and allow the interpretation of adrenal vein sampling data in most patients. PA subtyping spans a gradient of severities, marked by distinct steroid fingerprints.



Hypertension