The Journal of Clinical Hypertension

WILEY

ORIGINAL ARTICLE OPEN ACCESS

LC-MS/MS-Based Assay for Steroid Profiling in Peripheral and Adrenal Venous Samples for the Subtyping of Primary Aldosteronism

Xiuqing Chen[1,2,3,4,5,6] | Qinyi Li[7] | Linjing Huang[1,2,3,4,5,6] | Peiwen Wu[1,2,3,4,5,6]

[1]Department of Endocrinology, the First Affiliated Hospital, Fujian Medical University, Fuzhou, China | [2]Department of Endocrinology, National Regional Medical Center, Binhai Campus of the First Affiliated Hospital, Fujian Medical University, Fuzhou, China | [3]Clinical Research Center for Metabolic Diseases of Fujian Province, the First Affiliated Hospital, Fujian Medical University, Fuzhou, China | [4]Fujian Key Laboratory of Glycolipid and Bone Mineral Metabolism, the First Affiliated Hospital, Fujian Medical University, Fuzhou, China | [5]Diabetes Research Institute of Fujian Province, the First Affiliated Hospital, Fujian Medical University, Fuzhou, China | [6]Metabolic Diseases Research Institute, the First Affiliated Hospital, Fujian Medical University, Fuzhou, China | [7]Fujian Medical University, Fuzhou, China

Correspondence: Peiwen Wu (530556292@qq.com)

Received: 24 December 2024 | **Revised:** 28 March 2025 | **Accepted:** 12 April 2025

Funding: This research was funded by the Fujian Medical University Sailing Fund Project (No. 2021QH1068) and Joint Funds for the Innovation of Science and Technology, Fujian Province (Grant No.2021Y9106).

Keywords: adrenal venous samples | aldosterone-producing adenoma | hypertension | primary aldosteronism | steroid

ABSTRACT

Given the largely unexplored application of liquid chromatography-tandem mass spectrometry (LC-MS/MS) steroid analysis in primary aldosteronism (PA), we aimed to investigate its diagnostic utility in PA classification and to characterize steroid secretion patterns across PA subtypes. We retrospectively enrolled 67 patients with PA and collected samples from both peripheral and adrenal veins. We performed a steroid analysis to compare the steroid panel differences between aldosterone-producing adenoma (APA), bilateral adrenal hyperplasia (BAH), and unilateral adrenal hyperplasia (UAH). Analyses included steroid concentrations and secretion ratios, with the latter calculated as individual steroid concentrations divided by total steroid content. The concentrations of 18-hydroxycortisol (18-OHF) were higher in the peripheral veins of patients with APA than in those with BAH and UAH ($p < 0.01$). A threshold of 4.83 ng/mL for peripheral 18-OHF specifically identified APA cases. In APA cases, adrenal vein secretion ratios of aldosterone, 18-hydroxycorticosterone (18-OHB), and 18-OHF were significantly higher in dominant versus non-dominant adrenal veins ($p < 0.001$). A secretion ratio of 18-OHF ≥ 14.6‰ and 18-OHB ≥ 4.03‰ from the adrenal vein achieved 100% specificity for identifying the dominant secretory side in cases of APA. Collectively, our findings demonstrate that LC-MS/MS steroid profiling effectively differentiates APA from other PA subtypes. The biochemical criteria for the secretion ratios of 18-OHF and 18-OHB from the adrenal vein provide objective criteria for lateralization diagnosis in APA. These findings could refine diagnostic strategies for PA subtyping.

1 | Introduction

Hypertension is the primary cause of morbidity and mortality associated with cardiovascular disease, affecting over one billion adults worldwide, with prevalence rates reaching up to 30% in certain adult populations [1]. Primary aldosteronism (PA) is the most common cause of secondary hypertension, with an estimated prevalence of 6%–14% among patients with

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2025 The Author(s). *The Journal of Clinical Hypertension* published by Wiley Periodicals LLC.

hypertension in primary healthcare facilities and up to 20% in cases of resistant hypertension [2, 3]. Approximately one-third of patients with hypertension exhibit persistent aldosterone secretion during sodium loading tests, indicating that PA might be more common in patients with hypertension than has been reported in numerous studies [4]. Patients with PA are more prone to cardiovascular events and target organ damage than those with essential hypertension. The risk of stroke, atrial fibrillation, and heart failure is higher in patients with PA than in those with essential hypertension, and PA increases the risk of diabetes, metabolic syndrome, and left ventricular hypertrophy [5].

One-third of patients with PA present with a unilateral form; unilateral adrenalectomy not only effectively treats hypertension and electrolyte imbalances but also helps to repair target organ damage and reduce cardiovascular risks [6, 7]. Unilateral PA includes aldosterone-producing adenoma (APA) and unilateral adrenal hyperplasia (UAH) [8, 9]. Recent studies have revealed that, in terms of long-term surgical prognosis, typical APA has a lower recurrence rate after adrenalectomy than UAH. However, prior to APA surgery, an adrenal venous sampling (AVS) procedure is required to identify the aldosterone-dominant secretion side. The failure rate of AVS can reach 30% in certain centers, particularly in the right adrenal vein, where the complex anatomy, numerous branches, and small openings present significant challenges. This necessitates strict technical expertise and team support for successful AVS [10, 11]. Therefore, the surgical rate for unilateral PA remains low; in one Italian hospital, only 1.0% of patients with unilateral PA underwent adrenalectomy for PA treatment [12].

Recently, several studies have investigated other methods, including steroid analysis, for the classification of PA. A study conducted in Japan showed that the peripheral venous concentrations of 18-oxycortisol, 18-hydroxycortisol (18-OHF), and aldosterone are significantly elevated in patients with APA than in patients with bilateral adrenal hyperplasia (BAH) [13]. Similarly, a European study revealed that a panel encompassing all 15 steroid markers achieved an area under the curve (AUC) of 0.889, effectively distinguishing APA from BHA [14]. Nevertheless, there are only a few relevant studies in this area.

Furthermore, previous studies have predominantly used immunoassays for steroid measurement, with less use of mass spectrometry. Compared with traditional immunological methods, mass spectrometry effectively eliminates interference from structural analogs during steroid hormone detection, thereby offering superior accuracy and specificity [15]. In the United States, steroid concentrations determined by mass spectrometry are only clinically acceptable [16].

Given the potential application of mass spectrometry steroid analysis in the diagnostic subtyping of PA, which remains largely unexplored, we aimed to investigate its utility in simplifying the diagnosis of APA to improve the treatment rate of patients with APA. Therefore, we employed liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze steroids in peripheral and adrenal venous samples from PA patients, with the goal of exploring the characteristic steroid profile in APA.

