Morphologically Normal-Appearing Adrenal Glands as a Prevalent Source of Aldosterone Production in Primary Aldosteronism

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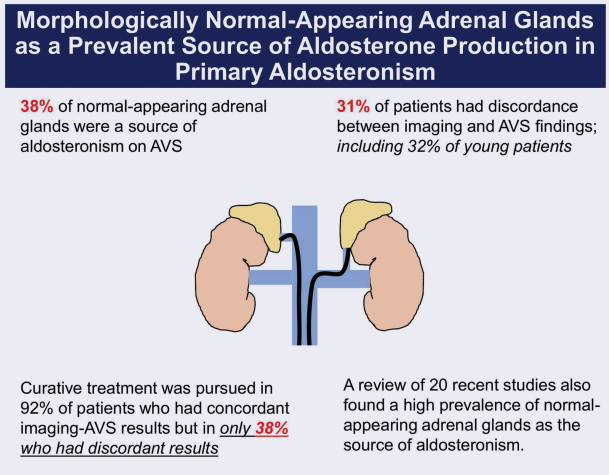
BACKGROUND
Normal-appearing adrenal glands on cross-sectional imaging may still be the source of aldosterone production in primary aldosteronism (PA).

METHODS
We evaluated the prevalence of aldosterone production among morphologically normal-appearing adrenal glands and the impact of this phenomenon on interpretations of localization studies and treatment decisions. We performed a retrospective cohort study of PA patients with at least 1 normal adrenal gland and reanalyzed contemporary studies to assess interpretations of imaging and adrenal venous sampling (AVS) at the individual patient and adrenal levels.

RESULTS
Among 243 patients, 43 (18%) had bilateral normal-appearing adrenals and 200 (82%) had a unilateral normal-appearing adrenal, for a total of 286 normal-appearing adrenal glands. 38% of these normal-appearing adrenal glands were a source of aldosteronism on AVS, resulting in discordance between imaging and AVS findings in 31% of patients. Most patients with lateralizing PA underwent curative unilateral treatment (80%); however, curative treatment was pursued in 92% of patients who had concordant imaging-AVS results but in only 38% who had discordant results ($P < 0.05$). In young patients, imaging-AVS discordance was detected in 32% of those under 45 years and 21% of those under 35 years. Among 20 contemporary studies (including 4,904 patients and 6,934 normal-appearing adrenal glands), up to 64% of normal-appearing adrenals were a source of aldosteronism resulting in 31% of patients having discordant results.

CONCLUSIONS
Morphologically normal-appearing adrenal glands are commonly the source of aldosterone production in PA, even among young patients. The lack of awareness of this issue may result in inappropriate treatment recommendations.

GRAPHICAL ABSTRACT



Keywords: adrenal gland; adrenal venous sampling; aldosterone; blood pressure; hypertension; primary aldosteronism.

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Primary aldosteronism (PA) is a common cause of hypertension.[1–3] The source of PA can originate from unilateral or bilateral adrenal glands.[4] The recommended treatment for patients with lateralizing PA is curative adrenalectomy, which has been shown to mitigate the risk of developing cardiovascular and kidney disease in several large observational studies.[5–8] In contrast, patients with bilateral PA are typically treated medically with mineralocorticoid receptor antagonists, which can also be very effective, though some studies have shown inferior outcomes compared with surgical adrenalectomy.[5,9]

Expert guidelines recommend cross-sectional imaging for all patients in whom PA is diagnosed.[10] Although computed tomography (CT) and magnetic resonance imaging can detect morphological abnormalities in the adrenal glands, they cannot differentiate functional adrenal foci from nonfunctional abnormalities, nor can they reliably detect aldosterone-producing micronodules.[11,12] Thus, the most reliable value of cross-sectional imaging is to evaluate the possibility of an adrenocortical carcinoma, and to provide an anatomical map prior to adrenal venous sampling (AVS) or surgery. The possibility of discordance between radiographic features and AVS results is known.[13] Therefore, the Endocrine Society recommends performing AVS in every patient who is a candidate for surgery to reliably differentiate lateralizing from nonlateralizing PA; however, it is suggested that young patients (age <35 years) with PA who have a unilateral adrenal mass may forego AVS and proceed directly to surgical adrenalectomy.[10]

Most studies have reported the concordance or discordance between imaging and AVS results based on patient-level metrics. To gain insights into the prevalence and impact of morphologically normal adrenal glands in the pathophysiology of PA, we investigated the discordance between imaging and AVS results both at the individual patient level and individual adrenal gland level using a large cohort of PA patients with at least 1 morphologically normal-appearing adrenal gland referred to our center for AVS. We subsequently assessed how this prevalence and discordance could impact treatment decisions. We also conducted a comprehensive literature review of studies in the past 10 years to determine the discordance rates between imaging and AVS in patients with at least 1 normal-appearing adrenal gland, to evaluate how frequently reliance on imaging findings could lead to inappropriate management.

