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Clinical Research Article

Impact of Conducting Adrenal Venous Sampling in the Morning Versus Afternoon in Primary Aldosteronism

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Abstract

Context: Adrenal venous sampling (AVS) is the gold standard technique for subtype differentiation of primary aldosteronism (PA) and to obtain aldosterone and cortisol measurements; however, their secretion patterns show fluctuations during the day.

Objective: We aimed to examine the effects of AVS timing on AVS results.

Methods: This multicenter, retrospective, observational study included a total of 753 patients who were diagnosed with PA and underwent AVS in 4 centers in Japan. Among them, 504 and 249 patients underwent AVS in the morning (AM-AVS) and in the afternoon (PM-AVS), respectively. The outcome measures were the impact of AVS timing and hormone fluctuations in a day on AVS results.

Results: There were no differences in the success rate of AVS, diagnostic rate of disease type, or frequency of discrepancy in PA subtypes between the AM-AVS and PM-AVS groups. Regarding patients with unilateral PA, aldosterone concentrations in adrenal venous blood did not differ between the 2 groups on the dominant or nondominant side. Conversely, regarding patients with bilateral PA, aldosterone concentrations in adrenal venous blood were significantly higher in the AM-AVS than in the PM-AVS group.

Conclusions: The timing of AVS did not seem to have a significant impact on subtype diagnosis. The aldosterone levels in adrenal venous blood were significantly higher in patients with bilateral PA in the AM-AVS group, but there was no such difference between patients with unilateral PA in the AM-AVS and PM-AVS groups. Each subtype may have a different hormone secretion pattern in a day.

Key Words: primary aldosteronism, adrenal vein sampling

Abbreviations: ACTH, adrenocorticotropic hormone; APA, aldosterone-producing adenoma; AVS, adrenal venous sampling; CLEIA, chemiluminescent enzyme immunoassay; LI, lateralization index; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PASO, Primary Aldosteronism Surgical Outcome; RIA, radioimmunoassay; SI, selectivity index.

Primary aldosteronism (PA) requires appropriate diagnosis and treatment as it is associated with a higher frequency of cerebral and cardiovascular complications than essential hypertension in patients of similar ages and with similar blood pressure levels [1]. Additionally, in previous studies, higher prevalence rates of obesity sleep apnea syndrome and impaired glucose tolerance have been reported among patients with PA than among patients with essential hypertension [2]. Although PA is partly characterized by hypokalemia, recent reports have shown a high frequency of normokalemia among patients with PA [3], making it difficult to differentiate it from essential hypertension. For these reasons, the clinical practice guideline of the Japan Endocrine Society recommends PA screening for all patients with hypertension. Patients with resistant hypertension are particularly recommended to undergo PA screening as it may be more cost-effective than lifelong medications [2].

Furthermore, the frequency of PA is estimated to be 3% to 10% in the population with hypertension [4–6]; therefore, it is considered a common disease. Treatment strategies include therapy with mineralocorticoid receptor antagonists or surgery, depending on the PA subtype [7, 8].

The most common procedure for determining the PA subtype is adrenal venous sampling (AVS), wherein blood is drawn directly from the vicinity of the adrenal gland using a catheter and the levels of cortisol and aldosterone secreted by the adrenal cortex are measured. Cortisol is regulated by adrenocorticotropic hormone (ACTH), which is secreted by the anterior pituitary gland. The cortisol level is generally the highest upon awakening and gradually declines over the

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course of the day. Chan and Debono found that in 33 healthy individuals who underwent 20-min cortisol profiling over a 24-hour period, the cortisol levels were the lowest around midnight, began to rise at around 2:00 to 3:00, and peaked at around 8:30 [9]. Aldosterone, similar to cortisol, is also regulated by ACTH and shows diurnal fluctuations, being the highest in the early morning and low in the late evening [10]. Thosar et al showed that the aldosterone levels in healthy individuals exhibit a significant endogenous rhythm, rising at night and peaking in the morning [11]. They also found that this early morning increase in aldosterone is attributed to circadian rhythms and increased morning activity, and it is not caused by presleep inactivity associated with sleep. Other factors that can cause aldosterone levels to fluctuate include body position, salt intake, aging, sex hormones, and medications.