2 | Materials and Methods

2.1 | Patients

We included patients with PA who underwent AVS for PA classification at the First Affiliated Hospital of Fujian Medical University between September 2021 and September 2024. PA was diagnosed according to the guidelines of the Endocrine Society [17]. An immunoassay was used to determine the plasma aldosterone concentration (PAC)/plasma renin concentration (PRC) ratio (ARR) to detect potential PA cases. When ARR $\geq$ 3.7 ng $\cdot$ dL^{-1}/μ IU $\cdot$ mL^{-1}, the diagnosis of PA was confirmed using a saline infusion test or captopril challenge test. For patients who were ARR negative and suspected to have PA, including those who were young, had low potassium levels, and suffered from refractory hypertension, confirmatory tests should also be conducted. Antihypertensive drugs were changed to nondihydropyridine calcium channel blockers or alpha blockers before screening. In AVS procedures, catheterization was considered successful when the selectivity index (SI = adrenal vein/peripheral vein cortisol concentration) was $\geq$2. PA was classified as unilateral or bilateral based on the lateralization index (LI = aldosterone/cortisol ratio between dominant and contralateral AVS), with LI $\geq$2 indicating unilateral PA and LI <2 indicating BAH. Five patients with PA were excluded from this study owing to unsuccessful AVS collection. Unilateral PA consists of the APA group and the UAH group. The APA group was defined as having imaging evidence of adenomas on the dominant adrenal vein based on AVS, which was verified by post-operative pathology showing typical adenoma and nodule features, and achieving complete biochemical remission after surgery; a total of 34 cases met the criteria. Imaging of the adrenal gland on the dominant side in 12 patients showed normal size or enlargement, and 5 patients had adrenal hyperplasia of surgical pathology; these 17 patients were defined as the UAH group. Patients with Cushing's syndrome and pheochromocytoma were excluded. In total, 67 patients with successful AVS were included: 16 of them were BAH, 34 were APA, and 17 were UAH. Owing to the lack of surgery, three patients with CT imaging suggesting adenomas in the dominant side could not be identified for PA subtypes; their data were only included in the analysis when comparing LC-MS/MS and immunological methods. All participants provided written informed consent, and the study protocol was approved by the Institutional Ethics Review Committee. Data supporting the results of this study can be obtained from the corresponding author upon reasonable request.

2.2 | AVS-Protocol and Data Interpretation

AVS was performed by a radiologist with over 5 years of experience in interventional vascular surgery. Blood samples were simultaneously collected from both the adrenal and peripheral veins. All AVS studies were conducted after adjusting for antihypertensive drugs based on recognized recommendations. The patients received potassium supplementation therapy to correct hypokalemia. No cosyntropin stimulation was administered. The use of rapid semi-quantitative cortisol testing during surgery aids in determining the success of blood sampling. We collected blood samples (2–8 mL) from the two adrenal veins using gravity or mild negative pressure and also collected peripheral

vein blood (6–8 mL) to evaluate the selectivity of AVS and the inhibition of contralateral aldosterone secretion. Samples were collected in test tubes containing EDTA as an anticoagulant and transported to the laboratory. We immediately used a portion of plasma for routine immunoassay of cortisol and aldosterone and stored the other portion at -80°C until further use for steroid determination by LC-MS/MS. The secretion ratio of individual steroids was calculated as individual steroid concentration/total steroid concentration.

2.3 | Immunoassay Determination

Serum cortisol levels were analyzed using a chemiluminescence immunoassay (Snibe, China). The coefficients of variation (CVs) for cortisol within and between detections were ≤5% and ≤10%, respectively, while the limit of detection (LOD) was 1.38 nmol/L. Plasma aldosterone levels were determined using the micromagnetic particle chemiluminescence method using an analytical kit, following the manufacturer's instructions (Snibe, China). The CVs of aldosterone within and between measurements were ≤5% and ≤10%, respectively, while the LOD was 0.5 ng/dL.

2.4 | Quantitative Analysis of Steroids Using LC-MS/MS

LC-MS/MS was used to detect the following 16 steroids in each sample: 17-hydroxyprogesterone, progesterone, cortisol, androstenedione, dehydroepiandrosterone sulfate, cortisol, aldosterone, dehydroepiandrosterone, pregnenolone, 17-hydroxypregnenolone, cortisone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisol, 18-OHF, and 18-hydroxycorticosterone (18-OHB).

2.5 | Sample Preparation

We transferred 400 μL of the serum sample to a labeled 1.5 mL EP tube, then added 20 μL of internal standard working fluid. This mixture was vortexed for 5 s. Subsequently, 1200 μL of succinate ester was added to it, the solution was shaken for 5 min, and centrifuged at 21 000 × *g* at 4°C for 5 min. Finally, 1000 μL of the supernatant was transferred into another EP tube and evaporated with nitrogen. The residue was dissolved in 80 μL of 50% methanol, shaken for 3 min, and left at 4°C for 3 min. The supernatant was used for LC-MS/MS analysis.

2.6 | LC-MS/MS Analysis

LC-MS/MS analysis was conducted using a QLife Lab 9000 Plus triple quadrupole mass spectrometry detection system (Pinsheng Medical). The Poroshell 120 EC-C18 (2.7 μm, 2.1 × 50 mm) column was selected for the analysis, with a column temperature of 40°C, flow rate of 0.3 mL/min, and injection volume of 10 μL. The mobile phase A consisted of 50 μM ammonium fluoride solution, while mobile phase B consisted of methanol. The gradient elution procedure was as follows: 0–1.5 min, 40% B; 1.5–3.5 min, 40% B to 60% B; 3.5–7.5 min, 60% B to 98% B; 7.6–10.0 min, 40% B.

The multiple reaction monitoring (MRM) mass spectrometric scanning mode was adopted, utilizing both positive and negative ionization modes of the electrospray ionization source (ESI). The following parameters were set: capillary voltage at 3500 V, nozzle voltage at 100 V (ESI+), and 1000 (ESI−). The drying gas temperature was maintained at 250°C, drying gas flow rate at 5 L/min, nebulizer pressure at 40 psi, sheath gas temperature at 350°C, and sheath gas flow rate at 11 L/min.

2.7 | Statistical Analysis

Data were analyzed using the Kolmogorov-Smirnov test to determine their distribution. Normally distributed data were presented as mean ± SD. Non-normally distributed data were presented as medians (interquartile ranges). Statistical analyses were performed using the *t*-test and One-way ANOVA for continuous variables, and the Mann–Whitney *U* test, Kruskal–Wallis test for nonparametric variables, and $\chi 2$ test for categorical variables. Receiver operating characteristic (ROC) analysis was used to determine the optimal variable cutoff values. All analyses were performed using SPSS version 17 (IBM Corp., Armonk, NY, USA). Graphics were created using GraphPad Prism 9 and SPSS version 17. For all statistical analyses, the bilateral p value was <0.05.

3 | Results

3.1 | Patient Characteristics

The demographic and clinical characteristics of the 34 patients with APA, 16 patients with BAH, and 17 patients with PAH are presented in Table 1. Because our center prefers patients with clearly visible adenomas and nodules for the AVS procedure, the number of unilateral PA cases exceeded that of BAH cases. There were no significant differences in gender, age, systolic blood pressure (SBP), diastolic blood pressure (DBP), PAC, PRC, or ARR among patients with APA, UAH, and BAH. Patients with UAH had higher blood potassium levels than patients with APA ($p < 0.05$). APA and UAH patients had higher LI levels compared to patients with BAH ($p < 0.001$) (Table 1).