METHODS

Study design and participants

This was a retrospective study including PA patients referred to Harvard Medical School hospitals for diagnosis and AVS (Beth Israel Deaconess Medical Center and Brigham and Women's Hospital, both in Boston, MA) between 2005 and 2019. All participants were confirmed to have PA based on criteria recommended by the Endocrine Society.[10,14,15]

Participants were eligible for this study if they had at least 1 morphologically normal-appearing adrenal gland on cross-sectional imaging and underwent a successful AVS procedure, defined as having a stimulated selectivity index of ≥3 (the mean adrenal vein cortisol divided by the inferior vena cava cortisol following a bolus of cosyntropin) in each adrenal vein. Cross-sectional imaging of the abdomen was performed with CT or magnetic resonance imaging, and radiology reports were reviewed to determine adrenal findings. Morphologically normal-appearing adrenal glands were defined as having no apparent nodule, mass, or abnormal thickening or hyperplasia on cross-sectional imaging. Participants with bilateral adrenal abnormalities on cross-sectional imaging were excluded from this analysis since the focus of this investigation was to assess how frequently morphologically normal-appearing adrenal glands were the source of PA. Of the 340 patients who underwent successful AVS and imaging during this time period, a total of 243 patients met these inclusion criteria.

The same interventional radiologist and staff conducted all AVS procedures. Patients were given potassium supplements, if needed, to normalize potassium levels prior to the AVS. Mineralocorticoid receptor antagonists were discontinued for at least 4 weeks prior to the procedure. All study participants gave informed consent, and all study procedures were conducted in accordance with institutional guidelines (protocol 2013P000564).

AVS protocol

As previously described,[14–16] catheters were introduced into the right and left femoral veins, and positioned in the infrarenal inferior vena cava to obtain baseline unstimulated measurements of aldosterone and cortisol. The 2 catheters were used to cannulate the right and left adrenal veins, with selective catheterization confirmed using adrenal venography and/or adjunctive cone-beam CT. Throughout the procedure, catheter position was confirmed via intermittent adrenal venography. Measurement of aldosterone and cortisol levels prior to the stimulation with cosyntropin was used to calculate the unstimulated lateralization index (LI).[16] Unstimulated, bilateral, and simultaneous aldosterone and cortisol levels were obtained in triplicate. LI was calculated for each patient, defined as the unstimulated mean aldosterone-to-cortisol (A/C) ratio from the dominant adrenal vein divided by the unstimulated mean A/C ratio from the contralateral vein.

After the third unstimulated measurement of aldosterone and cortisol was obtained in each adrenal vein, a single 250 μg bolus of intravenous adrenocorticotropic hormone (ACTH) was administered. ACTH-stimulated aldosterone and cortisol levels were obtained from each adrenal vein to enhance and calculate the stimulated selectivity index. ACTH-stimulated aldosterone and cortisol levels were collected from each adrenal vein, in triplicate, 5 minutes apart. A final infrarenal inferior vena cava sample was obtained immediately following the final postcosyntropin samples.

After AVS completion, patients were directed back to their referring physicians. The AVS results were evaluated by these respective physicians to determine the most reasonable and feasible treatment, which included unilateral adrenalectomy or unilateral radiofrequency ablation (RFA), or medical therapy.

Measurements

Aldosterone concentrations were measured using the Quest Diagnostic liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay with a laboratory-reported coefficient of variation ranging from 2.7% to 4.4%, depending on the aldosterone level. From 2017 to 2019, cortisol levels were measured using the Elecsys Cortisol II immunoassay (Roche) and Cobas e601 analyzer, with a laboratory-reported coefficient of variation ranging from 1.5% to 5.4%. From 2005 to 2017, cortisol levels were measured using the Elecsys Cortisol I immunoassay (Roche) with similar coefficient of variation values.[14,15]

Study outcomes and statistical analysis

The number of morphologically normal-appearing adrenal glands was characterized on cross-sectional imaging and correlated with AVS results. Lateralization on AVS was defined as an unstimulated LI of ≥2, and in addition, by a more conservative LI threshold of ≥4.[16]

Imaging-AVS "discordance" was defined as:

1) Lateralization on AVS to the contralateral morphologically normal-appearing adrenal in patients with a unilateral adrenal abnormality, or
2) Lack of lateralization on AVS in patients with a unilateral adrenal abnormality, or
3) Lateralization on AVS in patients with bilateral morphologically normal-appearing adrenal glands.