AVS is an invasive and challenging test; however, because of the high frequency of PA, it is currently performed at many institutions in Japan. AVS is generally performed during exogenous ACTH administration, in other words ACTH loading, to improve the success rate of the procedure [12]. However, AVS is also performed before and after ACTH loading. Considering the effects of diurnal variations in the levels of aldosterone and cortisol, the Endocrine Society guidelines recommend that AVS without ACTH loading should be performed in the morning [13]. However, owing to staffing issues and general limitations, AVS is performed in the afternoon in many facilities. Importantly, the impact of AVS timing has not been adequately studied to date. In this study, we examined the effects of AVS timing (morning/afternoon) on hormone secretion of the adrenal gland, as it is well known that cortisol level shows diurnal fluctuations and is elevated by stress. Furthermore, we evaluated the effect of AVS timing on PA diagnosis. The results of our evaluation of the impact of AVS timing could help more facilities to accurately diagnose patients with PA and help clarify the pathogenesis of each form of PA.

Materials and Methods

Patients

The study included 753 patients diagnosed with PA between 2011 and 2021 at 4 specialized institutions where AVS was performed. We followed the 2014 guidelines of the Japanese Society of Hypertension [14]. Screening was performed using a cut-off aldosterone:renin ratio (value of plasma aldosterone concentration [PAC] divided by plasma renin activity, pg/mL per ng/mL/hour) of 200 (a ratio >200 indicated a positive result). PA diagnosis was confirmed by the captopril challenge test, furosemide plus upright test, or saline infusion test. All the testing protocols adhered to the guidelines of the Japan Endocrine Society [15]. The 753 patients were divided into 2 groups according to the timing of the AVS procedure. The group classification of patients according to the time of the day when AVS was performed was not prospectively designed but rather determined on the basis of feasible conditions at each facility. The AM-AVS group consisted of 504 patients whose AVS was initiated between 9 AM and 11 AM. Of these, 123 were excluded: 118 had only basal AVS without ACTH stimulation, and 5 were not diagnosed with PA. The PM-AVS group included 249 patients whose AVS was initiated at 2:00 PM. A total of 40 patients were excluded because of insufficient data. The ACTH loading method involved bolus injection. The criterion for successful selective catheter insertion into the adrenal vein was the ratio of cortisol concentration in the adrenal vein to that in the inferior vena cava [13]. The same criteria were used, regardless of the implementation time. The lateralization index (LI) is defined as the ratio of the ipsilateral adrenal vein aldosterone to the cortisol concentration ratio over the contralateral aldosterone to cortisol ratio. In the basal AVS without ACTH stimulation, unilateral aldosterone overproduction was confirmed by LIs of >2. In ACTH-stimulated AVS, unilateral aldosterone overproduction was confirmed by LIs of >4, according to the Endocrine Society guideline [13]. For postoperative evaluation, the Primary Aldosteronism Surgical Outcome (PASO) study's consensus criteria for outcomes and follow-up of adrenalectomy for unilateral PA were used [16]. Concerning the steroid hormone measurements, 2 types of measurements exist owing to changes in hormone measurement methods in Japan. The PAC was measured by the radioimmunoassay (RIA) method. However, as of April 2021, PAC is measured by chemiluminescent enzyme immunoassay (CLEIA) owing to the discontinuation of the RIA method [2]. The timing of the change in measurement method varied among facilities. In our cohort, the CLEIA method was used in 9 out of all cases of AVS.

Ethics and Statistics

This clinical study was approved by the medical ethics committees of each of the 4 institutions. All the study procedures were performed in accordance with the Declaration of Helsinki. In addition, a waiver of consent was obtained from the ethics committee of the Kanazawa University. Statistical analyses were performed using PRISM software (OMS, Tokyo, Japan). The Mann–Whitney U test and chi-squared test were used to compare differences between the independent groups. The Wilcoxon test was used to evaluate the differences between the 2 conditions among the correlated samples. Spearman's rank correlation coefficients were used to determine the relationship between the 2 datasets. Statistical significance was set at $P < .05$. Box-and-whisker diagrams were used to display the results. Each box-and-whisker plot shows, from top to bottom, the maximum, 75th percentile, median, 25th percentile, and minimum values.

Results

Clinical Characteristics of the Patients

Table 1 shows the clinical characteristics of the patients in each group. There were no significant differences in age, systolic blood pressure, and diastolic blood pressure between the AM-AVS and PM-AVS groups. However, the serum potassium levels were significantly lower and the plasma aldosterone levels were significantly higher in the AM-AVS than in the PM-AVS group.