3.2 | Plasma Concentrations and Secretion Ratio of Major Steroids in Peripheral Veins

A comparison of peripheral venous steroid concentrations showed that 18-OHF was higher in patients with APA than in those with UAH ($p < 0.001$) and BAH ($p < 0.01$). The secretion ratio of individual steroids was calculated as the individual steroid concentration divided by the total steroid concentration. The secretion ratio of 18-OHF in peripheral veins was higher in patients with APA than in those with UAH ($p < 0.05$). There were no significant differences in the concentrations and secretion ratios of aldosterone and 18-OHB among patients with APA, BAH, and UAH (Table 2).

The differences in the concentrations of 18-OHF in the peripheral veins among patients with APA, BAH, and UAH are shown in Figure 1. The mean peripheral venous 18-OHF concentration in patients with APA demonstrated a 2.04-fold elevation compared

TABLE 1 | Demographic and clinical characteristics of patients with primary aldosteronism.

Item	APA	UAH	BAH	*p*
n	34	17	16	
Gender, M/F	20/14	12/5	7/9	0.294
Age, years	48.9 ± 11.8	53.1 ± 9.99	55.1 ± 11.9	0.170
Systolic BP, mmHg	145.8 ± 15.8	156.4 ± 18.0	149.8 ± 25.5	0.181
Diastolic BP, mmHg	88.8 ± 12.3	94.0 ± 11.8	89.6 ± 9.15	0.307
Potassium, mmol/L	3.10 ± 0.658	3.59 ± 0.624*	3.45 ± 0.589	0.028
PAC, ng/dL	30.7 (14.4, 43.5)	23.1 (15.1, 42.3)	22.3 (16.4, 29.4)	0.558
PRC, mU/L	3.55 (1.78, 7.22)	1.90 (1.35, 5.97)	2.32 (0.925, 5.18)	0.618
ARR	8.11 (4.74, 16.3)	9.88 (4.10, 18.3)	8.72 (4.59, 24.4)	0.914
LI	19.4 (5.25, 52.8)	9.93 (3.11, 35.7)	1.47 (1.19, 1.68)**,***	<0.001

Note: Normally distributed data are presented as mean ± SD, *p* values designate the presence of group differences by the One-way ANOVA. Non-normally distributed data are presented as medians (interquartile ranges), *p* values designate the presence of group differences by the Kruskal–Wallis test. $\chi 2$ test for categorical variables. Statistical significance was set at $p < 0.05$. *Difference ($p < 0.05$) from APA, **Difference ($p < 0.001$) from UAH, ***Difference ($p < 0.001$) from APA.

Abbreviations: APA, aldosterone-producing adenoma; ARR, plasma aldosterone concentration/plasma renin concentration ratio; BAH, bilateral adrenal hyperplasia; LI, lateralization index; PAC, plasma aldosterone concentration; PRC, plasma renin concentration; BP, blood pressure; UAH, unilateral adrenal hyperplasia.

TABLE 2 | Plasma concentrations and secretion ratio of major steroids in peripheral veins of patients with primary aldosteronism.

Variables	APA (*n* = 34)	UAH (*n* = 17)	BAH (*n* = 16)	*p*
Aldosterone concentration (ng/mL)	0.158 ± 0.104	0.139 ± 0.0875	0.104 ± 0.0535	0.148
18-OHF concentration (ng/mL)	3.57 ± 2.27	1.53 ± 0.698***	2.08 ± 1.57**	0.001
18-OHB concentration (ng/mL)	0.521 ± 0.431	0.407 ± 0.433	0.294 ± 0.263	0.164
Aldosterone secretion ratio (‰)	0.144 ± 0.109	0.129 ± 0.107	0.111 ± 0.0945	0.586
18-OHF secretion ratio (‰)	3.26 ± 2.77	1.37 ± 0.724*	2.72 ± 2.99	0.044
18-OHB secretion ratio (‰)	0.470 ± 0.487	0.407 ± 0.533	0.263 ± 0.184	0.320

Note: Data are presented as mean ± SD; *p* values designate the presence of group differences by the One-way ANOVA. Compared with APA; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Abbreviations: APA, aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia; UAH, unilateral adrenal hyperplasia.

to UAH cases ($p < 0.001$) and 1.49-fold higher than BAH cases ($p = 0.018$). Despite overlapping 18-OHF levels across groups, all BAH/UAH cases showed 18-OHF ≤ 4.83 ng/mL (interquartile range [IQR] 0.965–2.360 ng/mL), whereas 26.5% of APA patients (9/34) exceeded the 4.83 ng/mL diagnostic threshold (range 5.29–9.13 ng/mL). The specificity of this threshold for diagnosing APA was 100% (95% CI: 0.870–1.00). All nine patients exceeding the threshold exhibited unilateral APA with a normal contralateral adrenal gland.

3.3 | Plasma Concentrations and Secretion Ratio of Steroids in Adrenal Veins

To compare with the APA group, we defined the adrenal venous lateralization dominant in patients with BAH as a LI greater than 1.0. The plasma concentrations of steroids in the APA-dominant adrenal vein, APA-nondominant adrenal vein, BAH-dominant adrenal vein, and BAH-nondominant adrenal vein were compared. Aldosterone concentrations were lower in the APA-nondominant adrenal vein ($p < 0.001$), BAH-dominant adrenal vein ($p < 0.05$), and BAH-nondominant adrenal vein ($p < 0.01$) than in the APA-dominant adrenal vein. The concentrations of 18-OHF were lower in the APA-nondominant adrenal vein ($p < 0.01$) and BAH-nondominant adrenal vein ($p < 0.05$) than in the APA-dominant adrenal vein. The concentrations of 18-OHB were lower in the APA-dominant adrenal vein than in the APA-nondominant adrenal vein ($p < 0.01$) (Table S1).

The secretion ratios of steroids in the APA-dominant adrenal vein, APA-nondominant adrenal vein, BAH-dominant adrenal vein, and BAH-nondominant adrenal vein were compared. The secretion ratios of aldosterone were lower in the APA-nondominant adrenal vein ($p < 0.001$), BAH-dominant adrenal vein ($p < 0.05$), and BAH-nondominant adrenal vein ($p < 0.01$) than in the APA-dominant adrenal vein. The secretion ratios of 18-OHF were lower in the APA-nondominant adrenal vein ($p < 0.001$), BAH-dominant adrenal vein ($p < 0.05$), and BAH-nondominant adrenal vein ($p < 0.01$) than in the APA-dominant adrenal vein. The secretion ratios of 18-OHB were

lower in the APA-nondominant adrenal vein ($p < 0.001$) and BAH-nondominant adrenal vein ($p < 0.005$) than in the APA-dominant adrenal vein (Table 3). The differences in the ratios of aldosterone, 18-OHF, and 18-OHB in the adrenal vein in patients with APA and BAH are shown in Figure 2.