Imaging-AVS "concordance" was defined as:

1) Lateralization on AVS to the ipsilateral morphologically abnormal-appearing adrenal gland in patients with unilateral adrenal abnormalities on imaging, or
2) Lack of lateralization on AVS in patients with bilateral morphologically normal-appearing adrenal glands.

Post-treatment outcomes were analyzed for patients with at least 1 follow-up visit. Patients who underwent unilateral surgical adrenalectomy or unilateral RFA were classified as having absent, partial, or complete clinical and biochemical success as per the Primary Aldosteronism Surgical Outcomes study (PASO) criteria.[17]

Means are presented with standard deviations for normally distributed variables. Medians are presented with interquartile ranges for nonnormally distributed variables. Percentages of the total sample are presented for categorical variables. Student's *t* test was used to assess the difference between normally distributed data. Wilcoxon rank sum test was used for comparisons if data were nonparametric. Fisher's exact test or chi-square test was used for the comparison of binary variables. All statistical analyses were performed using STATA/SE 16.1 (College Station, TX).

Literature review and reanalysis of contemporary studies

In addition to this original research analysis, we performed a review of contemporary studies over the last 10 years that also investigated, or had data within the study to evaluate, the discordance between cross-sectional imaging findings and AVS results in patients with PA. The primary outcome of interest was the concordance of AVS results with imaging findings in patients with at least 1 morphologically normal-appearing adrenal gland. We searched PubMed for "adrenal venous sampling and normal imaging" to identify relevant studies, published in the English language, from the last 10 years to supplement the published studies already known to the authors. The references for each of the identified publications were then individually reviewed to identify other potentially relevant studies. Each study was evaluated for the number of patients, study design, definition of lateralization (AVS), and a summary of each study's findings were organized in tabular format.

RESULTS

Baseline characteristics

Baseline characteristics are shown in Table 1. On average, patients were hypertensive, hypokalemic, and had elevated aldosterone-to-renin ratios. Hypokalemia was corrected with potassium supplementation, and the mean serum potassium at the day of AVS was normal, 3.9 mmol/l. All patients had at least 1 morphologically normal-appearing adrenal gland on cross-sectional imaging. The majority of patients (82%) had a unilateral adrenal abnormality and a contralateral normal-appearing adrenal gland wherein the unilateral adrenal abnormality was more likely to be left sided, as has been previously described.[18–20] Among the total of 243 PA patients, 43 patients had bilateral morphologically normal-appearing adrenal glands and 200 patients had a unilateral morphologically normal-appearing adrenal gland, for a total of 286 morphologically normal-appearing adrenal glands. Among these 286 normal-appearing adrenal glands, 134 were left-sided (91 in patients with a unilateral left normal-appearing adrenal and 43 in patients with bilateral normal-appearing adrenals) and 152 were right-sided (109 in patients with a unilateral right normal-appearing adrenal gland and 43 in patients with bilateral normal-appearing adrenal glands) (Table 1).

Patient-level analyses: cross-sectional imaging findings and AVS results

Lateralizing PA, using an unstimulated LI ≥2, was detected in 203 of the 243 patients (84%); cross-sectional imaging findings showed a discordant adrenal abnormality corresponding to this lateralization in 31% of patients (26 patients with bilateral normal-appearing adrenals and lateralization on AVS; 23 patients with a unilateral normal-appearing adrenal and no lateralization on AVS, and 27 patients with a unilateral normal-appearing adrenal and lateralization to the contralateral normal adrenal) (Table 2). Similarly, when using an unstimulated LI ≥4, lateralizing PA was detected in 166 of the 243 patients (68%) with discordant imaging findings in 33% of patients (Table 2). Of the 43 patients with bilateral normal-appearing adrenals on imaging, there was no lateralization on AVS in 17 patients (hence, concordant imaging-AVS results), while lateralization was found in 26 patients (hence, discordant imaging-AVS results). Of the 200