Comparison of Cortisol Levels in the Inferior Vena Cava During AVS

In the AM-AVS group, the cortisol levels in the inferior vena cava obtained with ACTH-stimulated AVS were significantly higher than those obtained with basal AVS (Fig. 1). Similarly, in the PM-AVS group, the inferior vena cava cortisol levels obtained with ACTH-stimulated AVS were significantly higher than those obtained with basal AVS (Fig. 1). However, regarding basal AVS results, the AM-AVS group had a significantly

Table 1. Clinical characteristics of each group that underwent adrenal vein sampling (AVS) in the morning (AM-AVS) and afternoon (PM-AVS)

	AM-AVS	PM-AVS	
Number of patients, n	381	209	
Age, y, mean (range)	54 (23-81)	53 (28-82)	$P = .39$
Sex, males/females	193/188	97/112	$P = .34$
Systolic blood pressure, mmHg	137 ± 17	138 ± 16	$P = .37$
Diastolic blood pressure, mmHg	84 ± 14	86 ± 14	$P = .37$
Serum potassium, mEq/L	3.8 ± 0.5	3.9 ± 0.4	$P < .01$
Plasma aldosterone concentration, pg/mL	182 ± 140	160 ± 115	$P < .01$

Abbreviations: AVS, adrenal vein sampling; ACTH, adrenocorticotropic hormone; ACTH-s, ACTH-stimulated.

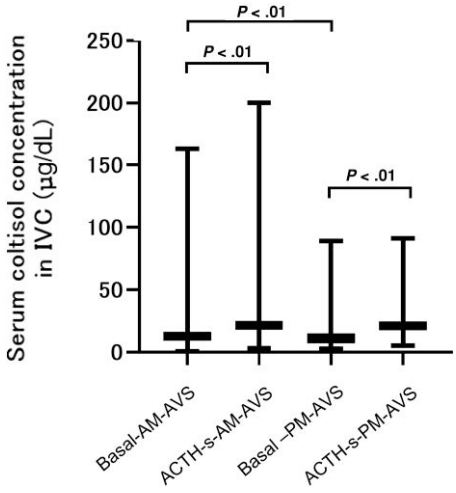

Figure 1. Comparison of the cortisol levels in the inferior vena cava blood during AVS. In both AM-AVD and PM-AVS groups, cortisol concentrations in the IVC significantly increased by ACTH stimulation. Regarding basal AVS, cortisol concentrations were significantly higher in the AM-AVS group than in the PM-AVS group. Data were compared between the 2 groups using the Mann–Whitney U test; data are presented in a box-and-hide diagram. ACTH, adrenocorticotropic hormone; ACTH-s, ACTH-stimulated; AVS, adrenal venous sampling; IVC, inferior vena cava.

higher cortisol level in the inferior vena cava; there were no significant differences between the 2 groups in the ACTH-stimulated AVS results.

Comparison of Selectivity Index Between AM-AVS and PM-AVS

There was no significant difference in the selectivity index (SI) of the right adrenal vein during basal AVS between the AM-AVS and the PM-AVS groups (Fig. 2). There was no significant difference in the SI of the right adrenal vein during ACTH-stimulated AVS between the AM-AVS and PM-AVS groups. In both the AM-VAS and PM-VAS groups, the SI of the right adrenal vein during ACTH-stimulated AVS was significantly higher than that during basal AVS.

There was no significant difference in the SI of the left adrenal vein during basal AVS between the AM-AVS and PM-AVS groups (Fig. 2). The SI of the left adrenal vein during

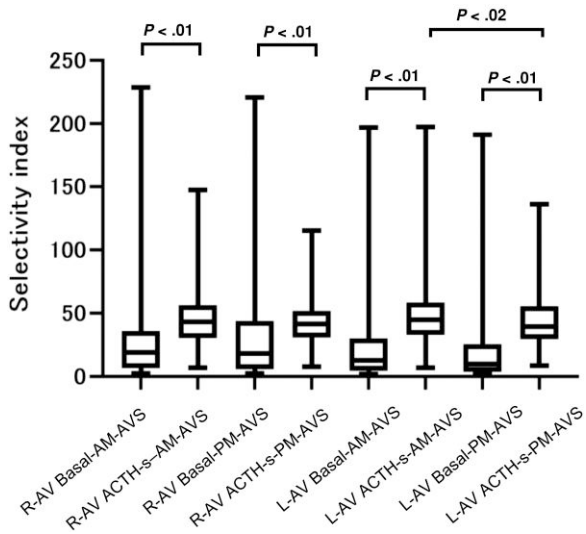

Figure 2. Comparison of SI of the left and right adrenal veins during basal and ACTH-s AVS in AM-AVS and PM-AVS groups. SI significantly increased by ACTH stimulation in both AM-AVS and PM-AVS groups, and SI of the left adrenal vein during ACTH-s AVS was significantly higher in the AM-AVS group. Data were compared between the 2 groups using the Mann–Whitney U test; data are shown in a box-and-whisker diagram. R-AV, right adrenal vein; L-AV, left adrenal vein; SI, selectivity index; ACTH, adrenocorticotropic hormone; ACTH-s, ACTH-stimulated; AVS, adrenal venous sampling.