To predict the APA-dominant adrenal vein in the APA cases, a ROC curve was constructed using the secretion ratios of aldosterone, 18-OHF, and 18-OHB in the APA adrenal vein (Figure 3). The optimal cutoff point value was obtained using the ROC curve and Youden index. For the prediction of APA-dominant adrenal veins, the best cutoff value for obtaining the aldosterone ratio was ≥0.567‰, with an AUC of 0.916 (0.843–0.987), sensitivity of 0.882 (0.716–0.962), and specificity of 0.852 (0.682–0.945). For the 18-OHF ratio, the best cut-off value was ≥14.6‰, with an AUC of 0.795 (0.683–0.907), sensitivity of 0.676 (0.494–0.820), and specificity of 0.941 (0.789–0.990). For the 18-OHB ratio, the best cut-off value was ≥4.03‰, with an AUC of 0.797 (0.689–0.906), sensitivity of 0.676 (0.494–0.820), and specificity of 0.941 (0.789–0.990). Based on these results, aldosterone and 18-OHB exhibited relatively high specificity in the diagnosis of APA-dominant adrenal vein. Therefore, we combined the cutoff values of aldosterone and 18-OHB. When combining the cut-off values of 18-OHF ≥14.6‰ and 18-OHB ≥4.03‰, the specificity reached 100% in diagnosing APA-dominant adrenal vein (Table 4).

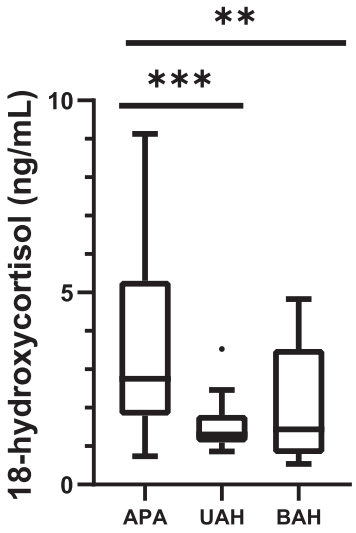

FIGURE 1 | Tukey box-whisker plot comparing 18-hydroxycortisol concentrations in the peripheral veins between patients with APA, BAH, and UAH. Compared with APA; $^{**}p < 0.01$, $^{***}p < 0.001$. APA indicates aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia; UAH, unilateral adrenal hyperplasia.

3.4 | Comparison Between Immunoassay and LC-MS/MS for Cortisol and Aldosterone Measurement

As the immunoassay (IA) method recommends a 1:10 dilution, we divided the serum cortisol concentration measured by the IA into three categories: (1) the upper LOD of IA (600 ng/mL), (2) greater than the upper limit of IA detection but less than 10 times the upper limit of IA detection (600–6000 ng/mL), and (3) >6000 ng/mL. Similarly, we divided the serum aldosterone concentration measured using the IA into two categories: (1) the upper LOD of IA (2 ng/mL) and (2) greater than the upper limit of IA detection (>2 ng/mL). We analyzed the correlation between serum cortisol and aldosterone concentrations measured using LC-MS/MS and IA and found significant correlations between the two methods (Tables 5 and 6; Figures 4 and 5).

3.5 | SI and LI Derived From Steroids

Of the 16 steroids measured, 6 steroids—corticosterone, pregnenolone, 17-hydroxypregnenolone, 11-deoxycorticosterone, 11-deoxycortisol, and deoxycortisol—had significantly higher SIs, compared with cortisol ($p < 0.01$; Figure 6). There was no significant difference in the LI for PA among the various steroids (Figure S1).

4 | Discussion

In this study, we analyzed steroids using LC-MS/MS and found that the concentration of 18-OHF in peripheral veins was higher in patients with APA than in patients with BAH and those with UAH. In the steroid analysis of adrenal veins, the unilateral secretion rates of aldosterone, 18-OHF, and 18-OHB were diagnostically valuable for the dominant secretion side in patients with APA. We also identified a correlation between cortisol levels measured by LC-MS/MS and immunoassay. Furthermore, our results suggest that certain steroids are superior to cortisol in determining the success of AVS catheterization.

A significant finding of our study is the observed differences in the secretion ratios of aldosterone, 18-OHF, and 18-OHB between the APA-dominant and APA-nondominant adrenal veins.

TABLE 3 | Comparison of steroid secretion ratios (‰) in dominant and nondominant adrenal veins of patients with APA and BAH.

Variables	APA-dominant adrenal vein ($n = 34$)	APA-nondominant adrenal vein ($n = 34$)	BAH-dominant adrenal vein ($n = 16$)	BAH-nondominant adrenal vein ($n = 16$)	*p*
Aldosterone (‰)	2.34 [1.15–7.53]	0.171 [0.0746–0.349]***	1.16 [0.267–2.59]*	1.14 [0.301–1.94]**	<0.001
18-hydroxycortisol (‰)	19.1 [9.34–34.4]	8.50 [5.01–12.2]***	10.1 [5.38–17.8]*	9.99 [5.85–13.1]**	<0.001
18-hydroxycorticosterone (‰)	5.15 [2.16–11.9]	1.29 [0.909–.48]***	2.81 [0.877–5.33]	2.40 [0.953–5.11]**	<0.001

Note: Data are presented as median (interquartile range); compared with APA-dominant adrenal vein; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.
Abbreviations: APA, aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia.