Table 1. Baseline characteristics of the study cohort

Characteristics	Total study population (n = 243)	PA patients with bilateral morphologically normal adrenal glands on imaging (n = 43)	PA patients with a unilateral morphologically normal adrenal gland on imaging (n = 200)
Age (years)	52.4 ± 10.9	47.1 ± 10.2	53.5 ± 10.7
Sex (% men)	61%	49%	64%
BMI (kg/m^2)	30.7 ± 5.8	32.4 ± 7.2	30.1 ± 5.2
SBP (mmHg)	148.5 ± 20.5	145.2 ± 22.1	149.3 ± 20.1
DBP (mmHg)	86.9 ± 12.1	86.5 ± 11.7	87.0 ± 12.2
Hypokalemia (%)	65%	56%	66%
Serum potassium at diagnosis (mmol/l)	3.4 ± 0.5	3.5 ± 0.6	3.4 ±0.5
PAC (ng/dl)	22.7 (16.7–34.0)	21.6 (17.0–26.0)	23.8 (16.6–38.0)
PRA (ng/ml/h)	0.4 (0.2–0.6)	0.6 (0.2–0.6)	0.4 (0.2–0.6)
ARR (ng/dl per ng/ml/h)	72.5 (35.0–152.0)	46.3 (31.7–86.0)	78.3 (36.4–168.4)
Number of antihypertensive medications (n)	3 (2–4)	3 (1–4)	3 (2–4)
Cross-sectional imaging findings			
Left normal adrenal, n (%)	134 (55%)	43 (100%)	91 (45.5%)
Right normal adrenal, n (%)	152 (63%)	43 (100%)	109 (54.5%)

Data are presented as mean ± SD, % or median (interquartile range). Abbreviations: ARR, aldosterone-to-renin ratio; BMI, body mass index; DBP, diastolic blood pressure; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PRA, plasma renin activity; SBP, systolic blood pressure.

Table 2. Cross-sectional imaging morphologies and interpretation of adrenal venous sampling (by patient-level data)

Definition of lateralization	AVS interpretation	PA patients with bilateral morphologically normal adrenal glands on imaging (n = 43)	PA patients with a unilateral morphologically normal adrenal gland on imaging (n = 200)		Total patients (n = 243)
			Normal left (n = 91)	Normal right (n = 109)	
LI ≥2	Bilateral PA	17	**9**	**14**	40
	Left lateralized	**12**	**7**	75	94
	Right lateralized	**14**	75	**20**	109
LI ≥4	Bilateral PA	28	**15**	**34**	77
	Left lateralized	**7**	**6**	65	78
	Right lateralized	**8**	70	**10**	88

Abbreviations: AVS, adrenal venous sampling; LI, unstimulated lateralization index; PA, primary aldosteronism. Bold values highlight patients with discordant results between cross-sectional imaging findings and AVS lateralization.

patients with a unilateral normal-appearing adrenal gland, an absence of lateralization on AVS was found in 23 patients, and lateralization to the contralateral normal adrenal was found in 27 patients (Table 2).

Adrenal-level analyses: prevalence of aldosterone production among morphologically normal-appearing adrenal glands

Among the 286 morphologically normal-appearing adrenal glands from 243 patients, 110 glands (38%) were a source of aldosteronism on AVS when using an unstimulated LI ≥2. Aldosterone production was found in 45 (34%) and 65 (43%) of the left and right morphologically normal-appearing adrenal glands, respectively (Table 3).

Similar results were observed when lateralizing PA was defined using an unstimulated LI ≥4; 136 normal-appearing adrenal glands (48%) were a source of aldosteronism, in which 56 and 80 glands were left and right, respectively (Table 3).

Treatment decisions and outcomes

Among the 203 patients with lateralizing PA on AVS, follow-up data regarding definitive treatments was available on 165 (81%). The majority of patients (80%; 132/165) who had lateralizing PA underwent unilateral intervention (surgical adrenalectomy or RFA) (Table 4). However, there was a significant discrepancy between the treatment for lateralizing PA depending on whether there was imaging-AVS concordance or discordance. The vast majority (92%) of patients with lateralizing PA who had concordant imaging-AVS results were referred to undergo curative surgical adrenalectomy or RFA. In contrast, only 38% of patients with lateralizing PA who had discordant imaging-AVS results were referred for a curative intervention ($P < 0.05$); these decisions were made by individual physicians with their respective patients using their interpretations of the AVS imaging findings.

Among patients with lateralizing PA who underwent a unilateral intervention, long-term clinical and biochemical outcomes were available in 80% patients, although not every patient had available data on clinical and biochemical outcomes (Supplementary Table S1 online). During a median follow-up of 5.5 months in which half of the patients followed up at least 6 months, most patients, irrespective of imaging-AVS concordance, achieved clinical and biochemical benefits after surgical adrenalectomy or RFA. Eighty-three percent of patients with available data achieved clinical benefits, albeit most had partial clinical success. Ninety-five percent of patients with available data achieved biochemical benefits, of which nearly all (90%) had complete biochemical success. Three patients with concordant imaging-AVS results did not have biochemical success. Analyses using LI ≥4 yielded similar results.