ACTH-stimulated AVS was significantly higher in the AM-AVS group than in the PM-AVS group ($P = 0.2$). In both the AM-VAS and PM-VAS groups, the SI of the left adrenal vein was significantly higher during ACTH-stimulated AVS than during basal AVS.

Comparison of AVS Success Rates

The success rate of basal AVS in the AM-AVS group was 81% (307/381) and that of ACTH-stimulated AVS was 92% (349/381) (Fig. 3). The overall success rate was 76% (288/381 patients). The success rate of basal AVS in the PM-AVS group was 74% (155/209) and that of ACTH-stimulated AVS was 88% (184/209) (Fig. 3). The overall success rate was 70% (147/209 patients). There was no significant difference in the AVS success rate between the AM-AVS and PM-AVS groups.

Comparison of Aldosterone Levels in the Inferior Vena Cava Blood

Regarding basal AVS, the aldosterone levels in the inferior vena cava of the AM-AVS group were significantly higher than those in the PM-AVS group (Fig. 4). Regarding ACTH-stimulated AVS, the aldosterone levels in the inferior vena cava of the AM-AVS group were significantly higher than those of the PM-AVS group. In both groups, ACTH stimulation significantly increased the aldosterone levels in the inferior vena cava.

Impact of AVS Time on PA Subtype Determination

In the AM-AVS group, both basal and ACTH-stimulated AVS were successful in 288 patients, of whom 44 (15%) were diagnosed with unilateral PA, based on both basal AVS and ACTH-stimulated AVS results (Fig. 5). In all, 120 patients

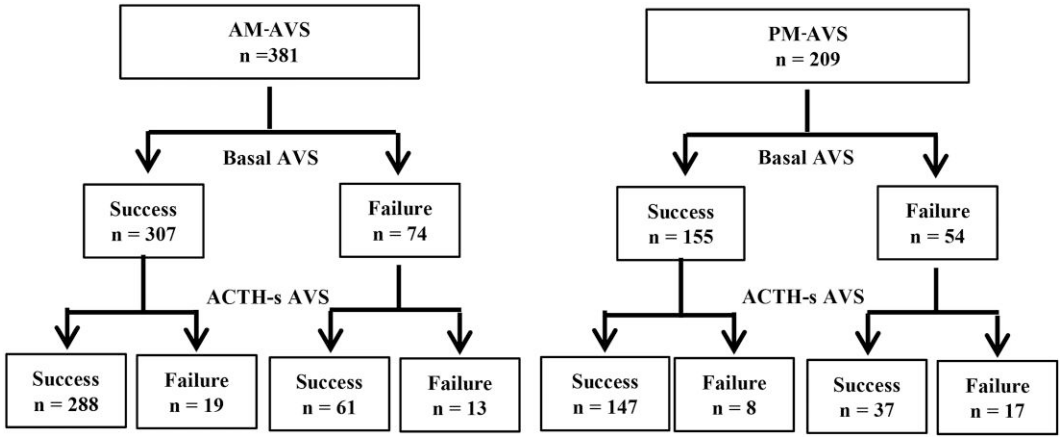

Figure 3. Success rate of AVS in AM and PM. SI <2 during basal AVS and SI <5 during ACTH-s AVS were excluded as failure. In the AM-AVS group, the success rates were 81% (307/381 patients) for basal AVS and 92% (350/381 patients) for ACTH-s AVS, with an overall success rate of 76% (288/381 patients). In the PM-AVS group, the success rates were 74% (155/209 patients) for basal AVS and 88% (184/209 patients) for ACTH-s AVS, with an overall success rate was 70% (147/209 patients). There was no significant difference in the AVS success rates between the AM-AVS and PM-AVS groups. SI, selectivity index; ACTH, adrenocorticotropic hormone; ACTH-s, ACTH-stimulated; AVS, adrenal venous sampling.

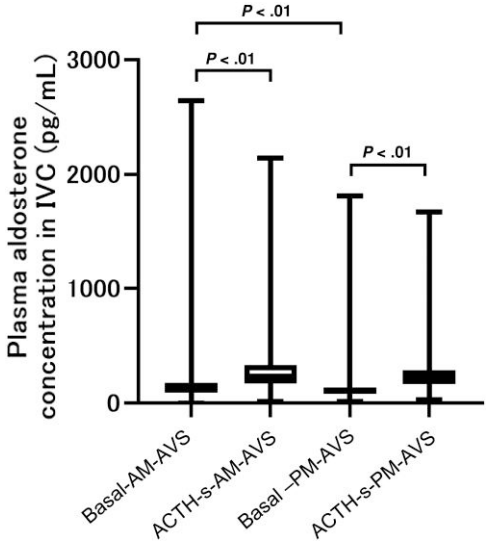

Figure 4. PAC in the inferior vena cava blood. The PAC of ACTH-s AVS was significantly higher than the PAC of basal AVS in both AM and PM groups. Regarding basal AVS, the PAC was significantly higher in the AM than in the PM. Data were compared between the 2 groups using the Mann–Whitney U test; data are shown in a box-and-whisker diagram. ACTH, adrenocorticotropic hormone; ACTH-s, ACTH-stimulated; AVS, adrenal venous sampling; PAC, plasma aldosterone concentration.