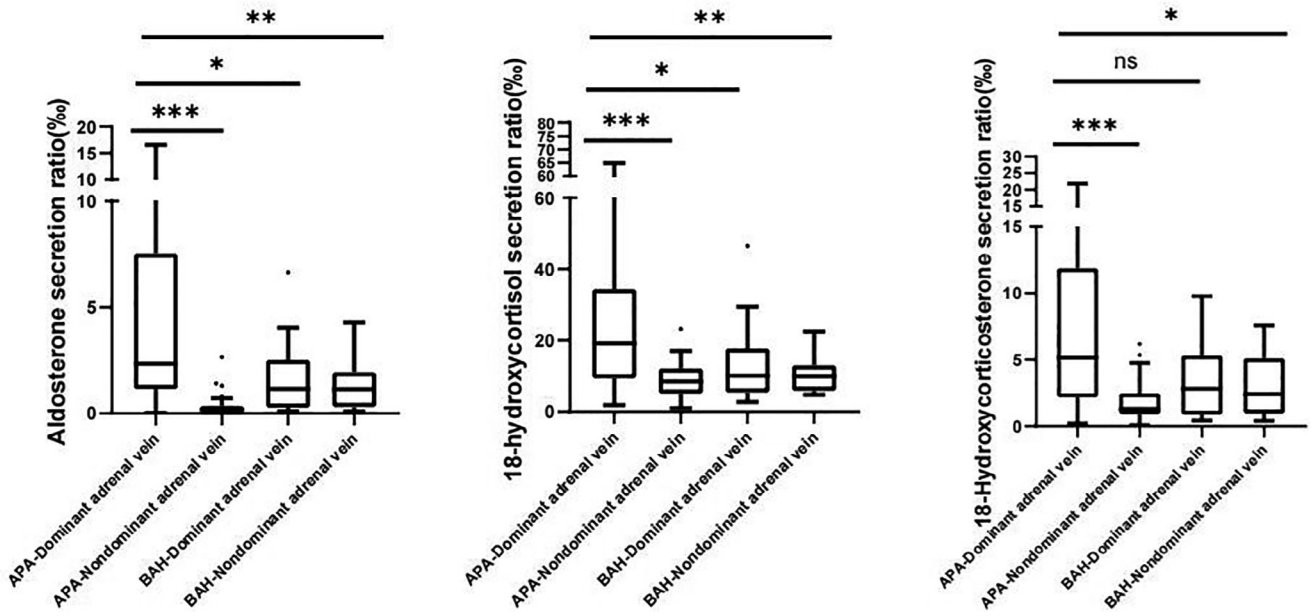

FIGURE 2 | Tukey box-whisker plot comparing the secretion ratios (‰) of aldosterone, 18-hydroxycortisol, and 18-hydroxycorticosterone in the adrenal vein between patients with APA and BAH. Compared with APA-dominant adrenal vein; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. APA indicates aldosterone-producing adenoma; BAH, bilateral adrenal hyperplasia.

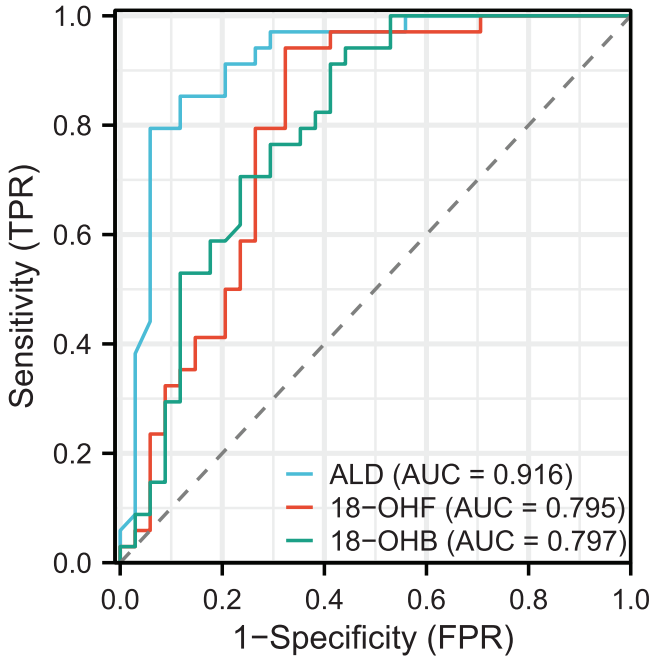

FIGURE 3 | ROC curves for the secretion ratios (‰) of aldosterone, 18-hydroxycortisol, and 18-hydroxycorticosterone in diagnosing APA-dominant adrenal of APA cases. APA indicates aldosterone-producing adenoma; ROC, receiver operating characteristic.

The diagnostic accuracy of the aldosterone secretion ratio for identifying the APA-dominant adrenal vein was relatively high, with an AUC exceeding 0.9. At the secretion ratios of 18-OHF ≥ 14.6‰ and 18-OHB ≥ 4.03‰ from the adrenal vein, the specificity in diagnosing the APA-dominant adrenal vein reaches 100%, suggesting that if this standard is met, no nondominant side can be misdiagnosed as dominant. Traditional AVS evaluations require the computation of cortisol and aldosterone concentrations in both adrenal veins, which precludes the determination of the APA-dominant adrenal vein in patients with AVS failure in one adrenal vein. Our findings indicate that even with AVS data from only one adrenal vein, the results could be analyzed to determine the dominant side. Chang et al. analyzed 15 types of adrenal steroid hormones in the adrenal veins of 75 patients with PA and found that the secretion rates of aldosterone and 18-OHF were higher on the dominant secretory side than on the nondominant side. They found that the sensitivity of aldosterone secretion rate in predicting the dominant side was 91.7%, with a specificity of 68.2%. The sensitivity of 18-OHF secretion rate in predicting the dominant secretion side was 62.5%, with a specificity of 90.7%, which is consistent with our findings, but their study did not include 18-OHB [18]. In our research, both 18-OHB and 18-OHF exhibit high specificity. When analyzed together, their specificity can reach 100%, which can further help avoid misdiagnosis.

18-OHF is a hybrid steroid because it possesses the structural features of both glucocorticoids and mineralocorticoids [19, 20]. In normal human adrenal cortex, 11β-hydroxylase (CYP11B1) activity may produce18-OHF [21]. In vitro experiments demonstrate that both CYP11B1 and aldosterone synthase (CYP11B2) can dose-dependently convert cortisol into 18-OHF [22]. The levels of 18-OHF in the peripheral veins and urine were higher in patients with APA than in patients with BAH. Patients with APA exhibit urine 18-OHF concentrations exceeding 510 μg/day, whereas patients with BAH exhibit concentrations below 130 μg/day [23]. Subsequent research revealed that the lateralization ratio for aldosterone and 18-OHF was greater in patients with APA with KCNJ5 mutations than in those without KCNJ5 mutations. Patients with KCNJ5 mutations in the APA group exhibited a 2.9-fold higher concentration of 18-OHF in the adrenal vein than that of the wild-type group [24]. Most APA cases are driven by somatic mutations, particularly KCNJ5 mutations, which are

TABLE 4 | Optimal cutoff values of secretion ratios (‰) of aldosterone, 18-hydroxycortisol, and 18-hydroxycorticosterone in the adrenal veins for predicting APA-dominant adrenal vein in APA cases.

Variables	AUC(95% CI)	Cutoffs (‰)	Sensitivity (95% CI)	Specificity (95% CI)
Aldosterone (‰)	0.916 (0.843–0.987)	≥0.567	0.882 (0.716–0.962)	0.853 (0.682–0.945)
18-hydroxycortisol (‰)	0.795 (0.683–0.907)	≥14.6	0.676 (0.493–0.820)	0.941 (0.789–0.990)
18-hydroxycorticosterone (‰)	0.797 (0.689–0.906)	≥4.03	0.588 (0.408–0.749)	0.912 (0.752–0.977)
18-hydroxycortisol ≥14.6‰ + 18-hydroxycorticosterone≥4.03‰	/	/	0.500 (0.328–0.672)	1.00 (0.874–1.00)

Abbreviations: APA, aldosterone-producing adenoma; AUC, area under the curve.

TABLE 5 | Correlation between cortisol concentrations detected by LC-MS/MS and immunoassay results.