Analyses among younger patients

There were 57 PA patients aged less than 45 years old; cross-sectional imaging findings showed a unilateral adrenal abnormality in 40 patients. Using an LI ≥2, lateralizing PA

Table 3. Cross-sectional imaging morphologies and interpretation of adrenal venous sampling (by adrenal-level data)

		Imaging morphology				
		Normal bilaterally		Unilateral abnormality		
Definition of lateralization	AVS interpretation	Normal left (*n* = 43)	Normal right (*n* = 43)	Normal left (*n* = 91)	Normal right (*n* = 109)	Total adrenal glands (*n* = 286)
LI ≥2	Bilateral PA	***17***	***17***	***9***	***14***	57
	Left lateralized	***12***	12	***7***	75	106
	Right lateralized	14	***14***	75	***20***	123
LI ≥4	Bilateral PA	***28***	***28***	***15***	***34***	105
	Left lateralized	***7***	7	***6***	65	85
	Right lateralized	8	***8***	70	***10***	96

Abbreviations: AVS, adrenal venous sampling; LI, unstimulated lateralization index; PA, primary aldosteronism. Bold and italicized values highlight aldosterone production in normal-appearing adrenal glands.

Table 4. Treatment decisions for patients with lateralizing primary aldosteronism (defined as an unstimulated LI ≥2)

	Imaging-AVS concordance (*n* = 128)	Imaging-AVS discordance (*n* = 37)	Total	P
Surgical adrenalectomy or RFA, *n* (%)	118 (92)	14 (38)	132 (80)	<0.05
Medical therapy, *n* (%)	10 (8)	23 (62)	33 (20)	

Abbreviations: AVS, adrenal venous sampling; LI, unstimulated lateralization index; RFA, radiofrequency ablation.

was detected in 43 of 57 patients, wherein the discordance between lateralizing PA on AVS and an imaging abnormality was 32% (Supplementary Table 2A online). Of the 17 young patients with bilateral normal-appearing adrenal glands, 9 patients had lateralization on AVS. Of the 40 patients with a unilateral normal-appearing adrenal gland, AVS did not lateralize in 6 patients and lateralized to the contralateral normal-appearing adrenal in 3 patients.

From the perspective of adrenal-level data, among the 74 morphologically normal-appearing adrenal glands from these 57 young patients, there was aldosterone production on AVS from 34 glands (46%) using an LI ≥2. Aldosterone production was found in 13 (42%) and 21 (49%) of the left and right normal-appearing adrenal glands, respectively (Supplementary Table 2B online).

Fourteen patients were younger than 35 years old. There were 3 patients with discordant results between cross-sectional imaging findings and AVS lateralization (21%). Two patients with bilateral normal-appearing adrenals had lateralizing PA, and 1 patient with a unilateral normal-appearing adrenal had no lateralization.

Reanalysis of contemporary studies

In a contemporary literature review, the PubMed search of "adrenal venous sampling and normal imaging" yielded 55 studies in the English language from 2011 to present. Nine studies with titles and abstracts fitting our inclusion criteria were reviewed in detail and 10 additional studies, including 1 systematic review, were added after an in-depth review of their references.[13,21–38] In Table 5, we present a summary of these 19 studies, plus the current study (total of 20 studies), which included AVS results in PA patients with at least 1 morphologically normal-appearing adrenal gland. Note that AVS interpretations were heterogeneous between studies, with some using unstimulated LI to make interpretations whereas others used ACTH-stimulated LI to determine lateralization, as well as different LI cutoffs. Despite this study heterogeneity, a consistent finding was that there was substantial discordance between cross-sectional imaging findings and AVS lateralization in every study. Among 2,030 patients with bilateral normal-appearing adrenals from these 20 studies, there was a lack of lateralization on AVS (hence concordance between imaging and AVS) in 1,462 patients (72%; range, 23%–89%), while lateralization (hence discordance between imaging and AVS) was found in 478 patients (24%; range, 11%–77%). Among 2,874 patients with a unilateral morphologically normal-appearing adrenal gland, lack of lateralization on AVS was found in 826 patients (29%; range, 6%–51%), and lateralization to the contralateral normal-appearing adrenal gland was found in 227 patients (8%; range, 0%–22%).

Among the 6,934 morphologically normal-appearing adrenal glands from these 20 studies, 4,455 (64%) were determined to be a source of aldosterone production on AVS.