A

Basal AVS	ACTH-s AVS: LI > 4	ACTH-s AVS: LI < 4
LI > 2	44	120
LI < 2	6	119

B

Basal AVS	ACTH-s AVS: LI > 4	ACTH-s AVS: LI < 4
LI > 2	16	54
LI < 2	4	73

Figure 5. Numbers of subtype diagnoses in AM (A) and PM (B) groups. Chi-squared tests were used. LI, lateralization index.

(42%) were classified as having unilateral PA according to basal AVS results and bilateral PA according to ACTH-stimulated AVS results. Six patients (2.1%) were classified as having bilateral PA according to basal AVS and unilateral PA according to ACTH-stimulated AVS results. Further, 119 patients (41%) were classified as having bilateral PA according to both basal AVS and ACTH-stimulated AVS results.

In the PM-AVS group, both basal and ACTH-stimulated AVS were successful in 147 patients. Sixteen (11%) of the 147 patients were diagnosed with unilateral lesions according to both basal AVS and ACTH-stimulated AVS results (Fig. 5); 54 (37%) were classified as having unilateral PA according to basal AVS and bilateral PA according to ACTH-stimulated AVS results. Four patients (3%) were classified as having bilateral PA according to basal AVS and unilateral lesions according to ACTH-stimulated AVS results. Further, 73 patients (50%) were classified as having bilateral PA according to both basal and ACTH-stimulated AVS results. There was no significant difference between the AM-AVS and PM-AVS groups concerning the rate of unilateral diagnosis by AVS before ACTH stimulation, the rate of unilateral PA diagnosis by AVS after ACTH stimulation, and the percentage of cases with discrepancies between the 2 AVSs.

Comparison of Adrenal Vein Aldosterone Levels and PASO Criteria in Unilateral PA

In the present study, there was a discrepancy in subtype diagnosis using basal AVS and ACTH-stimulated AVS results. Therefore, we considered cases diagnosed with unilateral PA in both AVSs as definite unilateral PA, and the aldosterone levels were compared between the dominant and nondominant adrenal veins (Fig. 6).

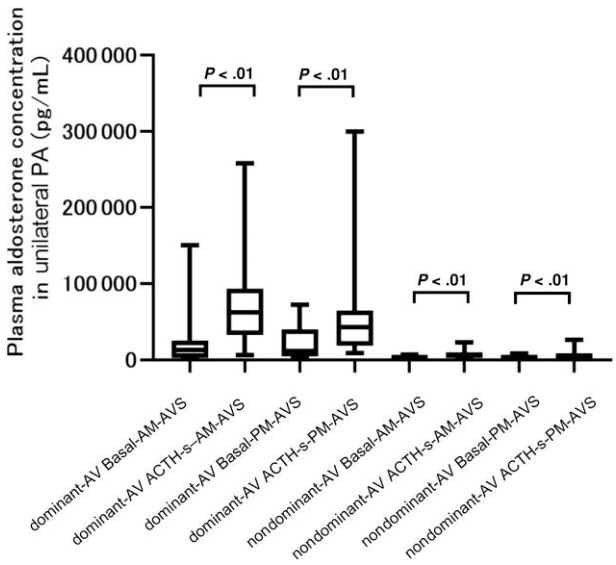

Figure 6. PAC in the adrenal veins of patients diagnosed with unilateral PA. Of the patients with successful AVS before and after ACTH stimulation, those with LI >2 before loading and LI >4 after loading were classified as having unilateral PA. In both dominant-side adrenal venous blood and nondominant-side adrenal venous blood, PAC significantly increased with ACTH stimulation; however, there was no significant difference in the PAC of dominant-side adrenal venous blood and nondominant-side adrenal venous blood between the AM and PM groups for both basal AVS and ACTH-s AVS. Data were compared between the 2 groups using the Mann–Whitney U test; data are presented in a box-and-whisker diagram. ACTH, adrenocorticotropic hormone; ACTH-s, ACTH-stimulated; AVS, adrenal venous sampling; PAC, plasma aldosterone concentration; PA, primary aldosteronism.