Immunoassay cortisol (ng/mL)	0–600	600–6000	> 6000
Cortisol IA	144.1 [99.6–229.5]	2670.5 [1409.1–4923.2]	10216.1 [7863.9–13587.1]
Cortisol LC-MS/MS	96.3 [65.8–134.6]	1259.5 [576.5–2182.1]	4018.9 [3131.8–5078.3]
R	0.961	0.762	0.519
R^2	0.924	0.580	0.269
p value	<0.001	<0.001	<0.001

Note: Data are presented as median (interquartile range).
Abbreviations: IA, immunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry.

TABLE 6 | Correlation between aldosterone concentration detected by LC-MS/MS and immunoassay results.

Immunoassay aldosterone(ng/mL)	0–2	> 2
Aldosterone IA	0.431 [0.288–0.887]	11.8 [6.14–30.4]
Aldosterone LC-MS/MS	0.340 [0.179–0.722]	9.61 [5.35–21.8]
R	0.855	0.716
R^2	0.730	0.513
p value	<0.001	<0.001

Note: Data are presented as median (interquartile range).
Abbreviations: IA, immunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry.

observed in as many as 80% of patients with APA in the East Asian population [25, 26]. Mutations in KCNJ5 can lead to an increase in the intracellular calcium concentration and the activation of calcium signaling, thereby promoting aldosterone production by increasing the expression of CYP11B2, which encodes aldosterone synthase. Additionally, adenomas carrying KCNJ5 mutations exhibit a higher number of cells expressing CYP11B1 than adenomas carrying other mutations [27]. This may explain the increased concentration and proportion of 18-OHF in the peripheral and adrenal veins of APA.

18-OHB is a precursor in the biosynthesis of aldosterone, formed from corticosterone by the action of CYP11B2 (partially catalyzed by CYP11B1) [28, 29]. P. Mulatero found that the concentration of 18-OHB in the peripheral vein was higher in APA than in BAH using immunoassays for steroid measurement [23]. Another study demonstrated that, in the supine position, 18-OHB levels can effectively differentiate between APA and BAH (mean 18-OHB: 142.1 vs. 38.8 ng/L) [30]. However, few studies used LC-MS/MS to simultaneously compare the differences in 18-OHB and 18-OHF in peripheral blood from patients with APA and those with BAH. Our study only revealed a difference in peripheral blood 18-OHF between APA and BAH, with no difference found in 18-OHB. We observed that the secretion of 18-OHF in adrenal venous from patients with APA was approximately 10 times higher than that of 18-OHB, suggesting that 18-OHB accounts for too small a proportion in steroid secretion. After dilution in peripheral venous, this may lead to the difference in 18-OHB not being apparent, suggesting that 18-OHF may be superior to 18-OHB in differentiating APA in peripheral venous.

Our findings propose a hierarchical diagnostic algorithm for PA subtyping. First, initial biochemical stratification via quantitative analysis of peripheral venous 18-OHF (>4.83 ng/mL threshold, 95% CI: 0.870–1.00 specificity for APA) holds promise as a non-invasive triage tool. For patients with radiologically confirmed unilateral adenoma and contralateral adrenal gland normality, these biochemical criteria may offer a viable means of circumventing technically challenging bilateral AVS, which is particularly valuable in centers with limited interventional radiology experience. Second, for cases requiring AVS, steroid profiling from adrenal veins can be used to determine the dominant side of APA, which resolves the persistent clinical dilemma of incomplete bilateral catheterization. This hierarchical approach synergizes biochemical precision with practical clinical implementation, optimizing diagnostic efficiency while maintaining diagnostic rigor across healthcare resource settings. Based on the above insights, we propose a clinical application: the development of an intraoperative rapid detection method

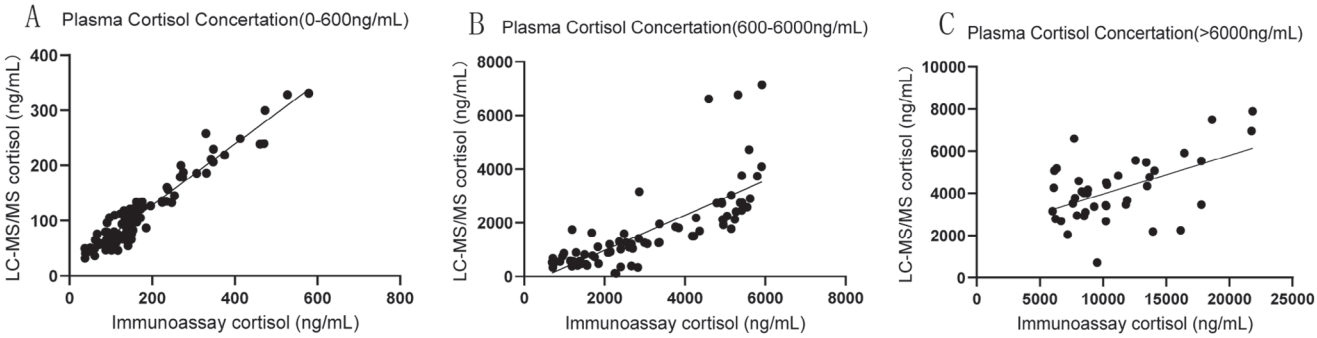

FIGURE 4 | Correlation between cortisol concentrations detected by mass spectrometry and immunoassay results.

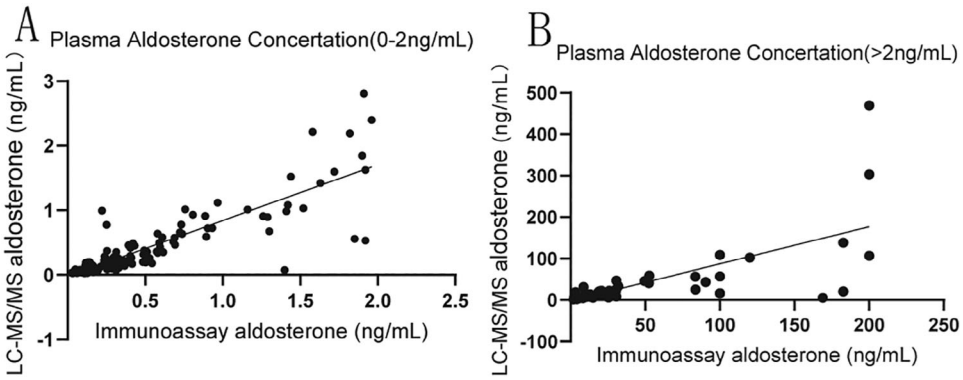

FIGURE 5 | Correlation between aldosterone concentration detected by mass spectrometry and immunoassay results.