DISCUSSION

The possibility of discordance between cross-sectional imaging and AVS findings in PA is known[13]; however, there is a lack of in-depth analyses into the frequency of aldosterone production from morphologically normal-appearing adrenal glands and how the discordance between imaging and AVS results can impact treatment decisions. In this study of PA patients with at least 1 normal-appearing adrenal, 31% of patients had discordant interpretations between

Table 5. Summary of recent studies comparing cross-sectional imaging findings and adrenal venous sampling results in patients with at least 1 morphologically normal-appearing adrenal gland

				Imaging findings and AVS interpretation					
				Bilateral normal-appearing adrenal glands			Unilateral normal-appearing adrenal gland		
	Participants	Type of study	Definition of lateralization	n	Bilateral PA, *n* (%)	Lateralization, *n* (%)	n	Bilateral PA, *n* (%)	Lateralization to contralateral normal adrenal, *n* (%)
Parksook *et al.* (this study)	PA patients	Retrospective	LI ≥2 pre-ACTH	43	17 (40)	26 (60)	200	23 (12)	27 (14)
Gkaniatsa *et al.*[21]	PA patients aged 26–40 years	Retrospective	LI >4 post-ACTH or LI 3–4 post-ACTH with CSI <1	14	12 (86)	2 (14)	25	5 (20)	0 (0)
Xu *et al.*[22]	PA patients	Retrospective	LI ≥2 pre-ACTH	—	—	—	89	23 (26)	15 (17)
Sam *et al.*[23]	PA patients with normal adrenal imaging	Retrospective	LI >3 pre-ACTH	174	104 (60)	70 (40)	—	—	—
Nagano *et al.*[24]	PA patients	Retrospective	LI >3 post-ACTH	54	48 (89)	6 (11)	103	42 (41)	3 (3)
Puar *et al.*[25]	PA patients	Retrospective	LI ≥4 post-ACTH or LI 2–4 post-ACTH + review	19	13 (68)	6 (32)	78	17 (22)	NA
Lee *et al.*[26]	PA patients	Retrospective	LI >2 pre-ACTH or >4 post-ACTH (or with CSI <1)	5	3 (60)	2 (40)	32	8 (25)	NA
Li *et al.*[37]	PA patients	Retrospective	LI ≥2 pre-ACTH	14	6 (43)	8 (57)	73	13 (18)	2 (3)
Aono *et al.*[27]	PA patients	Retrospective	LI ≥2 pre-ACTH	167	107 (64)	60 (36)	126	46 (37)	28 (22)
Sam *et al.*[28]	PA patients	Retrospective	LI >2 pre-ACTH[a]	104	62 (60)	42 (40)	191	43 (23)	31 (16)
Campbell *et al.*[29]	PA patients	Retrospective	LI ≥4 post-ACTH	4	3 (75)	1 (25)	63	32 (51)	2 (3)
Wachtel *et al.*[38]	PA patients	Retrospective	LI ≥4 post-ACTH	90	NA	NA	334	71 (21)	28 (8)
Umakoshi *et al.*[30]	PA patients	Retrospective	LI >4 post-ACTH	899	768 (85)	131 (15)	654	322 (49)	35 (5)
Kamemura *et al.*[31]	PA patients	Retrospective	LI >4 post-ACTH	228	204 (89)	24 (11)	156	NA	NA
Pedersen *et al.*[32]	PA patients	Retrospective	NA	13	3 (23)	10 (77)	31	2 (6)	NA
Raman *et al.*[33]	PA patients	Retrospective	LI >4 post-ACTH	7	5 (71)	2 (29)	35	8 (23)	4 (11)
Harvey *et al.*[34]	PA patients	Retrospective	LI >3 post-ACTH	24	20 (83)	4 (17)	49	10 (20)	3 (6)
Sarlon-Bartoli *et al.*[35]	PA patients	Retrospective	LI ≥2 pre-ACTH	6	2 (33)	4 (67)	47	16 (34)	7 (15)
Mathur *et al.*[36]	PA patients	Retrospective	LI ≥4 post-ACTH	5	2 (40)	3 (60)	57	6 (11)	5 (9)
Kempers *et al.*[13]	PA patients	Systematic review	Vary	160	83 (52)	77 (48)	531	139 (26)	37 (7)
Total, *n* (%)				2,030	1,462 (72)	478 (24)	2,874	826 (29)	227 (8)

Abbreviations: AVS, adrenal venous sampling; CSI, contralateral suppression index; LI, lateralization index; NA, not applicable; PA, primary aldosteronism.
[a]The study explored the discrepancy between imaging findings and AVS lateralization using varying thresholds for the lateralization index (from >2 to >5 pre-ACTH).