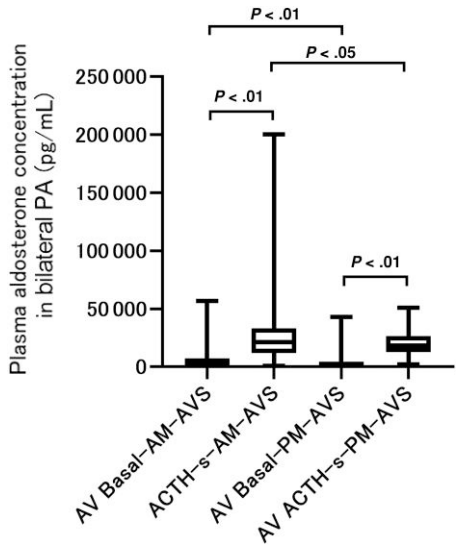

Figure 7. PAC in adrenal venous blood in patients with bilateral PA. PAC significantly increased with ACTH stimulation in both AM and PM groups. Those with LI values <2 during basal AVS and LI values <4 during ACTH-s AVS were classified as having bilateral lesions, respectively. PAC in adrenal venous blood was significantly higher in AM than in PM group for both basal AVS and ACTH-s AVS. Data were compared between the 2 groups using the Mann–Whitney U test; data are presented in a box-and-whisker diagram. AV, adrenal vein, ACTH, adrenocorticotropic hormone; ACTH-s, ACTH-stimulated; AVS, adrenal venous sampling; PAC, plasma aldosterone concentration.

Table 2. IVC aldosterone concentrations in patients with unilateral PA in the morning (AM-AVS) and afternoon (PM-AVS)

	AM-AVS	PM-AVS	
Basal-AVS, median (minimum-maximum)	205 (27-1209)	150 (64-505)	$P = .33$
ACTH-stimulated AVS, median (minimum-maximum)	354 (81-2140)	316 (130-1510)	$P = .74$

Abbreviations: AVS, adrenal venous sampling; IVC, inferior vena cava.

In the AM-AVS group, 44 patients were diagnosed with unilateral PA with both AVS results. Fifteen patients were not operated on or not evaluated after surgical treatment because of preoperative complications (n = 3), surgical treatment at another institution (n = 5), aldosterone overproduction from the contralateral side of a manifest adrenal tumor (n = 5), or other patient-related reasons (n = 2). Of the 29 patients in the AM-AVS group who underwent confirmed surgical treatment (including 2 patients with radiofrequency ablation), those with postoperative information were retrospectively evaluated using the PASO criteria.

In the PM-AVS group, 16 patients were diagnosed with unilateral PA with both AVS results. In the PM-AVS group, 12 patients with confirmed surgical treatment were evaluated retrospectively using the PASO criteria. In the comparison using the PASO criteria, the AM-AVS group had 12 complete, 13 partial, 1 absent, and 3 not evaluable cases in terms of clinical success, while the PM-AVS group had 7 complete, 4 partial, and 1 absent case, showing no significant difference between the AM-AVS and PM-AVS groups in the ratio of complete to partial success cases. In terms of biochemical success, the AM-AVS group had 21 complete, 4 partial, 2 absent, and 2 not evaluable cases. In contrast, the PM-AVS group had 10 complete, 1 absent, and 1 not evaluable case. There was no significant difference between the AM-AVS and PM-AVS groups in the ratio of complete to partial cases.

The aldosterone levels in the dominant adrenal vein of patients with unilateral PA according to basal AVS results were not significantly different between the AM-AVS and PM-AVS groups. Similarly, aldosterone levels in the dominant adrenal vein of patients with unilateral PA according to ACTH-stimulated AVS results were not significantly different between the AM-AVS and PM-AVS groups. There was also no significant difference between the AM-AVS and PM-AVS groups with respect to the aldosterone levels in the nondominant adrenal vein of patients with unilateral PA, according to both basal and ACTH-stimulated AVS results. In addition, there was no significant difference in the inferior vena cava cortisol levels between the AM-AVS and PM-AVS groups. In addition, the inferior vena cava aldosterone levels showed no significant differences between the AM-AVS and PM-AVS groups (Table 2).