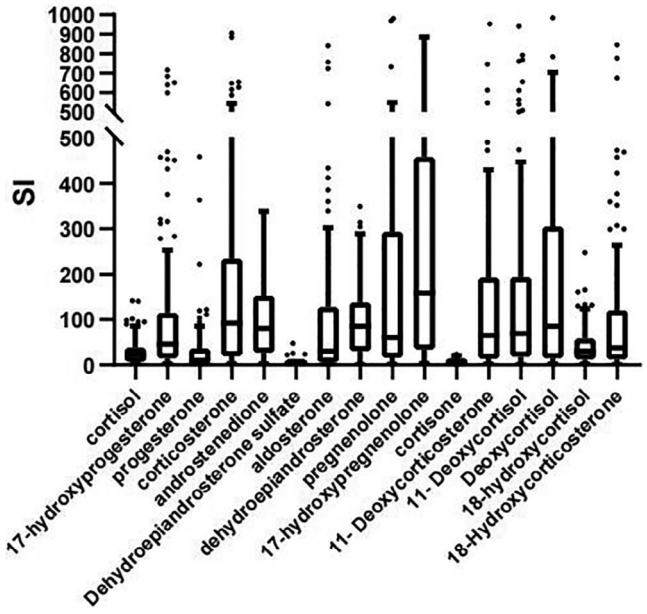

FIGURE 6 | Tukey box-whisker plots comparing selectivity index of LS-MS/MS steroids in a patient with primary aldosteronism. SI indicates selectivity index.

for real-time quantification of steroid ratios in adrenal veins. This method aims to simplify the standard process for unilateral dominant secretion, thereby reducing the complexity of AVS without compromising diagnostic accuracy.

Owing to their limited detection range, protein binding, and cross-reactivity with other steroids, immunoassay methods for steroid detection exhibit inherent biases in both accuracy and sensitivity [15]. An increasing number of clinical laboratories are adopting LC-MS/MS to measure steroid levels, a method known for its high accuracy and sensitivity. Our findings demonstrate a linear correlation between cortisol and aldosterone levels measured using IAs and LC-MS/MS within the detection range. However, the correlation between the two methods diminished when the laboratory detection limit exceeded 10 times or more. Using LC-MS/MS, our study revealed that six steroids (corticosterone, pregnenolone, 17-hydroxypregnenolone, 11-deoxycorticosterone, 11-deoxycortisol, and deoxycortisol) outperformed cortisol in assessing the success of AVS intubation, corroborating findings from other studies [31, 32], which indicates that steroid-based LC-MS/MS-derived SI enhances the success rate of AVS. Our results underscore the superiority of LC-MS/MS over IAs, allowing for the simultaneous measurement of multiple steroids in a single panel, thereby providing a comprehensive confirmation of the technical success of AVS.

Our study has several limitations. First, it is a single-center study with a small sample size, potentially limiting the applicability of the results to broader populations with diverse genetic backgrounds and environmental exposures. Larger-scale, multi-center trials are needed to validate the applicability of these results in diverse populations. Second, only hematoxylin and eosin staining was performed on the APA tissues, without CYP11B2 and CYP11B1 immunohistochemical staining. Furthermore, genetic mutation analysis was not conducted on the APA tissues.

5 | Conclusion

This study establishes LC-MS/MS steroid profiling as a transformative approach for PA subtyping. We identified divergent steroid metabolic profiles across PA subtypes, with peripheral venous 18-OHF concentrations (>4.83 ng/mL) demonstrating 100% specificity for APA identification. Crucially, adrenal venous 18-OHF ≥14.6‰ and 18-OHB ≥4.03‰ emerged as high-specificity biochemical criteria for lateralizing APA-dominant secretion, resolving the persistent diagnostic challenge of incomplete AVS. These findings enable a tiered diagnostic algorithm: initial non-invasive stratification followed by unilateral AVS optimization when required, reducing procedural complexity while maintaining diagnostic equivalence. Our results redefine PA management paradigms, offering resource-efficient solutions for clinical practice. Multicenter validation and exploration of molecular mechanisms underlying these steroid signatures are warranted to advance precision endocrinology.

Author Contributions

Xiuqing Chen: Conceptualization, investigation, visualization, writing—original draft, and writing– review & editing. Qinyi Li: Visualization, data curation, and writing—review & editing. Linjing Huang: Writing—review & editing and investigation. Peiwen Wu: Conceptualization, writing—original draft, and writing—review & editing.

Acknowledgments

The authors would like to thank all the research participants.

Disclosure

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Ethics Statement

The studies involving humans were approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University ([2015]084-2). The studies were conducted in accordance with the local legislation and institutional requirements.

Consent

All participants provided written informed consent in accordance with the national legislation and institutional requirements.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original contributions presented in the study are included in the article/Supporting Material, further inquiries can be directed to the corresponding author.

References

1. GBD 2013 Risk Factors Collaborators, M. H. Forouzanfar, L. Alexander, H. Ross Anderson, et al., "Global, Regional, and National Comparative Risk Assessment of 79 Behavioural, Environmental and Occupational, and Metabolic Risks or Clusters of Risks in 188 Countries, 1990–2013: A Systematic Analysis for the Global Burden of Disease Study 2013," *Lancet* 386, no. 10000 (2015): 2287–2323.

2. S. Monticone, J. Burrello, D. Tizzani, et al., "Prevalence and Clinical Manifestations of Primary Aldosteronism Encountered in Primary Care Practice," *Journal of the American College of Cardiology* 69 (2017): 1811–1820.

3. R. Libianto, G. M. Russell, M. Stowasser, et al., "Detecting Primary Aldosteronism in Australian Primary Care: A Prospective Study," *Medical Journal of Australia* 216, no. 8 (2022): 408–412.

4. A. Helber, G. Wambach, W. Hummerich, G. Bönner, K. A. Meurer, and W. Kaufmann, "Evidence for a Subgroup of Essential Hypertensives With Non-Suppressible Excretion of Aldosterone during Sodium Loading," *Klinische Wochenschrift* 58, no. 9 (1980): 439–447.

5. S. Monticone, F. D'Ascenzo, C. Moretti, et al., "Cardiovascular Events and Target Organ Damage in Primary Aldosteronism Compared With Essential Hypertension: A Systematic Review and Meta-Analysis," *Lancet Diabetes & Endocrinology* 6, no. 1 (2018): 41–50.

6. T. A. Williams, J. W. M. Lenders, P. Mulatero, et al., "Outcomes after Adrenalectomy for Unilateral Primary Aldosteronism: An International Consensus on Outcome Measures and Analysis of Remission Rates in an International Cohort," *Lancet Diabetes & Endocrinology* 5, no. 9 (2017): 689–699.

7. G. P. Rossi, M. Cesari, C. Cuspidi, et al., "Long-Term Control of Arterial Hypertension and Regression of Left Ventricular Hypertrophy With Treatment of Primary Aldosteronism," *Hypertension* 62, no. 1 (2013): 62–69.

8. J. Xie, C. Zhang, X. Wang, et al., "Exploration of KCNJ5 Somatic Mutation and CYP11B1/CYP11B2 Staining in Multiple Nodules in Primary Aldosteronism," *Frontiers in Medicine* 9 (2022): 823065.