cross-sectional imaging and AVS, wherein aldosterone production was attributable to 38% of normal-appearing adrenal glands. Our findings underscore the lack of awareness of this phenomenon; patients with lateralizing PA were significantly less likely to be referred for a curative intervention when AVS lateralized to a morphologically normal adrenal gland; we speculate because the role of cross-sectional imaging in subtyping was overvalued when compared with AVS results. These findings underscore the inaccuracy and misleading nature of cross-sectional imaging for the purposes of PA subtyping and suggest that it is best reserved for excluding the possibility of an adrenal malignancy and/or visualizing anatomy. When summarizing and reanalyzing data from 20 studies over the last 10 years, the rates of aldosterone production from morphologically normal-appearing adrenal glands were at least as high as our current study, but often even higher. Notably, we observed that these rates of imaging-AVS discordance were similar even among very young patients in whom it has traditionally been presumed that imaging abnormalities corresponded to the source of aldosterone production.

Our findings extend those of prior studies.[13,21–38] Although there was heterogeneity in AVS protocols and criteria in defining lateralizing PA between sites in our literature review, a consistent finding was demonstration of high rates of discordance between cross-sectional imaging findings and AVS results. In addition, a recent study by Rossi *et al.* showed that cross-sectional imaging (without AVS) failed to recognize 28% of surgically curable cases of unilateral PA.[20]

Importantly, we observed that when AVS results demonstrated lateralization to a morphologically normal adrenal gland, treatment decisions chosen by physicians and patients appeared to be impacted. Specifically, when AVS demonstrated lateralization to a morphologically normal-appearing adrenal gland, patients were much less likely to undergo a curative procedure (38% vs. 92%, respectively). This stark contrast strongly suggests that many physicians likely still think of PA using the classical paradigm of "unilateral adenoma versus bilateral hyperplasia" rather than considering confounding factors such as incidental and nonfunctional adrenal abnormalities,[39–41] and recognizing that aldosterone production can be driven by microscopic foci and somatic mutations that are not visualized on cross-sectional imaging.[42,43] Ohno *et al.* also observed similar findings in an international cohort of 4,818 PA patients wherein 12% of patients with unilateral PA did not undergo surgical adrenalectomy, in part due to the absence of a clearly visualized tumor on imaging or because the AVS results were discordant with the imaging results.[44] In this regard, a dedicated educational campaign to increase awareness is necessary to ensure clinicians recognize that the source of aldosterone production in PA may originate in morphologically normal-appearing adrenal glands; the possibility of curative interventions should not be dismissed when AVS reveals lateralization in these scenarios.

In the absence of a gold standard for subtype differentiation in PA, various studies have investigated the difference in treatment outcomes using CT- or AVS-guided unilateral adrenalectomy. Dekker *et al.* reported no short-term differences in the primary outcome between PA patients with CT- or AVS-directed adrenalectomy in the only randomized controlled trial ever published (SPARTACUS)[45]; the primary endpoint was the intensity of drug treatment to obtain target blood pressure after 1 year of follow-up. Importantly, AVS was conducted using cosyntropin stimulation which can decrease LI and underestimate surgically curable PA.[16] Subsequently, and in contrast, the PASO studies showed that AVS-guided surgical treatment resulted in more complete biochemical success compared with imaging-guided treatment alone.[27,46] In patients with normal-appearing adrenal glands, less is known regarding the treatment outcomes. Sam *et al.* reported biochemical and clinical improvement in the majority of patients with lateralizing AVS despite bilateral normal-appearing adrenal glands.[47] These findings were supported by a recent large international study by Rossi *et al.* that reported no substantial difference in clinical outcomes between patients with lateralizing PA on AVS regardless of the presence of adrenal neoplasia on imaging.[20] Wachtel *et al.* recently showed no significant difference in clinical benefits among patients with nonlocalized (bilateral normal or abnormal adrenal glands on imaging) and localized adrenal imaging who had lateralizing PA.[38]

The growing understanding of histopathologic and genetic mutations in aldosterone-producing lesions may explain why morphologically normal-appearing adrenal glands are often the source of PA. The recognition of aldosterone-producing cell clusters, nonneoplastic regions of excess aldosterone production that often harbor a somatic mutation in an aldosterone-driver gene, as a potential etiology of PA has changed the paradigm of PA histopathology.[48–51] Further, aldosterone-producing micronodules or diffuse hyperplasia have also been implicated as the source of excess aldosterone in normal-appearing adrenal glands.[12,52,53] Somatic aldosterone-driver mutations, namely *CACNA1D*, were detected in 81% of CYP11B2 immunopositive cells in micronodules.[12] Wannachalee *et al.* recently showed that PA patients with somatic *CACNA1D* mutations had high rates of imaging-AVS discordance.[54]