Comparison of Adrenal Vein Aldosterone Levels in Patients With Bilateral PA

Because of the discordance in subtype diagnosis between basal AVS and ACTH-stimulated AVS, the aldosterone levels in the adrenal veins of patients diagnosed with bilateral PA according to both AVSs were compared between the AM-AVS and PM-AVS groups (Fig. 7). The aldosterone levels in the adrenal veins were significantly higher in the AM-AVS group than in

Table 3. IVC-aldosterone concentrations in patients with bilateral PA in the morning (AM-AVS) and afternoon (PM-AVS)

	AM-AVS	PM-AVS	
Basal-AVS, median (minimum-maximum)	113 (13-396)	96 (13-875)	$P = .15$
ACTH-stimulated AVS, median (minimum-maximum)	221 (13-829)	192 (74-431)	$P = .13$

Abbreviations: AVS, adrenal vein sampling; ACTH, adrenocorticotropic hormone; IVC, inferior vena cava.

the PM-AVS group. However, there was no significant difference in the inferior vena cava aldosterone levels between the AM-AVS and PM-AVS groups. In addition, the inferior vena cava aldosterone levels of the AM-AVS and PM-AVS groups showed no significant differences (Table 3).

Discussion

Cortisol has 1 of the clearest and most fascinating circadian rhythms among the hormones involved in human physiology [9]. In the present study, cortisol concentrations in the inferior vena cava obtained with the basal AVS were significantly higher in the AM-AVS than in the PM-AVS group. This result is consistent with the circadian rhythm of cortisol. Moreover, cortisol is secreted by stimulation of ACTH, which is secreted by the pituitary gland; there was no difference in the cortisol levels in the inferior vena cava between the AM-AVS and PM-AVS groups in the AVS after ACTH bolus infusion. This indicates that ACTH stimulation during AVS can eliminate the effects of diurnal variations in cortisol levels due to the timing of AVS. Cortisol is used to determine the success or failure of vascular insertion into the adrenal vein in AVS. In the present study, which also examined the SI, the SI was significantly higher during ACTH-stimulated AVS in the AM-AVS than in the PM-AVS group only in the left adrenal vein. However, the absolute difference in the SI was not large. Examination of other factors showed no significant differences between the AM-AVS and PM-AVS groups. Furthermore, there was no significant difference between the AM-AVS and PM-AVS groups in terms of the success rate of AVS. Our study suggests that cortisol is as important as aldosterone in AVS, but diurnal variations in cortisol due to AVS timing do not significantly affect the AVS results.

Aldosterone is a hormone that exhibits diurnal fluctuations. PA is caused by autonomous production of aldosterone by adrenocortical adenomas and hyperplasia. Aldosterone secretion in patients with PA may also show diurnal variations. Kem et al reported that in 9 patients with hyperaldosteronism, the rhythm of PAC was similar to the circadian pattern of plasma cortisol, and normalization of these data showed an excellent correlation between the 2 hormones. In particular, their study of short-term suppression of ACTH with dexamethasone revealed that the circadian variation in plasma aldosterone disappeared in 4 of 5 patients, indicating that the circadian variation in plasma aldosterone levels is mediated by ACTH changes [17]. In our study, similar to cortisol levels during basal AVS, aldosterone concentrations in the inferior vena cava were significantly higher in the AM-AVS than in the PM-AVS group. Our results also suggest the possibility of diurnal variations in aldosterone concentrations in patients with PA.

There are other factors that cause fluctuations in cortisol concentration and PAC. Medication, such as antihypertensive agents, may change hormone secretion patterns. Previous studies have reported that angiotensin I receptor antagonist, calcium channel blockers, and angiotensin-converting enzyme inhibitor can reduce the amount of aldosterone in the plasma [18]. Hypokalemia also decreases PAC [19]. Furthermore, different methods (including posture, method of drawing blood, and catheter position) can result in hormone variations [18, 20, 21]. In the present study, all participants who underwent AVS were administered either a calcium channel blocker or alpha blocker. Therefore, considerable differences in hormone variation in individuals may not have been detected.

Various studies have suggested that the diurnal variation in aldosterone secretion in patients with PA varies by subtype. Schambelan et al examined each PA subtype and reported that the plasma aldosterone levels decreased in parallel with the circadian rhythm of cortisol in patients with aldosterone-producing adenoma (APA), while the aldosterone levels increased in parallel with a slight increase in renin and potassium in patients with idiopathic hyperaldosteronism [22]. Sonoyama et al also examined each subtype of PA and reported a prominent decrease in aldosterone concentration after dexamethasone administration in patients with APA, indicating that aldosterone secretion is more strongly dependent on endogenous ACTH in patients with APA than in patients with idiopathic hyperaldosteronism and non-PA [23]. Tezuka et al also reported that morning and night concentrations of aldosterone were significantly higher in patients with APA than in those with bilateral PA; furthermore, the APA group showed a significantly greater increase in aldosterone levels by ACTH stimulation [24]. However, these studies examined PACs in peripheral blood, while we examined the PAC in adrenal venous blood. Moreover, our study showed results opposite to those previously reported. The aldosterone concentrations in adrenal venous blood did not differ between patients with unilateral PA in the AM-AVS and PM-AVS groups on the dominant or nondominant side.