9. M. Omura, H. Sasano, T. Fujiwara, K. Yamaguchi, and T. Nishikawa, "Unique Cases of Unilateral Hyperaldosteronemia due to Multiple Adrenocortical Micronodules, Which Can Only Be Detected by Selective Adrenal Venous Sampling," *Metabolism* 51, no. 3 (2002): 350–355.

10. P. Elliott and D. T. Holmes, "Adrenal Vein Sampling: Substantial Need for Technical Improvement at Regional Referral Centres," *Clinical Biochemistry* 46, no. 15 (2013).

11. S. Zhong, T. Zhang, M. He, et al., "Recent Advances in the Clinical Application of Adrenal Vein Sampling," *Frontiers in Endocrinology* 13 (2022): 797021.

12. F. Buffolo, S. Monticone, M. Tetti, and P. Mulatero, "Primary Aldosteronism in the Primary Care Setting," *Current Opinion in Endocrinology, Diabetes and Obesity* 25, no. 3 (2018): 155–159.

13. F. Satoh, R. Morimoto, Y. Ono, et al., "Measurement of Peripheral Plasma 18-Oxocortisol Can Discriminate Unilateral Adenoma From Bilateral Diseases in Patients With Primary Aldosteronism," *Hypertension* 65, no. 5 (2015): 1096–1102.

14. G. Eisenhofer, T. Dekkers, M. Peitzsch, et al., "Mass Spectrometry-Based Adrenal and Peripheral Venous Steroid Profiling for Subtyping Primary Aldosteronism," *Clinical Chemistry* 62, no. 3 (2016): 514–524.

15. Z. Wang, H. Wang, Y. Peng, et al., "A Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)-Based Assay to Profile 20 Plasma Steroids in Endocrine Disorders," *Clinical Chemistry and Laboratory Medicine* 58, no. 9 (2020): 1477–1487.

16. T. Wannachalee and A. F. Turcu, "Developments in Primary Aldosteronism Subtyping Using Steroid Profiling," *Hormone and Metabolic Research* 52, no. 6 (2020): 373–378.

17. J. W. Funder, R. M. Carey, F. Mantero, et al., "The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline," *Journal of Clinical Endocrinology and Metabolism* 101, no. 5 (2016): 1889–1916.

18. Y. L. Chang, G. Y. Chen, B. C. Lee, et al., "Optimizing Adrenal Vein Sampling in Primary Aldosteronism Subtyping through LC-MS/MS and Secretion Ratios of Aldosterone, 18-Oxocortisol, and 18-Hydroxycortisol," *Hypertension Research* 46, no. 8 (2023): 1983–1994.

19. R. P. Lifton, R. G. Dluhy, M. Powers, et al., "Hereditary Hypertension Caused by Chimaeric Gene Duplications and Ectopic Expression of Aldosterone Synthase," *Nature Genetics* 2, no. 1 (1992): 66–74.

20. L. Pascoe, K. M. Curnow, L. Slutsker, et al., "Glucocorticoid-Suppressible Hyperaldosteronism Results From Hybrid Genes Created by Unequal Crossovers between CYP11B1 and CYP11B2," *Proceedings of the National Academy of Sciences of the United States of America* 89, no. 17 (1992): 8327–8331.

21. M. Ohta, S. Fujii, R. Miura, Y. Nonaka, and M. Okamoto, "Bovine Adrenal Cytochrome P-450(11 Beta)-Mediated Conversion of 11-Deoxycortisol to 18- and 19-Hydroxy Derivatives; Structural Analysis by 1H-NMR," *Journal of Steroid Biochemistry and Molecular Biology* 39, no. 6 (1991): 911–920.

22. E. M. Freel, L. A. Shakerdi, E. C. Friel, et al., "Studies on the Origin of Circulating 18-Hydroxycortisol and 18-Oxocortisol in Normal Human Subjects," *Journal of Clinical Endocrinology and Metabolism* 89, no. 9 (2004): 4628–4633.

23. P. Mulatero, S. M. di Cella, S. Monticone, et al., "18-Hydroxycorticosterone, 18-Hydroxycortisol, and 18-Oxocortisol in the Diagnosis of Primary Aldosteronism and Its Subtypes," *Journal of Clinical Endocrinology and Metabolism* 97 (2012): 881–889.

24. T. A. Williams, M. Peitzsch, A. S. Dietz, et al., "Genotype-Specific Steroid Profiles Associated with Aldosterone-Producing Adenomas," *Hypertension* 67 (2016): 139–145.

25. F. F. Zheng, L. M. Zhu, A. F. Nie, et al., "Clinical Characteristics of Somatic Mutations in Chinese Patients With Aldosterone-Producing Adenoma," *Hypertension* 65, no. 3 (2015): 622–628.

26. R. Taguchi, M. Yamada, Y. Nakajima, et al., "Expression and Mutations of KCNJ5 mRNA in Japanese Patients With Aldosterone-Producing Adenomas," *Journal of Clinical Endocrinology and Metabolism* 97, no. 4 (2012): 1311–1319.

27. K. De Sousa, S. Boulkroun, S. Baron, K. Nanba, M. Wack, W. E. Rainey, et al., "Genetic, Cellular, and Molecular Heterogeneity in Adrenals with Aldosterone-Producing Adenoma," *Hypertension* 75, no. 4 (2020): 1034–1044.

28. T. Kawamoto, Y. Mitsuuchi, K. Toda, et al., "Role of Steroid 11 Beta-Hydroxylase and Steroid 18-Hydroxylase in the Biosynthesis of Glucocorticoids and Mineralocorticoids in Humans," *Proceedings of the National Academy of Sciences of the United States of America* 89, no. 4 (1992): 1458–1462.

29. K. M. Curnow, P. Mulatero, N. Emeric-Blanchouin, et al., "The Amino Acid Substitutions Ser288Gly and Val320Ala Convert the Cortisol Producing Enzyme, CYP11B1, Into an Aldosterone Producing Enzyme," *Nature Structural Biology* 4, no. 1 (1997): 32–35.

30. J. L. Phillips, M. M. Walther, J. C. Pezzullo, et al., "Predictive Value of Preoperative Tests in Discriminating Bilateral Adrenal Hyperplasia From an Aldosterone-Producing Adrenal Adenoma," *Journal of Clinical Endocrinology and Metabolism* 85, no. 12 (2000): 4526–4533.

31. A. F. Turcu, T. Wannachalee, A. Tsodikov, et al., "Comprehensive Analysis of Steroid Biomarkers for Guiding Primary Aldosteronism Subtyping," *Hypertension* 75, no. 1 (2020): 183–192.

32. M. Peitzsch, T. Dekkers, M. Haase, et al., "An LC-MS/MS Method for Steroid Profiling during Adrenal Venous Sampling for Investigation of Primary Aldosteronism," *Journal of Steroid Biochemistry and Molecular Biology* 145 (2015): 75–84.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.