Recently, there has been increased awareness of cortisol cosecretion in neoplastic PA, especially with larger adenomas.[55–58] Reassuringly, O'Toole *et al.* reported no clinically relevant differences in lateralization indices on AVS among PA patients with and without cortisol cosecretion, suggesting that mild hypercortisolism is unlikely to significantly influence AVS interpretations.[56] However, there remains some debate as to whether cortisol cosecretion could substantially impact AVS interpretations.[55] This concern stems from the expectation that cortisol cosecretion from the dominant side could result in suppression of ACTH and decreased cortisol production from the contralateral (nondominant) adrenal gland, consequently an increase in the contralateral A/C ratio, and therefore a decrease in the LI. In this scenario, this decrease in the LI could change the AVS interpretation from unilateral or lateralizing PA to bilateral or nonlateralizing PA. In this regard, it is important to note 75% of patients in our study with a unilateral adrenal abnormality had concordant AVS lateralization, implying that the influence of any potential hypercortisolism did not influence interpretations in the vast majority of patients. Further, 60% of patients in our study who had bilateral normal-appearing adrenals on imaging exhibited clear lateralization on AVS;

a phenomenon that cannot be explained by any potential hypercortisolism (which generally requires adrenal neoplasia), and underscores the fact that morphologically appearing adrenal glands can indeed be the source of PA.

The primary concern regarding the use of cross-sectional imaging findings alone in subtype differentiation is that there were high rates of discordance between radiologic features and AVS lateralization, which may lead to inappropriate treatment. Curative unilateral interventions are associated with superior cardiometabolic, renal, and quality of life outcomes when compared with medical treatment[5–7,59,60]; therefore, it should be considered a missed opportunity when PA patients are eligible for curative therapy but deprived of this option. Our current study, and others,[21,61] have shown high discordance rates between imaging and AVS lateralization in patients with at least 1 normal-appearing adrenal gland, including young patients. Most patients with lateralizing PA, irrespective of concordant imaging-AVS status, achieved clinical and biochemical benefits after surgical intervention. Therefore, we recommend performing AVS in all patients in whom a curative intervention is considered, regardless of age, and recommend considering a unilateral intervention in lateralizing cases of PA regardless of adrenal morphology.

One limitation of this study is the heterogeneity of site-specific AVS protocols. We determined lateralization based on unstimulated LI, which was calculated from the average of triplicate measures. Due to variability of aldosterone levels during AVS procedures, we previously showed that using only 1 unstimulated A/C ratio could lead to misinterpretation of AVS results.[14] A second limitation is that our study was not designed to investigate the clinical and biochemical outcomes of patients who lateralized at AVS and underwent surgical intervention, nor did every patient have available outcome data. However, the majority of patients had post-operative outcomes available, and using PASO criteria, were comparable with previous published studies.[17,59] A third limitation is that we were not able to conduct histopathology, steroidogenic, proteinomic, or genetic analyses on surgical samples to better understand the factors underlying the source(s) of aldosterone production. Fourth, we did not routinely have measures of cortisol cosecretion as this was not standard practice for adrenal adenomas until recently. However, our data (discussed above), and prior studies,[56] suggest that the influence of any potential hypercortisolism would not substantially change the conclusions. Finally, our results may not be generalizable to the entire PA population at large since our participants represent patients with the most clinically apparent, and likely most severe forms of PA that were ultimately diagnosed and referred for AVS; PA is a highly prevalent condition and most cases of this syndrome are undiagnosed and not herein represented.[1,4,62,63] We speculate that the proportion of morphologically normal adrenal glands in milder cases of PA would be higher.

In conclusion, we observed a high prevalence of morphologically normal-appearing adrenal glands as the source of aldosterone production in PA. Discordance between cross-sectional imaging and AVS interpretations in patients with at least 1 morphologically normal-appearing adrenal was seen in over 30% of patients, in which nearly 40% of morphologically normal-appearing adrenal glands were a source of aldosteronism on AVS. Our reanalysis of prior publications suggests that these estimates may be in fact be a conservative underestimate of the true prevalence of this phenomenon. Importantly, these discordant results were also seen in very young patients. The lack of awareness of this issue may have resulted in inappropriate treatment recommendations wherein many patients with lateralizing PA were not referred for a curative intervention.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *American Journal of Hypertension* online.

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DISCLOSURE

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DATA AVAILABILITY

The data underlying this article will be shared on reasonable request to the corresponding author.

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