Conversely, regarding bilateral PA, aldosterone concentrations in adrenal venous blood were significantly higher in the AM-AVS than in the PM-AVS group. These results suggest that when adrenal venous blood is used, there is no diurnal variation in aldosterone secretion in patients with unilateral PA, as opposed to in patients with bilateral PA. Although the results of this study do not provide insights into the etiology of aldosterone autocrine secretion for each subtype, they provide a clue to the question of whether aldosterone secretion is a function of the diurnal variation in bilateral PA.

AVS has been reported to cause discrepancies in the determination of the disease type before and after ACTH loading. However, this factor is not yet well understood. ACTH stimulation during AVS is performed at many major centers worldwide [25]. The Japanese guidelines list 3 objectives of ACTH stimulation during AVS: (1) to minimize the effects of stress-induced fluctuations in aldosterone secretion, (2) to define the cortisol concentration gradient between the adrenal and inferior vena cava, and (3) to maximize aldosterone secretion in the APA [2]. In contrast, it has been reported that ACTH stimulation causes discrepancies in the final diagnosis between basal and ACTH-stimulated AVS in some cases [12, 26, 27]. In addition, a multicenter retrospective study in Japan

showed an increase in the proportion of patients diagnosed with bilateral PA after ACTH stimulation, wherein the changes in the LI before and after ACTH stimulation of the same patient were compared [28]. The discrepancy in the diagnosis of AVS before and after ACTH stimulation may be attributed to the presence of aldosterone-producing cell clusters [12]. A previous multicenter retrospective study showed that patients who fall under bilateral PA according to ACTH-stimulated AVS results have worse surgical outcomes than those who fall under unilateral PA according to both basal and ACTH-stimulated AVS [28]. However, in that report, among patients who changed from unilateral to bilateral before and after ACTH stimulation, the postoperative results were better in those with an LI of ≥ 8.3 during basal AVS. We also reported that some patients with bilateral PA according to ACTH-stimulated AVS also had APA with APA-specific genetic mutations. While ACTH stimulation improves the success rate of AVS, to our knowledge, there is no evidence suggesting that it improves localization. In the present study, there was no significant difference between the AM-AVS and PM-AVS groups concerning the rates of unilateral diagnosis by AVS before and after ACTH stimulation and the percentage of cases with discrepancies in the AVS results. The results of this study suggest that the variation in the hormonal levels throughout a day owing to AVS timing is not a cause of discrepancy in the diagnosis of the PA subtype with or without ACTH stimulation.

There are limitations to this study. First, in Japan, the PAC was measured by RIA since 2009; however, the use of this method was discontinued after April 2021, and it was replaced with CLEIA, which does not use isotopes. In fact, most patients enrolled in the study underwent RIA for screening, definitive diagnosis, and hormone measurements at the time of adrenal vein sampling. The results of CLEIA, which uses a specific monoclonal antibody against aldosterone, show a good correlation with the results of liquid chromatography with tandem mass spectrometry. In addition, the conventional RIA method yields higher PAC values than the CLEIA method, and evidence needs to be accumulated on this aspect in the future. Second, it has been reported that subtype diagnosis based on basal AVS and ACTH-stimulated AVS results favors a specific subtype, which was also the case in this study. Therefore, in the analysis of unilateral and bilateral PA, only the data of patients with concordant subtype diagnosis in both AVS were used. Third, although there were differences between the AM-AVS and PM-AVS groups according to the circadian rhythm of hormones, we cannot clearly state that the differences were related to the circadian rhythm of hormones. As we did not investigate the individual AVS outcome variation at different times, it is difficult to present the effect of diurnal hormone fluctuation directly.

This study evaluated the effect of AVS timing. Although this was a multicenter study, there were no differences in the success rate of AVS, diagnostic rate of subtype, or frequency of deviation in the subtypes between the AM-AVS and PM-AVS groups. In cases in which both AVSs showed unilateral PA, there was no significant difference in the postoperative results between the 2 groups using the PASO criteria. This was observed not only after ACTH stimulation but also after basal AVS. These results are relevant for facilities that can perform AVS only in the afternoon. Concerning aldosterone levels, no differences were observed between patients with unilateral lesions in the AM-AVS and PM-AVS groups, whereas its levels were different between patients with bilateral lesions in the AM-AVS and PM-AVS groups, suggesting the involvement of different mechanisms that regulate aldosterone secretion according to the circadian rhythm in patients with unilateral or bilateral lesions.

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Disclosure

The authors have nothing to disclose.

Data Availability

Availability restrictions apply to some or all of the data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. On request, the corresponding author will provide details of the restrictions and conditions under which access to some data may be provided.

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